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Marine Environmental Research 62 (2006) S327–S331
MARINE ENVIRONMENTAL RESEARCH
www.elsevier.com/locate/marenvrev

Short communication

Application of a suite of biomarkers in *Posidonia oceanica* (L.) delile to assess the ecotoxicological impact on the coastal environment

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Abstract

The main aim of this study was to develop and validate a suite of sensitive responses (biomarkers) for monitoring conservation status and ecotoxicological impact in *Posidonia oceanica* meadows. Analytical methods were developed for NADPH cytochrome *c* reductase, ethoxycoumarin-*o*-deethylase (ECOD), guaiacol peroxidase (GPOX) and superoxide dismutase (SOD) assays. A preliminary proteomic approach using 2-D electrophoresis was also proposed as a biomarker. These techniques were initially tested on samples of posidonia exposed experimentally to various contaminants. Once validated, this approach was applied to posidonia in a field study. Samples of the seagrass were collected at four sites with potentially different environmental impact along the northern Tyrrhenian coast. The results showed that reductase activity was significantly induced in the various sampling areas with respect to the reference site. GPOX and SOD showed a similar trend; the highest activities were found in samples collected off a chlor-alkali plant and near a river estuary. Analysis of trace elements, polycyclic aromatic hydrocarbons (PAHs) and organochlorine compounds (OCs) in posidonia leaves showed differences between sites. A significant correlation was found between Hg concentrations and GPOX activity and between Cr, Al and As concentrations and reductase activity. The results validated these biomarkers in posidonia for the assessment of ecotoxicological impact on the coastal ecosystem.
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0141-1136/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.marenvres.2006.04.014

Keywords: *Posidonia oceanica*; Biomarkers; NADPH cytochrome reductase; ECOD; Peroxidase; Superoxide dismutase; 2-DE; Trace elements; OCs; PAHs

Decline of the endemic seagrass *Posidonia oceanica* due to human activities has been repeatedly documented in the Mediterranean. Several phenological and morphological studies indicate its high sensitivity to pollution. In the present research, posidonia was studied from an ecotoxicological point of view with the main aim of developing and validating a suite of biochemical responses for monitoring conservation status and pollution impact in posidonia meadows. Analytical methods were developed for MFO activities (NADPH cytochrome reductase and ethoxycoumarin-O-deethylase, ECOD) and antioxidant enzyme assays (guaiacol peroxidase, GPOX and superoxide dismutase, SOD). These techniques were initially tested on samples exposed experimentally to trace elements, benzo-a-pyrene and crude oil (Leonzio et al., 2002). Once validated, these biomarkers were applied to posidonia in a field study to investigate ecotoxicological impact on this seagrass. A preliminary proteomic approach using two-dimensional electrophoresis (2-DE) was also proposed as a biomarker.

Samples of posidonia were collected in November 2003 from four differently contaminated sites between the Arno and Ombrone estuaries (NW Tyrrhenian coast, Italy): (1) Secche della Meloria near the Arno estuary; (2) Rosignano, off a chlor-alkali plant known as a source of mercury pollution, (3) Cala di Forno, near the Ombrone estuary and (4) Porto Santo Stefano as reference site. Immediately after collection, the basal parts of the leaves was removed, frozen in liquid nitrogen and stored at -80°C until analysis.

NADPH-cytochrome *c* reductase activity was assayed in S9 homogenate by the method of Livingstone and Ferrar (1984). ECOD activity was measured in the microsomal fraction by the method of Werck-Reichhart et al. (1990). GPOX was assayed spectrophotometrically using guaiacol as substrate as described in Putter (1975). SOD was determined spectrophotometrically by monitoring inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm (Beauchamp and Fridovich, 1971). Protein extraction from leaves for 2-DE was developed using an adaptation of the protocol of Wang et al. (2003) for olive leaves. Concentrations of trace elements were determined by atomic absorption spectrophotometry, levels of PAHs by HPLC analysis (Griest and Caton, 1983) and concentrations of OCs by GC analysis.

In a previous experimental study posidonia showed high sensitivity of response towards different contaminants (trace elements, crude oil, PAHs). Specifically, NADPH cytochrome *c* reductase activity was sensitive to treatment with metals and crude oil, POX and SOD showed similar responses, showing greatest induction with trace elements (Leonzio et al., 2002).

In the field study (Fig. 1) the enzyme responses analysed showed differences between the various sites and the control site. NADPH cytochrome *c* reductase activity was significantly induced (Kruskall–Wallis test, $p < 0.05$) with respect to control (70.13 ± 71.02 nmol min^{-1} mg^{-1} protein) in samples from Cala di Forno and Rosignano (395.47 ± 198.76 and 365.73 ± 135.47 nmol min^{-1} mg^{-1} protein, respectively). ECOD activity was higher near the Arno estuary (Secche della Meloria). These findings suggest the presence of cytochrome P450 inducers (hydrophobic contaminants) in the sampling

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