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Ecotoxicological evaluation of marine sediments using free and immobilized phytoplanktonic algae

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Abstract

Marine sediments play an important role in the accumulation-storage and/or release of contaminants in seawaters; sediment bioassays provide for general information on pollutant bioavailability. This work points out the importance to utilize free and immobilised (Na-alginate) marine algae for the ecotoxicological evaluation of metals (Cd, Zn, Cu) as well as of elutriates and whole sediment samples collected in harbour sites. The bioassay was carried out with the marine microalga* Tetraselmis suecica*; algal growth inhibition was determined after 24 and 48 hours as percent growth in comparison to controls. After 24 hours a lower toxicity of metals for the immobilised algae than for free algae was observed; this trend decreased in time. Algae exposed to whole sediment showed a greater reduction of growth than algae exposed to the relative elutriate. This result emphasizes that the whole sediments seem to be suitable to detect the toxicity of such complex environmental matrix.

Introduction

Marine sediments are a reservoir of organic and inorganic pollutants, that can be adsorbed in suspended particulate matter, and can occur in higher concentration than in the water column. The ecotoxicological evaluation of sediments must give information about determined end-points, include the use of different matrices (elutriate and whole sediment) and employ adequate test-organisms able to detect the occurrence of pollutants [1]. The guidelines for the ecotoxicological assessment, the application of bioassays and the ecotoxicological characterization of sediments have been indicated recently [2]. Aim of this work was to study the employment of free and immobilized phytoplanktonic algae *Tetraselmis suecica*, to date recommended only for the elutriate matrix, also for ecotoxicological studies on whole sediments. The technique of the immobilization in Na-alginate has been utilized.

Materials and methods

Both free and Na-alginate immobilized algae were used to evaluate the ecotoxicity of metals and elutriates of sediments; only immobilized algae were used for whole sediments evaluation. Free algae were exposed to different metal concentrations or elutriate dilutions in a multi-plate system. The immobilization of algae was carried out by using a 3% p/v sodium alginate solution (Sigma-Aldrich, Milano, Italy) in deionized water at pH 8.00±0.02. Exponential growing algal cells were added to this solution; cell immobilization was achieved through dropping in a solution 0.03M $CaCl_2$ until complete gelification, obtaining 2-4 mm diameter alginate beads [4]. Immobilized algae were placed in polystyrene inserts fitted with polyester mesh bottoms (Netwell™ Ø=24 mm, membrane mesh size 74 µm) and brought into contact with a thin layer (2 mm) of whole sediments that were placed in multi-well plates. For each sediment 3 replicates and a control test were prepared. The plates were kept in thermostatic room at 20±2 °C with 16:8 light:dark period at 6000-10000 lux [5]. Algal growth was determined after 24 and 48 hours by counting with Thoma haemocytometer; the growth of immobilized cells was determined after treatment of beads with 5% sodium hexametaphosphate. The percent variation of algae growth in comparison to the control was considered as end-point for tests with metals, elutriates and whole sediments.

Results

The tests carried out with metals showed an initial (24 hours) lower toxicity for the immobilized algae than for free ones; this was seen particularly for Cd and Cu. After 48 hours exposition only copper showed a minor toxicity for the immobilized algae (Tab. 1).
The tests carried out with elutriates showed low variability between free and immobilized algae (data not shown); cell growth decrease of immobilized algae was more substantial after exposition to whole sediments than to elutriates (Fig. 1).

EC_{50} (mg/l)	Time exp.	$ZnSO_4$	$CdNO_3$	$CuSO_4$
Free algae	24 hours	0.95	0.37	1.28
	48 hours	0.40	0.25	0.40
Immobilized algae	24 hours	1.17	1.09	2.60
	48 hours	0.36	0.25	1.17

Table. 1 EC_{50} values after exposition of free and immobilized algae to metals.

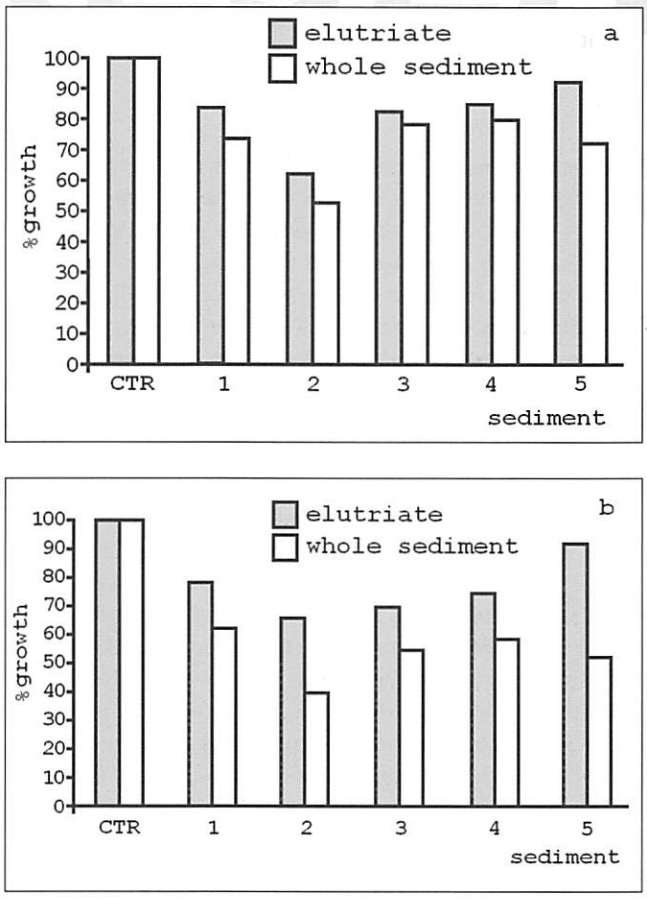

Figure 1. Percent growth of immobilized algae compared to the control (CTR) in the tests carried out with elutriates and whole sediments after 24 (a) and 48 (b) hours.

Discussion

The results show that some pollutants occurring into the sediment are not found in the elutriate and therefore their ecotoxicity can not be detected; this emphasizes the importance to apply new methods able to detect underestimated ecotoxicological effects of sediments. Overall, the bioassay with the marine microalga *Tetraselmis suecica* and the immobilization in Na-alginate could be a good estimation tool for the ecotoxicological assessment of marine sediments, in particular to evaluate the toxicity of whole sediments; the employment of polystyrene inserts allowed to improve the procedures.

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