NOTE

Sediment toxicity assessment using bacterial bioluminescence: effect of an unusual phytoplankton bloom

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ABSTRACT: The toxicity of selected sediments in the Adriatic Sea and the effect of phytoplankton bloom on sediments in the vicinity of Rovinj, Yugoslavia, using bacterial bioluminescence toxicity assay, have been investigated. Sediments under the influence of urban and industrial wastes tend to be more contaminated than those in the open sea. The toxic effect of decayed material derived from sinking mucus aggregates was higher at locations influenced by local pollution. The time course study at 1 station suggested that increases in toxicity of sediment extracts is mainly due to changes in organic matter derived from decayed products of mucus aggregates.

During summer 1988, an intense nonseasonal phytoplankton bloom occurred in the Northern Adriatic (Degobbis in press) forming large mucus aggregates. The Istrian coast of the Northern Adriatic was fouled with large quantities of this mucus material. The nature of these aggregates is unknown, but most have associated pelagic and benthic organisms, empty shells, much detritus and bacteria. This event has been previously described in the waters of Rijeka Bay (Zanon 1931) and the Gulf of Trieste (Herndl 1988). Sinking of these aggregates transfers nutrients and decayed material from the surface mixed layer to the seafloor, causing a drastic decrease in the number of benthic organisms caused by mechanical events or anoxic conditions (Zavodnik 1977, Stachowitsch 1984). Sediments in the vicinity of Rovinj were covered (80 to 90 % of whole area) with a 1 to 2 cm thick layer of decayed material.

Since 1987 our laboratory has dealt with a program for monitoring the local pollution. In order to determine any secondary effects of decayed material on sediments at 4 locations in the vicinity of Rovinj, the

bioluminescence toxicity assay was used. It is a rapid method of comparing and ranking the toxicity of organic extracts of contaminated sediments (Schiewe et al. 1985) and is a reliable screening technique for a variety of pollutants in sediments (Williams et al. 1986). The results of our assay exhibited a high degree of similarity with other sediment toxicity tests (Giesy et al. 1988) and hence this technique can be used as an exploratory screening test.

Materials and methods. The surface layer of sediment samples were collected by SCUBA divers from 11 locations (Fig. 1). About 500 g wet sediment was frozen at $-20\,°C$ and transported to laboratory. Dry weight was determined by heating 10 g aliquots at $105\,°C$ for 24 h. The presence of total organic material (TOM) was estimated by weight loss after heating at $500\,°C$. Extractable organic material (EOM) was determinated according to Meyers et al. (1984). For the bioluminescence assay, 100 g wet wt of sediments were extracted with dichloromethane-methanol (2:1) as previously described by Schiewe et al. (1985). After several washings to remove methanol, dichloromethane extracts were evaporated to dryness and dissolved in dimethylsulfoxide (DMSO). Extraction blanks were prepared by the same procedure but without sediment. Water samples (2 l) were extracted with dichloromethane, evaporated to dryness and dissolved in 1 ml DMSO. Extraction blanks were prepared with distilled water.

Bacterial luminescence of a laboratory grown, healthy culture of the marine bacterium *Photobacter phosphoreum* will be inhibited upon exposure to toxic substances (Bulich & Isenberg 1981). The luminescence of cultures exposed to a series of dilutions of sediment and water extracts was measured in a Packard-Tri-Carb

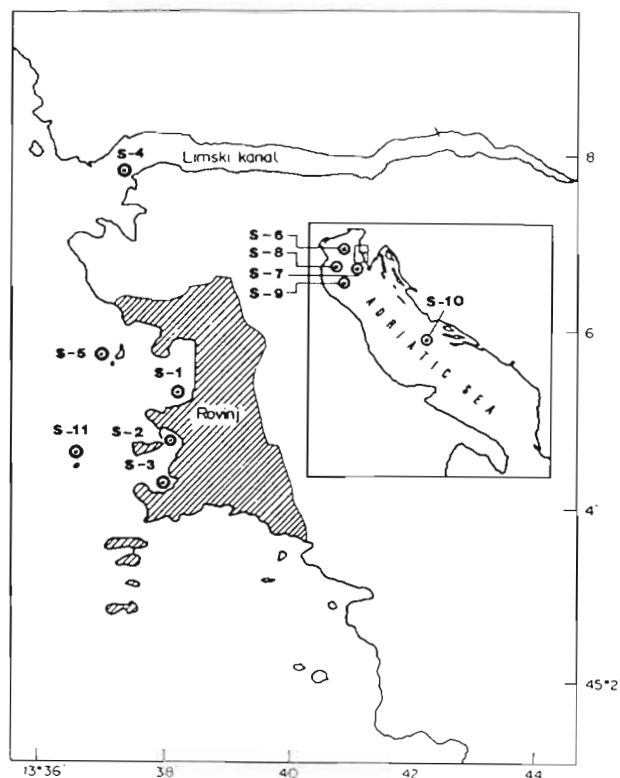* Addressee for correspondence

Fig. 1 Map of Rovinj, Northern Adriatic, showing sampling sites (S-1 to S-11)

Table 1. Comparison of toxicities of organic extracts of selected sediments (Sed., mg⁻¹ dry wt) and waters (mg). ED_{50}: amount (mg dry wt or ml H_2O) causing 50 % reduction of bioluminescence in 15 min; ND: not determined; CI: confidence interval at 95 % significance in parentheses

Station	ED_{50}	
	Sed.	Water
S-1	0.9 (0.6–1.3)	1.1 (0.8–1.3)
S-2	1.5 (1.2–2.9)	0.3 (0.2–0.7)
S-3	3.2 (1.9–3.8)	0.6 (0.4–1.0)
S-4	5.6 (3.8–7.5)	ND
S-5	2.4 (1.3–2.5)	2.9 (2.1–5.7)
S-6	22.0 (10–30)	ND
S-7	29.0 (10–30)	4.8 (2.6–5.2)
S-8	13.0 (10–20)	ND
S-9	80.0 (50–100)	2.6 (1.4–3.4)
S-10	560.5 (410–830)	6.1 (5.4–6.8)
S-11	3.0 (1.8–3.5)	ND

The range in toxicity of sediment extracts is comparable to results obtained for Detroit River sediments (Giesy et al. 1988) and for Puget Sound sediments, Washington (Schiewe et al. 1985). Using our extraction procedure it was possible to analyse toxicity derived only from organic extractable pollutants. The contribution of some polar substances e.g. some classes of toxins produced by bacteria living in the sediments (Simidu et al. 1987, Kogure et al. 1988), as well as the bulk of metallic compounds, to sediment toxicity was excluded by the extraction procedure itself.

The toxicity of mucus material increased 15-fold after 3 d incubation at 25 °C (ED_{50} decreased from 4.2×10^{-2} to 2.8×10^{-3} ml of mucus material), suggesting formation of toxic products during decay and biodegradation of mucus material in the laboratory. In order to investigate the possible influence of decayed material in the field, the toxicity of sediments at 4 locations, in the vicinity of Rovinj, were investigated after the mucus material sank. Two of the investigated locations (S-2 and S-3) which are directly influenced by urban sewage and industrial runoff were more impacted (Table 2). Since no toxic plankton bloom was detected in the investigation area, toxicity arose by either single classes of products (derived from large quantities of natural decayed material), or by contaminants scavenged from the water column. Extracts of water samples from heavily polluted areas showed 5 to 10 times higher toxicity (S-2 and S-3). However, at locations not directly influenced by local pollution (S-5 and S-11) the increase in sediment toxicity could be explained by the decay of mucus aggregates. A further explanation could be the increased remobilisation of contaminants from sediments caused by the additional impact of decayed material. Moreover, areas that have sediment of smaller grain size and higher organic con-

liquid scintillation spectrometer. Briefly, in 0.5 ml saline suspensions containing 10^6 exponentially growing luminescent bacteria, 0.5 ml of subsequent dilutions of sediment and water extracts in 2 % saline were added. After 15 min incubation at 15 °C the bioluminescence was measured, compared with blank samples, and corrected for spontaneous decline in photoactivity. The results are expressed as mg of dry weight of extracted sediments or ml of water causing a 50 % reduction of bioluminescence in 15 min (ED_{50}). Estimates of ED_{50} were obtained using linear regression analysis, as described by Schiewe et al. (1985) and 95 % confidence intervals for each estimate of ED_{50} were calculated based on Fieller's theorem (Finney 1964).

Results and discussion. Three of the 11 sample stations (Fig. 1), i.e., S-1, S-2, and S-3, are under the direct influence of industrial and/or urban runoff from a fish cannery, tobacco factory and urban waste, respectively. Comparison of toxicities of organic extracts of selected sediments and waters from the stations are presented in Table 1. Sediments influenced by urban sewage and industrial runoff tend to be more contaminated. The toxicity of waters directly influenced by industrial and urban wastes (S-1, S-2, S-3) were also higher than those of the open sea. However, this correlation, between the toxicity of sediment and water extracts was not observed at all stations.

Table 2. Comparison of toxicities or organic extracts of selected sediments before and after sinking of decayed material. ED_{50}: amount (mg dry wt or ml H_2O) causing 50 % reduction of bioluminescence in 15 min; CI: confidence interval at 95 % significance (in parentheses); Increase: x-fold increase in toxicity

Station	Sediment type	ED_{50} (mg^{-1} dry wt)		Increase
		Before	After	
S-2	Sandy	1.5 (0.9–1.9)	9.2 (7.1–14) $\times 10^{-2}$	16
S-3	Sandy	3.2 (1.9–3.8)	4.7 (4.3–8.6)$\times 10^{-2}$	68
S-5	Sandy-silt	2.4 (1.3–2.5)	3.2 (1.8–3.7)$\times 10^{-1}$	7.5
S-11	Muddy sand	3.0 (1.8–3.5)	6.8 (5.1–10) $\times 10^{-1}$	4.4

tent tend to become more contaminated (Knezovich et al. 1987), since these often correspond to the areas of greatest deposition.

In order to obtain a better understanding of such processes, the influence of decayed material on sediment from one location (S-11) over a period of 10 mo was investigated. A 10-fold increase in the toxicity of sediments followed an increase in EOM (Fig. 2). This is in agreement with the measured increase in TOM caused
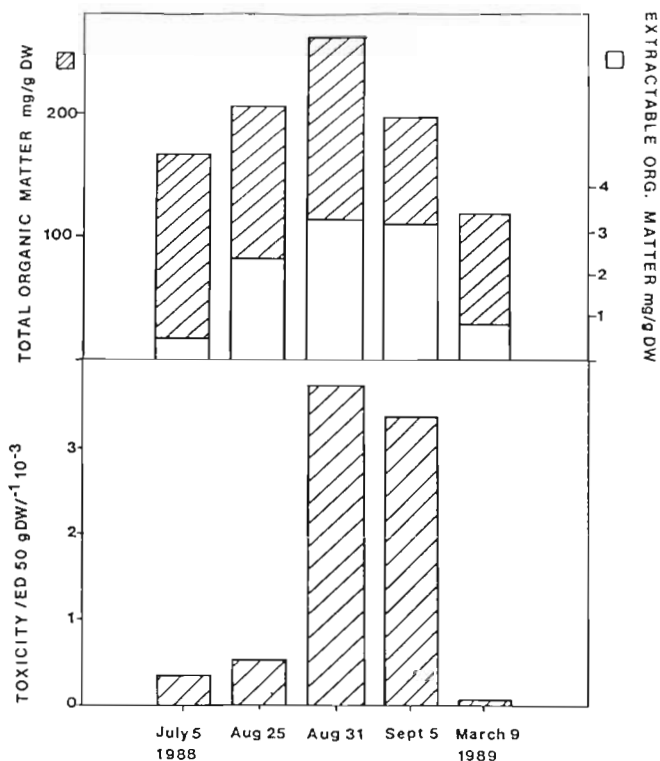

Fig. 2. Total and extractable organic matter content and toxicity of sediment extracts at Station S-11 during the phytoplankton bloom

by continuous sedimentation of decayed material. At the end of August the bulk of decayed material had settled into the sediment surface. No further increase in extractable organic matter and toxicity was observed. Six months later, both the EOM and toxicity decreased

to the level measured prior to the bloom. These results suggested that the increase in toxicity of sediment extracts could be explained mainly as a consequence of changes in organic matter derived from the decayed products of mucus aggregates. The contribution of contaminants, scavenged from the water column and/or by remobilisation from sediments, to the increase in sediment toxicity has yet to be established.

Sediment meiofauna, in the vicinity of Rovinj, are sensitive to local point pollution (Vidaković 1983) as further indicated by our results. Extrapolation of the increase in sediment toxicity, due to the decay of mucus material, to organisms living in the sediment is complex. The primary effect of mucus aggregates on living organisms in the sediment is on mechanical processes and anoxic conditions (Zavodnik et al. 1989). However, the secondary effect, i.e. of an increase in the toxicity of sediment organic matter as demonstrated here, must also be considered.

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