integrated in a multimarker approach for the early detection of biological responses at several cellular targets. Induction of metallothioneins, peroxisomal proliferation and activity of acetylcholinesterase were measured as markers of specific classes of chemicals. Special attention was given to oxyradical metabolism and appearance of oxidative-mediated toxicity. Besides individual antioxidants, the total oxyradical scavenging capacity (TOSC) allowed to quantify the overall capability to neutralize specific forms of ROS, i.e. peroxyl and hydroxyl radicals. Cellular damages were evaluated as lysosomal destabilization (membrane stability, accumulation of lipofuscin and neutral lipids), lipid peroxidation processes (malondialdheyde) and DNA integrity (strand breaks and micronuclei). Trace metals (As, Ba, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Zn) revealed only limited variations in transplanted mussels during various experimental periods, partly related to natural fluctuations. Among biological responses, variations of antioxidants and lysosomal stability were confirmed as sensitive early warning signals of environmental disturbance. The presented protocol with caged mussels allowed to exclude marked biological effects of produced waters and represented an useful approach for monitoring the impact of off-shore activities in the Adriatic sea.

A biomarker approach to assess the C10-2-linear alkylbenzene sulfonate effect on benthic marine fish, *Solea senegalensis*, using a continuous exposure flow system

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Abstract

Linear alkylbenzene sulfonates (LAS) is a surfactant which is between the most relevant used and produced chemical organic compounds in the world. It has many different applications being mainly found in detergents formulation due to its foaming and tensioactives properties. Although it is greatly biodegraded at the wastewater treatment plants, its presence has been observed in the aquatic environment, and can exert a possible risk to organisms.

The aim of this study was to evaluate the toxicity effects of 2-phenil C10 LAS at environmental exposure levels and the organism chosen was the benthic fish *Solea senegalensis* that is an edible species very common in the zone.

The experiment has been carried out in a continuous flow through system using sea water LAS spiked at different concentrations and consisting in two phases: exposure (120 h) and depuration (72). Specimens were sampled at the end of both phases, dissection of tissues were performed and stored until their processes at –80 °C. The tissue chosen for the study was the gills. It is the first place of contact between organism-pollutant and the effects observed are more significant than in other tissues like alimentary canal (data not showed). The biomarkers utilized in this work were Phosphatase acid and alkaline (AcP y AlP), Catalase (KAT), Glutathione Transferase (GST), Glutathione reductase (GR) and Glutathione Peroxidase (GPX).

In exposed organisms, GR decreased at the end of the exposure phase whereas KAT showed an increase in relation to control organisms. Nervetheless, both enzymatic activities did not show significant differences between control and exposed organisms in the depuration phase. It is indicated that the enzymatic activity modifications after 2-phenil C10 LAS exposure were reversible.

A new non-lethal protocol for the ecotoxicological study of the Mediterranean loggerhead turtle *Caretta caretta*

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Abstract

The goal of this pilot study is to develop and validate a series of "diagnostic" and "prognostic" tests based on "non-lethal" sampling techniques to evaluate exposure and effects of environmental pollutants in a Mediterranean long lived organism *Caretta caretta*. Particular attention was paid to potential toxicological effects of EDCs.

Sampling (blood and excreta) was performed on 53 specimens from four sites along Italian coasts. Blood was analyzed for vitellogenin, AChE and BChE activity, 17-b-estradiol levels and several parameters of blood biochemistry (c-GT, AST, ALT). Excreta samples were analyzed to evaluate porphyrin concentrations. Several biomarkers showed high sensitivity of response. Monoclonal antibody *mouse anti-alligator* (ND-1E8 Biosense, Norway), used in the ELISA test to assess plasma vitellogenin levels, showed high cross-reactivity with turtle proteins. This result was validated in blood from two female turtles during laying. AChE, BChE and c-GT activities showed significant differences between sampling sites. Levels of ALT were particularly high compared to those in the literature. Porphyrin profile showed the presence of uro-, copro- and proto-porphyrins.

In conclusion, biomarker responses showed considerable differences between the various specimens analyzed, both in relation to sex and maturity stage and to the area of collection. The present results represent the base of a draft protocol for ecotoxicological studies in this species, potentially susceptible to the negative effects of environmental contaminants in the Mediterranean environment.

Integrated responses to copper and benzo[*a*]pyrene exposure in a non-model bivalve species, *Mytilaster minimus*