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Contaminant accumulation and biomarker responses in caged mussels, *Mytilus galloprovincialis*, to evaluate bioavailability and toxicological effects of remobilized chemicals during dredging and disposal operations in harbour areas

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ABSTRACT

Remobilization of chemicals from contaminated sediments is a major risk associated with dredging and disposal operations in harbour areas. In this work caged mussels, *Mytilus galloprovincialis*, were chosen as bioindicator organisms to reveal the impact and recovery of organisms from these activities in the harbour of Piombino (Tuscany, Italy) where approximately 100,000 m^3 of sediments were removed and disposed in a local confined disposal facility (CDF). Organisms were deployed before, during and after the end of operations, selecting sites differently impacted by these activities. Temporal changes in environmental bioavailability and biological effects of pollutants were assessed by integrating analyses of trace metals and polycyclic aromatic hydrocarbons (PAHs) accumulated in tissues of caged mussels with a wide array of biomarkers reflecting exposure to specific classes of pollutants and different levels of cellular unbalance or toxicity. Such biological responses included levels of metallothioneins, activity of acyl CoA oxidase (AOX) as a marker of peroxisome proliferation, oxidative stress biomarkers (content of glutathione, enzymatic activities of catalase, glutathione *S*-transferases, glutathione reductase, glutathione peroxidases), total oxyradical scavenging capacity (TOSC) toward peroxyl and hydroxyl radicals, lysosomal membrane stability and genotoxic effects measured as DNA strand breaks and frequency of micronuclei. Obtained results indicated that a general disturbance was already present in the whole harbour area and especially in the inner site before the beginning of operations, when caged mussels exhibited a significant accumulation of PAHs and Pb, lower TOSC values and higher levels of both lysosomal and genotoxic damages. Bioavailability of trace metals and PAHs markedly increased during dredging activities with values up to 40 μg/g for Pb and up to 2200 ng/g for PAHs in tissues of caged mussels, a significant inhibition of antioxidant efficiency and increase of oxidative damages. While bioavailability of trace metals returned to the pre-dredging values after the end of operations, the accumulation of PAHs, oxidative effects and genotoxic damages remained elevated in mussels caged in the inner area and in front of CDF. Overall this study confirmed the utility of caged mussels to assess the remobilization of chemicals from dredged sediments and the onset of potentially harmful biological effects.

1. Introduction

Harbour areas represent critical environments in the Mediterranean with strategic economic importance but also pose major concerns because of the presence of toxic chemicals and their harmful effects on the marine ecosystems. Several industrial and urban sources (i.e. shipping, loading and bunkering operations, shipyards, accidental spills, wastewater emissions) are responsible for typically elevated concentrations of pollutants which accumulate in sediments due to the limited hydrodynamic energy on the inside portions of harbours. In this respect, dredging operations, which are regularly needed to maintain accessibility and navigational depths, require a proper assessment and management of contaminated sediments.

In Italy dredging activities are regulated by Legislative Decree n. 152/2006 and specific guidelines support management decisions on the disposal of harbour-dredged sediments (ICRAM-APAT, 2007). According to results of chemical–physical characterization of dredged materials integrated with acute and chronic toxicity bioassays, disposal options may include beneficial use (i.e. beach

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nourishments, construction of fill, landscaping, landfill cover, etc.), specific treatments or clean-up procedures, storage in confined disposal facilities (CDF). The direct open water discharge can be considered as the last option only for noncontaminated sediments when there is no technical or economical possibility for their reutilization (ICRAM-APAT, 2007).

The chemical and toxicological evaluations of sediments can indicate the hazard and the most appropriate disposal strategy to some degree, but they do not reflect the risks during the dredging operations. Remobilization of pollutants from sediments can greatly affect their mobility, bioavailability and effects on organisms, which should be adequately monitored, especially when the disposal option is the confinement of dredged materials within onsite CDFs. In fact, the more frequent concern about these artificial structures is their efficacy in isolating materials from recontamination of surrounding environments.

The measurement of bioaccumulation and biological responses in sentinel organisms is thus of great utility and a similar approach has already been reported in various harbours as a valuable tool to assess the impact and toxicological effects of chemicals remobilized from sediments (Stephensen et al., 2000; Regoli et al., 2002b, 2004; Stronkhorst et al., 2003; Frenzilli et al., 2004; Almroth et al., 2005; Sturve et al., 2005; Barsiene et al., 2006).

In this work, an active biomonitoring strategy with transplanted mussels, *Mytilus galloprovincialis*, has been applied during various phases of dredging in the harbour of Piombino (Tuscany, Italy) where approximately 100,000 m^3 of sediments were removed between January and May 2005 and disposed in a local CDF. Organisms were deployed before, during and after the end of dredging and disposal operations, selecting sites differently influenced by these activities.

Chemical analyses of trace metals and polycyclic aromatic hydrocarbons (PAHs) in caged mussels were expected to reflect changes of their bioavailability during different phases and in different areas of the harbour. These results were integrated with a multi-biomarker approach, measuring a wide array of biological responses at the molecular and subcellular levels. Such biomarkers are the earliest warning signals of chemical disturbance, being often very sensitive or specific toward particular classes of pollutants, and reflecting different levels of cellular unbalance and toxicity (Cajaraville et al., 2000). The induction of metallothionein-like proteins and peroxisome proliferation were chosen as specific responses toward exposure to trace metals and organic compounds, respectively. Metallothioneins are low molecular weight, cytosolic proteins rich in –SH groups, normally involved in homeostasis of trace metals and over-expressed by elevated intracellular concentrations of Hg, Cu, Cd and Zn (Viarengo et al., 1997). Peroxisomes are heterogeneous organelles containing various oxidases and antioxidant enzymes with a metabolic function associated to lipid metabolism and inactivation of ROS (Cajaraville et al., 2003). Peroxisome proliferation in mussels exposed to PAHs and polychlorinated biphenyls (PCBs) has been shown by the increased number and volume of peroxisomes, and by the induction of enzymes involved in fatty acid oxidation, such as acyl CoA oxidase (Cajaraville et al., 2003; Orbea and Cajaraville, 2006).

A typical pathway of chemical toxicity is mediated through the increased intracellular generation of reactive oxygen species (ROS) and modulation of antioxidant defences (Regoli et al., 2002a). Although variations of antioxidants can be difficult to predict and often exhibit contradictory results in field conditions, nonetheless such oxidative biomarkers have also been shown to be sensitive in revealing a prooxidant chemical challenge in Mediterranean mussels (Regoli and Principato, 1995; Regoli, 1998, 2000; Frenzilli et al., 2001; Roméo et al., 2003; Petrović et al., 2004; Regoli et al., 2004; Gorbi et al., 2008; Viarengo et al., 2007). In this study, prooxidant effects of chemicals potentially released from dredging activities were assessed by measuring enzymatic activities of catalase, glutathione *S*-transferases (GST), glutathione reductase, glutathione peroxidases and levels of glutathione. These data were integrated with the analyses of total oxyradical scavenging capacity (TOSC) to quantify the overall cellular resistance toward peroxyl radicals, $ROO^•$, and hydroxyl radicals, $HO^•$ (Regoli and Winston, 1999; Regoli, 2000). Compared to individual antioxidants, TOSC is less sensitive but has a greater prognostic value since an impaired capability to neutralize ROS has been associated with the onset of various forms of oxidative damage like lysosomal alterations and genotoxic damages (Regoli, 2000; Frenzilli et al., 2001; Gorbi and Regoli, 2003; Regoli et al., 2004).

Toxic effects on lysosomal systems are generally included in all the ecotoxicological studies with mussels; these organelles are widely developed in digestive tissues and haemocytes, and have a fundamental role in cell physiology, food digestion, intracellular turnover, immune function, sequestration and excretion of harmful compounds (Moore, 1988). On the other hand, lysosomes are also the target of several contaminants which can act directly on the membranes or, indirectly, through generation of ROS or signalling pathways (Regoli, 1992, 2000; Canesi et al., 2004; Regoli et al., 2004; Viarengo et al., 2007). In the present work, lysosomal membrane stability in harbour-caged mussels was selected as a sensitive biomarker already validated in several field studies and well characterized in terms of natural variability and seasonal fluctuations for Mediterranean organisms (Regoli, 1992, 2000; Lowe et al., 1995; Bocchetti and Regoli, 2006; Viarengo et al., 2007; Gorbi et al., 2008).

The presence of a genotoxic risk during dredging and disposal operations was evaluated by the loss of DNA integrity in caged mussels. While DNA strand breaks reflects a sensitive and potentially repairable effect caused by chemicals and/or enhanced prooxidant challenge, the frequency of micronuclei indicates an irreversible genetic damage from chromatin breakage or anueploidy during cell division (Bolognesi et al., 2004).

The overall results of this study were expected to demonstrate the utility of an ecotoxicological approach with caged mussels as an additional tool to better assess the impact and risks during dredging and disposal operations in harbour areas, allowing to measure temporary variations in bioavailability of chemicals released from dredged and disposed sediments, and the early onset of harmful effects for the organisms.

2. Materials and methods

2.1. Sites and experimental design

The harbour of Piombino, located along the Tyrrhenian coast, is characterized by the presence of one of the largest European metallurgy and steel complex, carbon coke production, thermoelectric energy plant and intensive maritime traffic related to industrial activities, commercial trade and tourism. In the period January–May 2005 approximately 100,000 m^3 of sediment was dredged from the industrial area of the harbour and discharged in an artificial confined disposal facility, CDF (Fig. 1).

Mussels, *M. galloprovincialis* (6 ± 0.5 cm shell length), were obtained from a commercial farm and translocation experiments were performed in September 2004, February, April and July 2005 to monitor variations in the bioavailability and biological effects of pollutants before, during and after the end of dredging and disposal operations. In these periods mussels were caged for 4 weeks in the inner part of the harbour affected by the activities, in front of the CDF and in a more external location separated by a quay