USE OF CAGED MUSSEL *MYTILUS GALLOPROVINCIALIS* IN AN ECOTOXICOLOGICAL APPROACH TO ASSESS ENVIRONMENTAL IMPACT IN OFF-SHORE ACTIVITIES

S. Gorbi [1], M. Benedetti [1], R. Bocchetti [2], C. Virno-Lamberti [2], G. Moltedo [2], R. Di Mento [1], D. Fattorini [1], B. Pisanelli [1], F. Regoli [1] *

[1] Istituto di Biologia e Genetica, Universitï£¡ Politecnica delle Marche, Ancona, Italy

[2] ICRAM, Istituto Centrale per la Ricerca Scientifica e Tecnologica Apllicata al Mare, Roma, Italy - f.regoli@univpm.it

Abstract

Mediterranean mussels, *Mytilus galloprovincialis*, are well recognized bioindicator organisms which can be easily caged in investigated areas to assess the impact of anthropogenic activities. In this work a monitoring protocol was developed for off-shore installations in the Adriatic sea. Integration of chemical analyses with a wide range of biomarkers analysed in mussels caged at 2 platforms, allowed to evaluate the biological disturbance and confirmed the utility of the ecotoxicological approach for monitoring off-shore activities.

Keywords : Bio-indicators, Adriatic Sea.

Several environmental issues are associated with the off-shore oil and gas industry, from the impact caused during installation to various form of disturbance related to daily ship traffic, extraction activities, maintenance of structures and, finally, decommissioning of old platforms. During the last year a monitoring protocol with caged mussels, *Mytilus galloprovincialis*, has been developed, to evaluate the potential ecotoxicological effects caused from the off-shore platform "Giovanna" in the Adriatic sea. Obtained results allowed to exclude marked biological disturbance and demonstrated the suitability of this approach. In this respect considering "Giovanna" as model platform, the monitoring protocol with caged mussel has been extended including also another off-shore installation, the "Emilio" platform.

In this work native mussels were collected on a seasonal basis from a reference site on the Adriatic coast (Portonovo, Ancona) and transplanted for 4-6 weeks in both the sampling area and to the investigated platform "Giovanna" (42° 46' 060N, 14° 27' 750E) and Emilio (42° 56' 305 N; 14° 13' 915 E). After the translocation period, mussels were recovered dissected tissues frozen in liquid nitrogen and maintained at -80°C until analyses.

Translocation procedures have been performed in Autumn 2005 (November-December, I sampling period), Winter 2006 (February-March, II sampling period), Spring 2006 (May-June, III sampling period), Autumn 2006 (September-October 2006) in order to characterize seasonal variability of analyzed parameters. Chemical analyses on trace metals (arsenic, cadmium, chromium, copper, iron, mercury, manganese, nickel, lead, zinc) in mussels tissues [1] were integrated in a multimarker approach for the early detection of biological responses at several cellular targets. Induction of metallothioneins-like proteins, acetylcholinesterase activity and peroxisomal proliferation were measured as typical responses to metals and organic aromatic pollutants [2]. An important role in environmental toxicity of both metals and organic contaminants is certainly assumed by the enhancement of intracellular reactive oxygen species (ROS) which modulate the onset of several deleterious effects and cell damages [3]. Analysed antioxidants in caged mussels included the activities of superoxide dismutase, catalase, glutathione S-transferases, glutathione reductase, Se-dependent and Se-independent glutathione peroxidases and the levels of total glutathione. Variations of the redox status were further investigated with the total oxyradical scavenging capacity (TOSC) assay which, measuring the overall capability to neutralise various oxyradicals, provides a quantitative index of the susceptibility of biological tissues to oxidative stress [4]. Lysosomal impairment is largely recognised as a sensitive marker of chemical disturbance in mussels where this compartment is highly developed both in digestive tissues and in haemocytes: lysosomal alterations were measured through the analysis of membrane stability, accumulation of lipofuscin and neutral lipids, and content of malondialdehyde which reflect the intensity of lipid peroxidation [5] . The appearance of genotoxic alterations in caged mussels was investigated as loss of DNA integrity; the Comet assay, measuring the early onset of strand breaks was integrated with the frequency of micronuclei (MN). A general index of physiological status, was finally measured in caged mussels as their capability to survive in air [6].

An overall evaluation of results confirmed the absence of marked biological effects caused by the activities of "Giovanna" platform, as already demonstrated during the previous monitoring project. More variations were observed in mussels translocated to "Emilio", i.e. higher activities of glutathione S-transferases, catalase and peroxisomal proliferation decrease of oxyradical scavenging capacity toward hydroxyl and peroxyl radicals and lysosomal destabilization (inhibition of neutral red retention time), indicating an onset of impairment condition in the organisms. Compared to mussels transplanted at the reference site, those from "Emilio" platform did not exhibit more elevated concentrations for the various metals and only for zinc and cadmium an higher bioavailability was detected close to the platform, suggesting the influence of galvanic anodes for cathodic protection.

The overall results of this work confirmed the utility of using caged mussels as an additional contribution for monitoring off-shore activities and provided an ecotoxicological protocol based on cellular biomarkers for the early detection of biological disturbance.

Acknowledgements

Alessandra Notti, Maria Ferraro, Anna Maria Cicero.

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