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Environmental hazards from natural hydrocarbons seepage: Integrated classification of risk from sediment chemistry, bioavailability and biomarkers responses in sentinel species

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ABSTRACT

Potential effects of natural emissions of hydrocarbons in the marine environment have been poorly investigated. In this study, a multidisciplinary weight of evidence (WOE) study was carried out on a shallow seepage, integrating sediment chemistry with bioavailability and onset of subcellular responses (biomarkers) in caged eels and mussels. Results from different lines of evidence (LOEs) were elaborated within a quantitative WOE model which, based on logical flowcharts, provide synthetic indices of hazard for each LOE, before their integration in a quantitative risk assessment.

Evaluations of different LOEs were not always in accordance and their overall elaboration summarized as Moderate the risk in the seepage area. This study provided first evidence of biological effects in organisms exposed to natural hydrocarbon emissions, confirming the limit of chemical characterization as stand-alone criteria for environmental quality assessment and the utility of multidisciplinary investigations to determine the good environmental status as required by Environmental Directives.

1. Introduction

Crude oil and gas seepage are natural phenomena responsible for a significant contribution to the release of hydrocarbons in the marine environment. Recent global estimates indicate that about 47% of crude oil currently entering the oceans is from natural seeps while 53% derives from anthropogenic activities (NAS, 2003). Gas seeps have been firstly reported in the Adriatic Sea since 1940, and numerous shallow, gas-enriched layers of sediments and gas vents have then been described in various areas of the basin (Conti et al., 2002). Some of them have been commercially exploited by oil and gas companies, while others appear like scattered and small fields showing evidences of surface oil and/or gas release. An example of micro-seepage is present in Central Adriatic, in front of the town Civitanova Marche (site Fontespina) approximately 30 Km south of Ancona. The seep is characterized by a slight surface structure exposing Quaternary strata covered by a thin overburden of sand and, despite the limited evidence of such emissions, some concern is often arisen especially during summer seasons by the frequent observations of oil and gas bubbles close to touristic beaches.

The environmental and ecotoxicological effects of hydrocarbons released from natural sources have generally received less attention compared to anthropogenic inputs, with a few case-studies generally limited to seeps releasing massive amounts of polycyclic aromatic hydrocarbons (PAHs) (Roy et al., 2003; Seruto et al., 2005; Spies et al., 1996). The Coal Oil Point (COP) is located off the coast of Goleta/Santa Barbara (California) and it has been active for thousands of years at 19,000–95,000 l/day of petroleum (Kvenvolden and Cooper, 2003); concentrations of water-soluble PAHs ranged from 1 up to several μg l^{-1} while values of weathered hydrocarbons in sediments were often higher than 1000 mg kg^{-1} (Kvenvolden and Cooper, 2003). This continuous release of hydrocarbons caused limited changes in benthic and microbial communities (Montagna et al., 1987), and a slightly higher incidence of histopathological lesions was documented in feral fish with benthic feeding and limited movements (Spies et al., 1996). Laboratory exposures to COP sediments confirmed a few variations of typical biochemical and physiological responses to PAHs in Horneyhead turbot (*Pleuronichthys verticalis*) and California halibut (*Paralichthys californicus*), suggesting that the reduced responsiveness to natural PAHs could represent a possibly unique

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response to similar emissions, generally dominated by low molecular weight hydrocarbons (Roy et al., 2003; Seruto et al., 2005).

Fate and biological effects of natural hydrocarbons have never been investigated in areas with small or scattered natural seepage where released compounds might potentially cause different biological effects, ranging from molecular targets to organisms or communities. While it is widely recognized that changes of populations and communities are of greater ecological relevance, similar variations can be recalcitrant to appear especially in low-impacted areas where counteracting responses or adaptation mechanisms can arrest the propagation of biological disturbance and prevent clear cause–effect relationships. Biochemical or cellular biomarkers represent more sensitive warning signals toward the onset of adverse effects at an early stage, even in the absence of changes at higher levels of biological organization (Moore et al., 2006; Regoli, 2011).

Recent European Directives (i.e. 2000/60/CE and Marine Strategy Directive) recommend the use of multiple quality indicators to evaluate and classify the ecological status of water bodies. The weight of evidence (WOE) is a classical approach which combines data from different typologies of investigations, or lines of evidence (LOEs), and typically integrates chemical data with assessment of various biological effects (Chapman et al., 2002). The WOE assessments can use either qualitative or quantitative criteria and individual LOEs are independently elaborated before their final integration when a different weight is given according to their ecological relevance. Among the advantages of a similar approach is the possibility to discriminate differences between evaluations from various typologies of studies: it is quite common that elevated chemical loads in sediments are not bioavailable and, at the same time, that unexpected biological effects are caused by low levels of pollutants acting with synergistic mechanisms. Despite the choice of more appropriate LOEs depends on local objectives and specificities, WOE studies have been increasingly adopted for environmental quality characterization and as a fundamental component within Ecological Risk Assessment (ERA) strategies (Benedetti et al., 2012).

In the present work, a WOE investigation was applied to evaluate the potential impact of chemicals released by a natural seepage in Central Adriatic Sea (Fontespina, Civitanova Marche, Fig. 1). Investigated LOEs were selected to address the presence of specific classes of pollutants in sediments, their potential bioavailability and early effects at subcellular level in model sentinel species. Chemical characterization of sediments included polycyclic aromatic hydrocarbons (PAHs), aliphatic hydrocarbons and trace metals which are typically associated to deposits of oil and gaseous hydrocarbons. Bioavailability of these compounds was assessed with caging experiments in tissues of two typical bioindicator organisms, the European eel (*Anguilla anguilla*) and the Mediterranean mussel (*Mytilus galloprovincialis*). The use of translocation procedures is a common monitoring strategy which limits the influence of genetic variability and adaptive mechanisms to chronic disturbance (Brooks et al., 2012; Gorbi et al., 2008; Nigro et al., 2006). Both eels and mussels are suitable species for caging, being highly tolerant to both environmental conditions and handling, able to accumulate organic and inorganic chemicals, and widely characterized in terms of molecular and cellular responses to environmental pollutants (Benedetti et al., 2012; Brooks et al., 2012; Giuliani et al., 2013; Gorbi et al., 2005; Van der Oost et al., 2003). In this study, a wide battery of biomarkers was analysed in caged organisms, including the main mechanisms of biotransformation/detoxification of chemicals (cytochrome P450 pathway, peroxisomal proliferation, metallothioneins, acetylcholinesterase), cellular response to reactive oxygen species (individual antioxidants and total oxyradical scavenging capacity), and onset of cellular and DNA damages (lysosomal stability, lipid peroxidation, and loss of DNA integrity).

Results from sediment chemistry, bioaccumulation and biomarkers were elaborated within a recently developed and software-assisted WOE model (Sediqualsoft); different typologies of data are initially evaluated within specific modules which, through logical flowcharts and mathematical algorithms, provide synthetic indices of hazard for each of considered line of evidence, before their final integration in a quantitative WOE evaluation (Piva

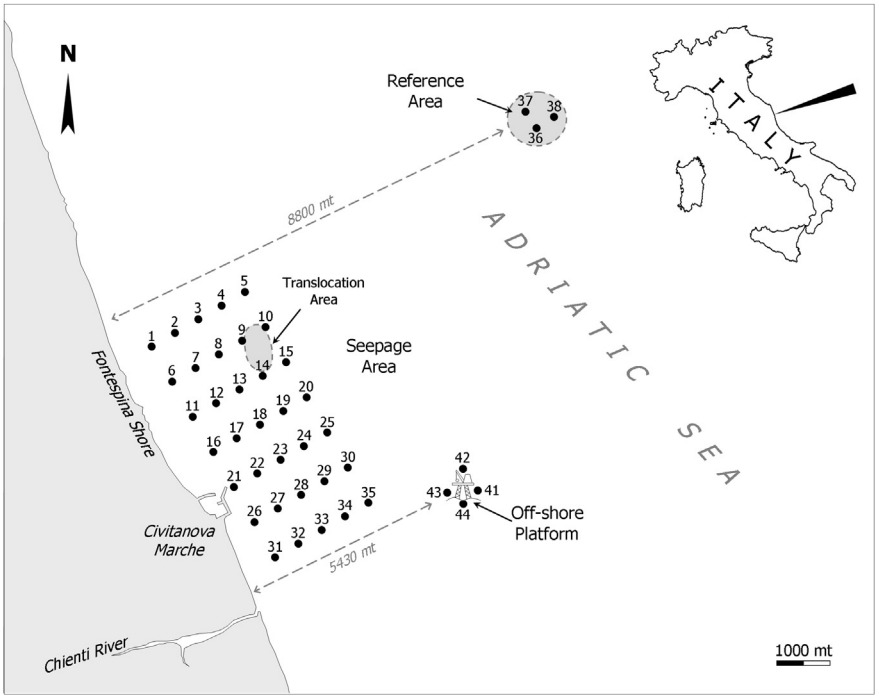

Fig. 1. Localization of sampling and translocation sites in the seepage area, off-shore platform, and reference area.