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Assessment of biological effects of environmental pollution along the NW Mediterranean Sea using mussels as sentinel organisms

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The biomarker approach is suitable for assessment of environmental pollution in the NW Mediterranean Sea.

Abstract

With the aim of assessing the biological effects of pollution along three gradients of pollution in the NW Mediterranean Sea, a biomonitoring survey was implemented using a battery of biomarkers (lysosomal membrane stability, lysosomal structural changes, metallothionein (MT) induction and peroxisome proliferation) in mussels over a period of two years as part of the EU-funded BEEP project. Mussels from the most impacted zones (Fos, Genova and Barcelona harbours) showed enlarged lysosomes accompanied by reduced labilisation period of lysosomal membranes, indicating disturbed health. MT levels did not reveal significant differences between stations and were significantly correlated with gonad index, suggesting that they were influenced by gamete development. Peroxisomal acyl-CoA oxidase (AOX) activity was significantly inhibited in polluted stations possibly due to interactions among mixtures of pollutants. In conclusion, the application of a battery of effect and exposure biomarkers provided relevant data for the assessment of biological effects of environmental pollution along the NW Mediterranean Sea.

Keywords: Biomonitoring NW Mediterranean Sea; *Mytilus galloprovincialis*; Lysosomal responses; Metallothioneins; Peroxisome proliferation

1. Introduction

The Mediterranean Sea is a semi-enclosed basin covering an area of 2.5 million km^2 and containing 3.7 million km^3 of water. The water exchange time is of about 80 years and this slow turnover rate results in a high anthropogenic impact. Urbanisation has been particularly growing along the coastline, to accommodate both permanent and temporal population (the Mediterranean is the greatest tourism destination in the world), with the result of a substantial modification of the coast and adverse effects on the quality of the environment. There is a large range of industrial activities widespread all along the Mediterranean basin, and a number of highly industrialised spots that are concentrated mainly in the NW part of the region. All these activities constitute sources of pollution through direct disposal, continental runoff and atmospheric transport (UNEP Chemicals, 2002). The presence of these pollution hot spots, located generally in semi-enclosed gulfs and bays near important harbours, big cities and industrial areas, is probably the major problem in the Mediterranean Sea (EEA, 1999). Regarding only petroleum hydrocarbon pollution, between 1987 and the end of 1996 an estimated 22 223 tonnes of oil entered the Mediterranean Sea as the result of shipping incidents causing localised damage to the Mediterranean marine and coastal environment (EEA, 1999), and 250 000 tonnes of petroleum hydrocarbons are discharged per year due to shipping operations (UNEP Chemicals, 2002). Other chemicals such as polychlorinated biphenyls (PCBs) and derivates, pesticides and metals are also continuous sources of pollution.

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In view of these problems, the conservation of the Mediterranean Sea environment has become a matter of growing concern. In 1975, the United Nations Environment Programme (UNEP) launched the Mediterranean Action Plan (MAP) and, since then, several programmes covering scientific, socio-economic and legal aspects of the protection of the Mediterranean environment have been implemented. The first monitoring activities were initiated with the MED POL programme that is the scientific and technical component of the MAP (UNEP, 1997). Afterwards, the UNEP recommended a series of biomarkers for the MED POL biomonitoring programme (UNEP/RAMOGE, 1999). These measurements of biological response might be used to identify sources of pollution and biological effects of a wide range of pollutants (Cajaraville et al., 2000). The use of a battery of different biomarkers is essential in biomonitoring programmes, as the complexity of environmental contaminants can induce varieties of responses in organisms that are not necessarily correlated (Viarengo et al., 2000). Biomarkers are measurements at the molecular, biochemical or cellular level, which indicate that the organism has been exposed to pollutants (exposure biomarker) and/or the magnitude of the organism's response to the pollutants (effect biomarker) (McCarthy and Shugart, 1990). Owing to the rapid response of biomarkers, they are used as early warning signals of the biological effects of environmental pollutants, in order to forecast changes at higher levels of biological organisation.

In the framework of the EU-funded BEEP project (''Biological Effects of Environmental Pollution in marine coastal ecosystems'') a biomonitoring programme was performed along the NW Mediterranean Sea using mussels (*Mytilus galloprovincialis*) as sentinels (Beliaeff and Bocquené, 2004; Shaw et al., 2004; Narbonne et al., 2005; Auffret et al., 2006). Mussels have been employed worldwide since Goldberg (1975) proposed the ''Mussel Watch'' concept, firstly for chemical monitoring but afterwards also for the assessment of biological effects of pollution. These sessile and filter-feeding organisms are very suitable as sentinels because they accumulate contaminants to a great extent and respond significantly to pollutant exposure (Cajaraville et al., 2000). The aim of the present study was to apply a battery of effect and exposure biomarkers in mussels to monitor the biological impact of pollutants at sites particularly exposed to domestic and industrial wastes in the NW coast of the Mediterranean Sea. For this purpose, mussels were sampled at the beginning of the spawning period (May) and after spawning was completed (September) over a period of two years (2001–2003) from three pollution gradients in the Gulf of Fos/Marseille area (France), the Ligurian Sea (Italy), and the Catalonian coast (Spain). In the past decades the Gulf of Fos was considered as one of the most polluted areas in Europe attending to the metal concentration in the water column (Benon et al., 1978). More recent studies also reported the presence of high levels of organic pollutants in sediments from this area (Baumard et al., 1998). These levels of exposure to contaminants seemed to have effects on the biota as demonstrated by the high levels of ethoxyresorufin-*O*-deethylase (EROD), catalase and glutathione-*S*-transferase (GST) activities in fish (Burgeot et al., 1996). Similarly, chemical analyses performed in native and caged mussels have revealed the presence of moderate to high levels of polycyclic aromatic hydrocarbons (PAHs) in the Ligurian Sea (Burgeot et al., 1996; Piccardo et al., 2001). The Genova harbour located in this area is heavily polluted with PAHs and trace metals, which results in significant genotoxic effects on mussels (Bolognesi et al., 2004; Regoli et al., 2004). In 1991, the shipwreck of the oil tanker the ''Haven'' resulted in the spillage of a large amount of crude oil into the Ligurian coast. As to the Catalonian coast, a wide range of pollutants can be found in the area. The Barcelona harbour presents a high concentration of PAHs whereas Cala Montjoy can be considered as relatively clean (Burgeot et al., 1996; Porte et al., 2001). Ebro Delta is a highly contaminated site with high concentrations of pesticides.

The biomarkers selected for the study, namely, lysosomal membrane stability and lysosomal structural changes (effect biomarkers), metallothionein (MT) induction (biomarker of exposure to metals) and peroxisome proliferation (biomarker of exposure to organic xenobiotics), are all recommended by UNEP/MAP (2005) for biological effects monitoring in the Mediterranean Sea. Lysosomal alterations in the digestive cells of molluscs have been used as general markers of pollutant-induced stress in several laboratory (Moore, 1988; Marigómez and Baybay-Villacorta, 2003) and field studies (Marigómez et al., 1996, 2005; Domouhtsidou and Dimitriadis, 2001). The evaluation of MT levels has been widely used to verify the presence of metal pollution in laboratory experiments (Roesijadi, 1992; Bolognesi et al., 1999) and in field studies using molluscs (Geret and Cosson, 2000; Bebianno and Serafim, 2003), crabs (Pedersen et al., 1998) and fish (Hylland et al., 1992). In mussels collected in the Eastern Mediterranean Sea a significant negative correlation between lysosomal membrane stability and MT content was found (Domouhtsidou et al., 2004). Peroxisome proliferation, measured in terms of acyl-CoA oxidase (AOX) activity induction and/or increased peroxisomal density, has been demonstrated to respond to changes in the bioavailability of PAHs and PCBs in mussels both in laboratory as well as in field conditions (Fahimi and Cajaraville, 1995; Krishnakumar et al., 1997; Cancio et al., 1998; Porte et al., 2001; Cajaraville et al., 2003; Orbea and Cajaraville, 2006). Gender and gamete development stage were also determined histologically with the aim of assessing possible disturbances in reproduction. It is well known that some environmental pollutants can cause alterations in gametogenesis, gender determination, differentiation and development in aquatic animals (WHO/IPCS, 2002).

2. Materials and methods

2.1. Sampling and chemical analysis

Mussels were sampled in September 2001, May and September 2002 and May 2003. Sampling was performed on the French oceanographic vessel ''L'Europe''. No mussels were sampled from the Spanish area in May 2002 due to technical problems, and no mussels were found in Cortiou in May 2003. The cruises followed a route starting from the Gulf of Fos/Marseille area in France towards the Ligurian Sea (Italy) and finishing in the Catalonian