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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