Changes in sediment toxicity to sea urchin sperm as a function of sample processing. I. Toxicity of solid phase vs. water extracts

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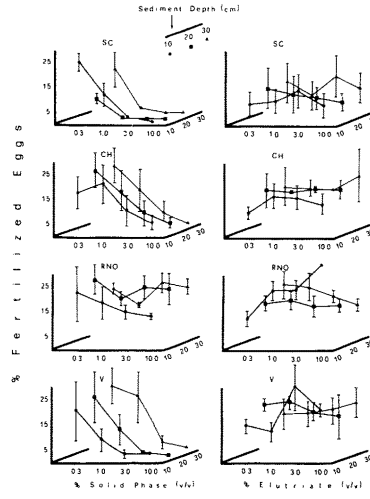
In a previous study of water and sediment toxicity on sea urchin embryos and sperm (PAGANO *et al.*, 1992), we have observed that water samples from polluted water bodies displayed only minor toxicity, whereas sediment samples from a number of river, estuary and coastal sites exerted toxic outcomes to fertilization and early development with clear-cut results, consistent with the pollution status of the samples. Thus, it may be suggested that testing sediment toxicity may provide comprehensive information on the environmental health status of a water body, also in relation with the difficulties of obtaining reliable data on the toxicity of the water column. In this study, we have tested sediment toxicity from some selected sites of a brackish water body located in Southern France (Etang de Berre). The study has been conducted by utilising sea urchin sperm and embryo bioassays on *Paracentrotus lividus* (PAGANO *et al.*, 1986).

The question is raised whether the best conditions for a realistic bioassay should be obtained in the presence of whole, untreated sediment (including solid phase), or by testing water extracts ("elutriate") from sediment samples. Another relevant question in sediment toxicity testing relates to which depths in a sediment core may provide the most realistic information on sediment toxicity. Thus an appropriate sampling procedure should be standardised concerning the most suitable depth(s) of sediment core.

We conducted two series of experiments testing the spermiotoxic action of sediment samples collected from four sites at the Etang de Berre, labeled SC, CH, RNO and V. Each sample was divided into three subsamples corresponding to the depths of 0-10cm, 10-20cm and 20-30cm from surface, which were processed separately. An aliquot from each subsample was then suspended (10%) in seawater, stirred and maintained overnight at + 4°C; the water extract (elutriate) was obtained by centrifugation (350xg, 10min). Solid phase sediment aliquots were maintained in the same conditions as those designed to obtain elutriate (except for centrifugation). Both solid phase suspensions and elutriate were tested in v/v dilutions ranging from 0.3% to 10%. Sea urchin (*P. lividus*) sperm suspensions (1% dry sperm) were added gently to filtered seawater containing solid phase sediment (without resuspending the pellet) or elutriate; after a 15-min exposure, sperm was used to inseminate untreated egg suspensions (1:100 sperm:eggs; approx. 100eggs/ml); thereafter, zygotes were washed by decantation with control seawater. Changes in fertilising capacity were determined by reading fertilization rate (FR = % fertilised eggs) in live embryos starting from early cleavage. In a series of experiments on developmental toxicity, *P. lividus* zygotes (10min after fertilization) were exposed to sediment or elutriate throughout embryogenesis up to the larval stage of pluteus. Developmental defects were scored according to PAGANO *et al.* (1986) both in the cultures exposed during development and in the offspring of pretreated sperm.

As shown in Fig. 1, solid phase sediment from three sites (SC, CH and V) showed dramatic spermiotoxicity, with a drop in fertilization rate (FR) to zero between 1% and 3%; on the other hand, sediment from the site RNO only showed minor effects. Elutriate samples invariably showed a lesser spermiotoxicity than solid phase, without any dose-response trend and, at sites RNO and V, a slight increase in FR was observed. As observed in Fig. 1 and confirmed in a subsequent series of experiments, a clear-cut depth effect was observed at site SC and, to a lesser extent, CH, in that the 10-cm sample showed the least spermiotoxicity as compared to 20-cm and 30-cm samples; no such effect could be detected at site V, where spermiotoxicity appeared to be evenly distributed across the three depth segments. Again, RNO site only showed minor spermiotoxicity, if any, at all depths considered. The offspring quality of sediment-pretreated sperm (either solid phase or elutriate) in no case appeared to be affected. The exposure of embryos throughout development failed to provide any evidence of an embryotoxic/teratogenic action at 1% sediment (solid phase only).

These results confirm the suitability of solid phase in sediment toxicity testing. Moreover, our present data suggest that water extraction of sediment samples may result in non-realistic observations or false negatives.



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