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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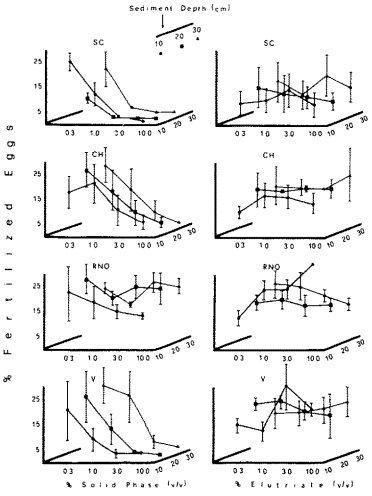
In a previous study of water and sediment toxicity on sea urchin embryos and sperm (PAGANO *et al.*, 1992), we have observed that water samples from polluted water bodies displayed only minor toxicity, whereas sediment samples from a number of river, estuary and coastal sites exerted toxic outcomes to 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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Changes in sediment toxicity to sea urchin sperm as a function of sample processing. II. Effects of sample storage temperatures

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When testing sediment toxicity, some problems may arise from the facts that: a) immediate; testing may not always be feasible, and b) in many cases a delayed replicate of test(s) may be needed. On the other hand, storing sediment samples for later bioassays meets the difficulties related to the alteratic induced by sediment-associated microorganisms that can affect sample toxicity by a number mechanisms. Thus the question is raised about the most suitable storage conditions for sediment samp in toxicity testing. If a chemical preservation technique is excluded *a priori*, then a basic choice may confined to either storing sediment samples at +4°C or at -20°C. In both cases some inconveniences in occur, since refrigeration does not prevent microbial activity, whilst freezing-and-thawing may result undesired artifacts in the structure and/or composition of sediment samples, turning into changes of th toxicity.

Seeking an at least partial answer to the above questions, we tested sediment toxicity samples from four freshwater sites in Campania, Italy. The sites were labeled S.1 (Solofrana Stream), (Sarno River), and V.6 (mouth of Regi Lagni drainage system), all being affected by a number of pollution sources, and V.3, a reference site in the Volturno River. All sites were characterised both for a series inorganic and organic pollutants, and for granulometry (MELLUSO *et al.*, 1991; PAGANO *et al.*, 1992). Each sediment sample was carried refrigerated to the laboratory, and thereupon divided into three subsamples, each stored overnight as follows: a) at -20°C; b) at +4°C, and c) dessiccated at +60°C (for dry weight determination). Thereafter, the samples (a) and (b) were suspended at the concentration of 1 (dry weight equivalent) in seawater and allowed to settle in containers. Bioassays were carried out on c urchin sperm and embryos (*Paracentrotus lividus* and *Sphaerechmus granularis*), processed according PAGANO *et al.* (1986), and applying the same schedules described in the first section of this paper.

Following a 15-min sperm pretreatment (*P. lividus*) in sediment-containing seawater spermiotoxicity was affected according to sample storing conditions, in two out of four sampling sites shown in Table 1, samples from sites S.3 and V.6 displayed a dramatic shift in their spermiotoxicity, in that refrigerated samples were significantly more spermiotoxic than freeze-thawed samples. *Vice versa*, sample was spermiotoxic, and V.3 was non-spermiotoxic, both following refrigeration and freeze-thawing of samples. These effects were consistently observed in a subsequent series of sediment sampling a experiments on *P. lividus* (totalling 10 sperm lots from 45 males). A triplicate experiment on *S. granula* sperm, limited to three sites, partially confirmed the above data (in two out three sites).

When developing embryos were reared in sediment samples stored at +4°C or -20° embryogenesis was affected by each sample independently of sediment storage temperature; this held true both for the kind of effect and for the frequencies of abnormalities. Namely, S.1 sample caused an early embryonic mortality (arrest at zygote), S.3 and V.6 samples induced developmental abnormalities, and failed to affect development, as observed previously (PAGANO *et al.*, 1992). In each case, these effects was observed independently of whether sediment samples had been stored at +4°C or -20°C.

Regarding spermiotoxicity experiments, one may speculate about the apparently contrastion behaviour of site S.1 (thawing insensitive, mainly affected by industrial, inorganic pollutants) vs. sites and V.6 (thawing sensitive, characterised by agricultural and domestic pollution). Among possible interpretations, it might be suggested that spermiotoxic agents being present at sites S.3 and V.6 might biopolymers or other organic contaminants, whose toxicity could be affected by thawing-associated changes.

The lack of any differences in sediment embryotoxicity according to storage temperature may be partly related to the different exposure of embryos, as compared to sperm; unlike sperm, only being exposed in suspension for 15min, the embryos had a longer (72hrs) and partly direct contact with sediment (approx. 10hrs up to hatching); moreover, the toxicants and/or the mechanisms involved in spermio-embryotoxicity are expected to be different, thus accounting for the observed outcomes.

Independently of the possibility to observe or not an effect of storage temperatures sediment toxicity, it should be stressed, however, that freeze-thawing procedures should be avoided was storing sediment samples, in order to prevent artifacts in toxicity endpoints. Moreover, since microbial activity may as well affect any toxic outcomes in refrigerated sediment samples, these should be tested toxicity as shortly as possible after their collection.

Table 1. Fertilisation Rate (% fertilised eggs) in *P. lividus* embryos following a 15-min sperm exposure to seawater containing 1% (dry wt. equivalent) sediment stored at + 4°C or -20°C. Quintuplicate experiment on a total of 25 males.

Site	Fertilisation Rate +4°C	-20°C
Control	88.7 + 3.5	
S.1	47.6 ± 11.7	45.4 ± 8.5
S.3	9.4 ± 3.3	88.2 ± 3.2
V.3	89.4 ± 1.8	72.2 ± 9.9
V.6	6.0 ± 1.5	77.4 ± 6.1

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