Toxicity of sewage sludge to *Crangon crangon* and *Artemia salina*, with reference to other marine Crustacea

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Abstract

The toxicity of sewage sludge to adult and larval sand shrimp, *Crangon crangon*, and larval brine shrimp *Artemia salina*, was studied. Temporal changes in toxicity, ammonia and pH of sewage sludge in seawater were determined. The feasibility of sediment choice bioassays using adult *Crangon* were examined. The 24 h LC50 of sewage sludge at 20°C was 0.4% sludge for *Crangon* larvae, and 10.0% for *Artemia* larvae. Toxicity to *Crangon* larvae decreased with decreasing ammonia and increasing pH as sludge decayed over 7 days. In adult *Crangon*, a dose response with greater variation at <3% sewage sludge (wet volume in seawater) was found. For adult *Crangon* at 10°C, the LT50 at 1% sludge was 80-90 h and 96 h LC50 0.8-1.7% sludge. No clear differences were apparent between 96 h LC50's in static and 24 h renewal tests. Adult *Crangon* avoided sediments mixed to concentrations of 6.25% and 10% sewage sludge, but buried at similar frequencies in uncontaminated and 1.57% sludge sediments. In addition to possible chemical effects from ammonia and other sewage sludge components (e.g. metals, organochlorines, hydrocarbons), sludge solids may have physical effects on Crustacea. Bioaccumulation is possible if persistent contaminants occur in the sludge. From estimated field concentrations of sewage sludge, and the results of this and other toxicity studies, lethal effects at sewage sludge disposal sites are believed unlikely. However, further sediment studies (longer-term, life-cycle, bioaccumulation, and mesocosm) are required to evaluate the role of sediment tests in understanding the effects of sewage sludge on benthic Crustacea.

Keywords: Sewage sludge, marine Crustacea, toxicity, sediments.

Toxicité des boues de station d'épuration sur Crangon crangon *et* Artemia salina *en référence à d'autres Crustacés marins.*

Résumé

La toxicité des boues de station d'épuration sur les adultes et les larves de la crevette grise, *Crangon crangon*, et sur les larves d'*Artemia salina*, a été étudiée. Des modifications de la toxicité de l'ammoniaque, du pH des boues dans l'eau de mer ont été déterminées. La faisabilité des tests dans le choix du sédiment en utilisant des adultes de *Crangon crangon* a été examinée. La concentration létale LC50 (24 h) de boue à 20 °C est de 0,4 % de boue pour des larves de *Crangon*, et 10 % pour des larves d'*Artemia*. Pour les larves de *Crangon*, la toxicité diminue avec la baisse de l'ammoniaque et l'augmentation de pH lorsque la décomposition des boues excède 7 jours. Chez les adultes de *Crangon*, une réponse est observée avec de grandes variations à une proportion inférieure ou égale à 3 % de boue (volume humide en eau de mer). Pour des adultes de *Crangon* à 10 °C, le temps létal, LT50, à 1 % de boue est de 80-90 h et 96 h pour LC50 0,8-1,7 % de boue. Aucune différence apparente des LC50 (96 h) entre les tests en milieu statique et ceux où le milieu est renouvelé toutes les 24 h n'a été observée. Les crevettes *Crangon* adultes évitent des sédiments mélangés à des concentrations de 6,25 et 10 % de boue, mais elles s'enfouissent à des fréquences similaires dans des sédiments non contaminés et à 1,6 % de boue. Aux effets chimiques de l'ammoniaque et d'autres composantes des boues (ex. métaux, composés organochlorés, hydrocarbures),

(2) The late Karen Fretwell died on 9 January 1993, in a tragic canoeing accident. She was only 26 years old.

des aggrégats peuvent avoir des effets physiques sur les crustacés. La bioaccumulation est possible si des contaminants rémanents se trouvent dans les boues. A partir des concentrations estimées sur le terrain, et les résultats de cette étude, et d'autres études de toxicité, les effets létaux de boues sur les sites d'épandage sont considérés comme peu probables. Cependant, d'autres études de sédiment (à plus longs termes, sur des cycles biologiques, sur la bioaccumulation et sur le mésocosme) sont nécessaires pour estimer le rôle des tests de toxicité du sédiment pour la compréhension des effets des boues d'épandage sur les Crustacés benthiques.

Mots-clés : Boue de station d'épuration, Crustacés marins, toxicité, sédiments.

INTRODUCTION

Sewage sludge is disposed of in the sea via outfalls and by dumping from barges. It generally ranges from 1-7% dry solids, and usually contains a wide range of contaminants from industrial, domestic and agricultural sources (e.g. metals, organoclorines, pesticides, hydrocarbons, synthetic organics, detergents).

In a review of the sensitivity of marine fish and crustaceans to a variety of toxicants, Suter and Rosen (1988) found that crustaceans tended to be more sensitive. Indeed, the lowest toxic concentrations published for sewage sludge for any marine organisms are 0.001% (24 h LC50) and 0.005% (96 h LC50) dilutions in seawater for the larvae of *Crangon crangon* (Franklin, 1983) and juvenile *Mysidopsis bahia* (Santoro and Fikslin, 1987) respectively. Not only may crustaceans be particularly sensitive to sewage sludge toxicity, but they are unique amongst marine animals in their species richness and abundance in both the benthos and plankton. Furthermore, they occupy all stages in the food chain through which pollutants are transfered and bioaccumulated (predators, herbivores, scavengers suspension and deposit feeders), and in turn are important prey of commercial fish species.

The present study repeated some previously published tests, notably that on *C. crangon* larvae, but also conducted sublethal sediment choice tests, and examined changes in toxicity, pH and ammonia with sludge decay. These results are reported in full and the results of other published studies on the toxicity of sewage sludge to marine crustaceans are reviewed.

METHODS

Test animals

Adult shrimp, *Crangon crangon* of up to 8.4 cm in length (tip rostrum to tip telson) were collected from Firemore Bay, Loch Ewe, north-west Scotland in a 2 m beam trawl (4 mm mesh), in June and July 1988. Before use in experiments, the shrimp were held for two weeks at 10 ± 1 °C on a 11 : 13 h light : dark cycle, at densities of *c.* 100 individuals in 210 l (75 cm deep, 80 cm diameter), black polyethylene aquaria, with 1-2 cm of sand (median diameter 220 μm, < 125 μm diameter and *c.* 35% >250 μm, sorting coefficient 1.2). The sand and flow-through seawater (salinity 34 $g\,l^{-1}$) were also from Firemore Bay, and the shrimp were fed chopped squid *ad libitum* three times a week. Four days before an experiment 4.5-8.0 cm shrimp (*table* I) were placed into 10-L perspex aquaria with sand and 20 μm filtered, aerated seawater at 10 ± 1 °C, before being transfered to the test containers.

Egg bearing females were held at 10-11°C in 2-l beakers until the eggs hatched and larvae were removed by pipette. Stage one yolk-sac larvae (*ca.* 2 mm in length) were transfered to triplicate petri (5.7 cm^2) dishes with filtered seawater for experiments.

Brine shrimp, *Artemia salina*, larvae were hatched from cysts (San Francisco Bay brand) at 25°C in filtered seawater, and transfered to triplicate petri dishes for experiments. Only stage 2 and 3 larvae were used as they are more sensitive to pollutants than other larval stages (Sorgeloos *et al.*, 1978).

Sewage sludge

A single batch of sewage sludge from the Shieldhall treatment works in Glasgow, as in dumped off Garroch Head in the Clyde estuary, was stored at 4°C for the duration of the studies (8 weeks). The sludge samples used in experiments ranged from 0.85%-3.84% dry solids (*table* I). As with most sewage sludge of urban orgin, the sludge contains a wide range of contaminants from domestic and industrial sources. Toxicity may be influenced by both synergistic and antagonistic interactions between contaminants and cannot be attributed to any particular constituent (*see* review by Costello and Read, 1993). Although the contaminants determined are not given here, the chemical composition (including metals and organochlorines) of the sludge has been published by Costello and Gamble (1992).

Design of tests

All tests had controls replicated as for treatments. Tests with *Crangon* larvae for 24 h were conducted at 10°C, 15°C and 20°C with sludge of 0.85 g dry solids, and with *Artemia* larvae at 20°C with sludge of 3.84 g dry solids. Neither *Crangon* nor *Artemia* larvae were fed but they may have eaten particulate material from the sludge during the experiments. The experiments at 10°C and 15°C were conducted on a 11 : 13 h dark-light cycle, and experiments at 20°C on a natural 18 : 6 h cycle.

An unfiltered sample of sludge was taken, following thorough mixing of the sludge in the storage container, and added to the test containers on a percent wet volume basis. In the toxicity decay test, 0.3% sludge was added to triplicate petri dishes at 20°C over 7 days and then a 24 h toxicity test was conducted with *Crangon* larvae in each dish at 20°C. The controls consisted of filtered seawater similarly added to triplicate petri dishes each day.

Changes of pH and ammonia were determined following the decay of 0.3% sewage sludge at 10 ± 1 °C over 7 days in triplicate, aerated, 2 l beakers covered in polyethylene film. Therefore, by the seventh day there was sludge in beakers for 24, 48, 72, 96, 120, and 144 hours (as for the toxicity decay test). On the seventh day samples were analysed from all beakers for total ammonia-nitrogen by the phenol-hypochlorite method (Greenberg *et al.*, 1985) and for pH at 20 ± 2 °C using a combined glass electrode (Digiphase meter), calibrated with buffers made from Trizma acid and base mixed with artificial seawater at 34 $g\,l^{-1}$ salinity (Hansson, 1973; Sigma, 1979). From the total ammonia, pH, temperature and salinity, the proportion of unionised ammonia was calculated following Bower and Bidwell (1978) and Spotte and Adams (1983).

There were four tests with adult *Crangon* in sewage sludge treated seawater at 10°C. These tests overlapped in their range of concentrations of sludge in an attempt to both repeat and more accurately define the toxic concentrations The test concentrations were: 1%, 0.3%, 0.1%, 0.03% in Experiment I; 30%, 10%, 3%, 1% in Experiment II; 3%, 1%, 0.3%, 0.1% in Experiment III; 10%, 3%, 1%, 0.3%, 0.1% in Experiment IV. All were static and aerated, but test IV had a 24 h renewal of the test solution. The tests were conducted in aerated (oxygen saturation was always >75%), randomly arranged, 2-l acid-washed borosilicate glass beakers, covered in polyethylene "cling" film. There were four shrimps per beaker and five replicates, including controls. Shrimps were of similar size (*table* I), randomly allocated to beakers, not fed during a test, and dead (no response to prodding) shrimps removed four times daily.

There were two sediment test designs. Nine adult *Crangon* were placed in triplicate 10 l perspex aquaria (1060 cm^2) with randomly arranged 9 cm diameter petri dishes containing sand. The sand was mixed with 0, 1, or 4 ml of sewage sludge, resulting in nominal 0%, 1.57% and 6.25% sludge concentrations. There was no sand between the dishes, and the aquaria were gently aerated. The positions of the *Crangon* were recorded at 0.5, 1.5, 3, 4, 5, 6, 7, 22 and 24 h after the addition of the shrimp. In the second design, the base of two of the same aquaria were equally divided by a partition and half covered in clean sand and half with sand premixed with 10% sludge. The sediment partition was removed and the surface smoothed with a glass rod. On eight occasions with different *Crangon*, two individuals were simultaneously added to the centre of the aquarium and their positions recorded every 15 min for 1 h. After 5 h the sediment and water was replaced as it was impossible to see the shrimp due to their burying activity and resulting opacity of the water.

Table I. – Details of *Crangon crangon* (size in cm, % egg bearing females) and sewage sludge used in experiments.

Experiment	II	III	IV and Sediment tests
Number of *Crangon*	80	80	96
Body length ($x \pm SD$)	5.21 ± 0.61	6.40 ± 0.81	6.20 ± 0.68
% egg bearing	6	43	54
% Dry solids	0.85	3.84	3.51
Specific gravity	1.029	0.995	1.017

RESULTS

Over 24 h there was no toxicity to *Crangon* larvae up to 5% sewage sludge at 10°C, and up to 2.0% at 15°C. At 20°C the 24 h LC50 was 0.38% (95% CL 0.36 and 0.42) for *Crangon* larvae, and 10.0% (95% CL 8.5 and 11.75) for *Artemia* larvae.

Examination of the decay rate of sludge at 10°C found ammonia decreased and pH increased, and both reached background levels in 96 h. Sludge toxicity to *Crangon* larvae with sludge decay at 20°C reached control levels within 48 h (*fig.* 1). Unionised ammonia showed a closer correlation ($r^2 = 0.42$) to toxicity than total ammonia ($r^2 = 0.32$), but neither correlation was significant ($p > 0.05$).

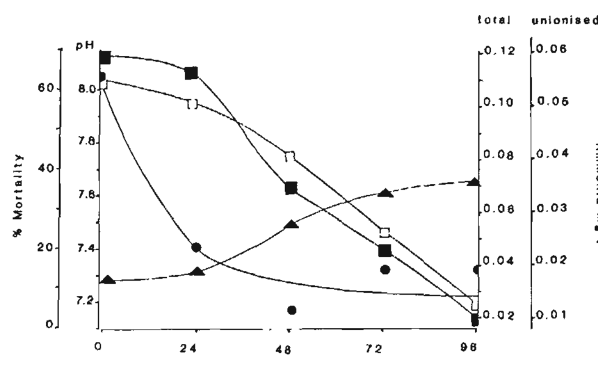

Figure 1. – Changes in total (□) and unionised (■) ammonia, pH (▲) and toxicity (●) to *Crangon* larvae with sludge decay over time. Curves fitted by hand and toxicity curve considers unplotted data at 120 h and 144 h.

A preliminary test (I) found only 12% mortality of adult *Crangon* in 1% sewage sludge. In further tests (II, III, IV) with adult *Crangon*, there is considerable overlap in LT50 values (*fig.* 2*a*), and variation in toxicity was greater at low (<3%) sludge concentrations (*fig.* 2). However, a dose response was apparent (*fig.* 2*a*, *b*). While tests using sludge with a higher dry solids content produced higher toxicity, this was not significant ($p>0.5$) (*fig.* 2*a*). Neither was there a significant difference between the static and 24 h renewal test results. Tests III and IV had 96 h LC50 values of 0.79 (95% CL, 0.53 and 1.50) and 1.69 respectively.

The *Crangon* spent significantly more time (62%, $p<0.05$) on the petri dish sediments than not on sediments, and significantly less time on the high (6.35%) sludge treatment sediments ($p<0.05$) (*fig.* 3). In the second sediment test, *Crangon* did not move position after 15 min, and there were over three times more shrimp on the clean sand than sand with 10% sludge (25.6 ± 1.33 versus 8.20 ± 2.23, mean $\pm$ SE) ($p<0.001$).

DISCUSSION

This study found a 24 h LC50 at 20°C of 0.4% sewage sludge to *Crangon crangon* stage 1 larvae, 24 h LC50 at 20°C of 10.0% to *Artemia salina* stages 2 and 3 larvae, and 96 h LC50 at 10°C of 0.8% and 1.7% to adult *Crangon*. These levels were within the range of 0.01% to 8.40% found in other 24-96 h LC50 studies for Crustacea (Franklin, 1983; Chapman, 1985; Fava *et al.*, 1985; Miller *et al.*, 1987; Santoro and Fikslin, 1987). Sewage sludge concentrations at dumping grounds have been estimated to be generally less than 0.1% within 30 min and 0.01% within 1 h (Costello and Read, 1993). Considering these LC50 results and the LT50 results (e.g. 80-90 h LT50 at 1% sludge, *fig.* 1), lethal effects on benthic Crustacea due to sewage sludge appear unlikely at disposal sites.

However, bioaccumulation of sewage sludge components can occur and perhaps have longer term effects. Franklin (1983) found accumulation of zinc and copper in adult *Crangon* exposed to 0.03% and 0.1% sludge over 10 days, but Maciorowski *et al.* (1985) found no accumulation of cadmium, mercury, DDT, PCB's, or petroleum hydrocarbons in grass shrimp, *Palaemonetes pugio*, exposed to 0.033% to 0.0042% sludge over 10 days. Chapman *et al.* (1988) found that filtering feeding barnacles, *Elminius modestus* Darwin, and mysids, *Schistomysis spiritus* (Norman) accumulated a radioactive silver marker in sludge, and *Crangon* feeding on the contaminated mysids additionally accumulated the silver. Elevated levels of metals and organochlorines have been recorded in Crustacea in field surveys, notably *Crangon* sp., *Cancer pagurus* L., and *Eupagurus bernhardus* L. in Liverpool Bay (Murray and Norton, 1982; Norton *et al.*, 1984), and metals in *Crangon allmani* Kinahan and *Pandulus montagui* Leach in

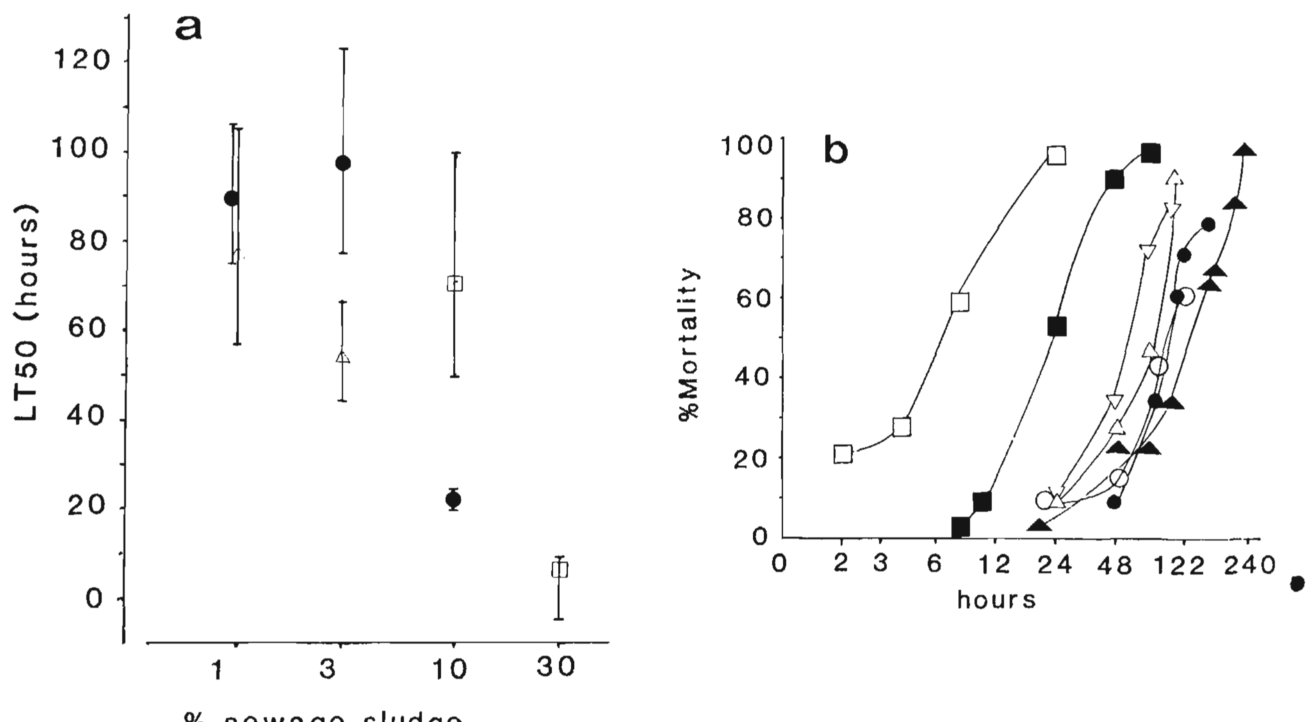

Figure 2. – Toxicity of sewage sludge to adult *Crangon crangon*. (a) LT50 in 24 h renewal (●, exp. IV), and static tests with low (□, exp. II) and high (△, exp. III) sludge solids content. (b) Mortality at 30% (□), 10% (■), 3% (▲, △, ▽), and 1% (●, ○) sludge concentrations, in experiments II (□, △), III (□, ○) and IV (■, ▲, ●).

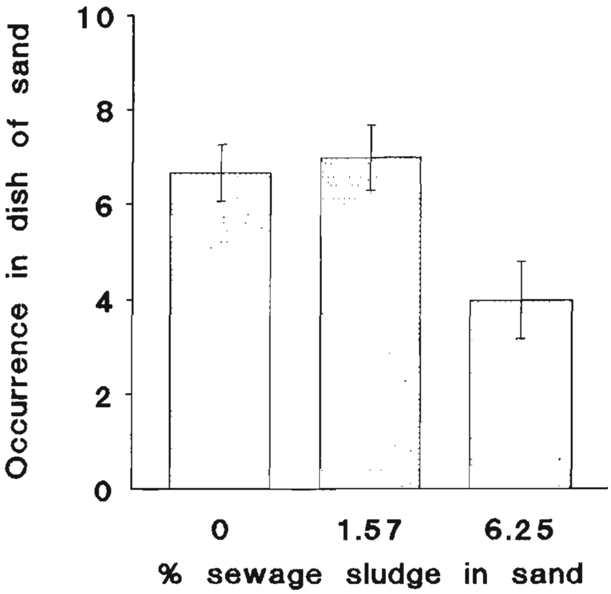

Figure 3. – Occurrence (mean ± standard error) of *Crangon* buried in clean sand and sand mixed with sewage sludge at two different concentrations.

Garroch Head (Mackay *et al.*, 1972). Hence, while laboratory studies may indicate that lethal effects are unlikely in the field over days, longer term sublethal effects may occur. The elimination of persistent and bioaccumulating substances from sewage sludge to be disposed of at sea should be a priority in pollution control.

Considering the high levels of ammonia and its decay with time (*fig.* 2), an increased toxicity in 24 h sludge renewal tests in comparison to non-renewal static tests may have been expected. However, Miller *et al.* (1987) similarly found no difference in sludge toxicity with and without sludge renewal. It may be that microbial communities increased on the sides of the test container and quickly utilised any further ammonia added.

A significantly higher toxicity of sewage sludge at higher solids concentrations was found for Crustacea, but not for fish, by Fava *et al.* (1985). Fava *et al.* (1985) suggested that particulate matter in the sludge may have interfered with feeding, respiration and orientation in the mysid *Mysidopsis bahia*. Entrappment of a planktonic copepod, *Temora longicornis* (Müller), and planktonic cod, *Gadus morhua* L., and herring, *Clupea harengus* L., larvae by sludge has also been observed (Chapman, 1985; Costello and Gamble, 1992). Physical factors may therefore be more important in the toxicity of sludge to Crustacea than for other taxa.

The decrease in toxicity and unionised ammonia with sludge decay support the suggestions in other studies (Read, 1977; Fava *et al.*, 1985; Costello and Gamble, 1992), that at least in short-term tests, ammonia has an important role in toxic effects of sewage sludge. The absence of a statistically significant correlation between ammonia and mortality in the present sludge decay experiment may be due to the limited number (5) of samples available for comparison.

Lethal toxicities (96 h LC50) of unionised ammonia-nitrogen to Crustacea have been found for adult shrimp (*Palaemonetes pugio*) at 2.57 $mg.l^{-1}$ NH_3-N and mysids (*Mysidopsis bahia*) at 0.86 $mg.l^{-1}$ NH_3-N (Fava *et al.*, 1985). The copepods *Acartia tonsa* and *A. hudsonica* were more sensitive, with a 48 h LC50 of 0.12 $mg.l^{-1}$ NH_3-N (Sullivan and Ritacco, 1985). Miller *et al.* (1990) found a 96 h LC50 of 0.54 $mg.l^{-1}$ NH_3-N for the mysid *Mysidopsis bahia*, and suggested levels of 0.03 and 0.09 $mg.l^{-1}$ NH_3-N were necessary for the survival and growth of this species respectively. Chin and Chen (1987) proposed a safe level of 0.01 $mg.l^{-1}$ NH_3-N for the culture of the tropical shrimp *Penaeus monodon*. Levels of unionised ammonia in the present study (<0.06 $mg.l^{-1}$ NH_3-N) were low, and close to the levels reported necessary for the survival and growth of some crustaceans in the literature. To clarify the role of ammonia in sewage sludge toxicity to test organisms, the sensitivity of the test population to ammonia should be determined in parallel with the sewage sludge toxicity tests.

The sublethal sediment choice-tests conducted indicate the feasability of such techniques. However, further work is required to determine the role of physical and chemical characteristics of the sludge in the choice of sediment by the *Crangon* and determine the sensitivity of the test. Field surveys often find a change to a more Polychaeta dominated benthic community (e.g. MacKay *et al.*, 1972; Norton *et al.*, 1981; Talbot *et al.*, 1982; MacKay, 1986; Topping, 1987). Whether such changes are due to increased mortality (present study suggests this unlikely in short-term), emigration or reduced recruitment of Crustacea is unclear. Furthermore, whether reductions in Crustacea numbers are a response to the physical, organic or toxic effects of sewage sludge are unknown. Sediment tests, as attempted in this study, may be more useful if considered as part of life-cycle, bioaccumulation, and mesocosm studies, and designed to relate to field studies.

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