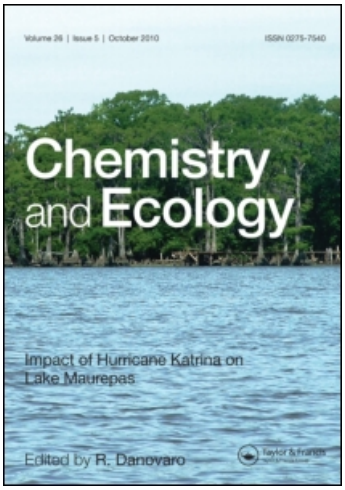

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R. Butler[a]; B. D. Roddie[a]; C. P. Mainstone[a]; Tsen C. Wang[a]; J. W. Tucker Jr.[a]
[a] Water Research Centre (1989) plc Medmenham Laboratory, Marlow Bucks, U.K.

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THE EFFECTS OF SEWAGE SLUDGE ON TWO LIFE-HISTORY STAGES OF *MYTILUS EDULIS*

R. BUTLER, B. D. RODDIE and C. P. MAINSTONE

Water Research Centre (1989) plc
Medmenham Laboratory
Henley Road, Medmenham
P.O. Box 16 Marlow
Bucks. SL7 2HD U.K.

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Adult and embryos of the common mussel *Mytilus edulis* were exposed to dilutions of, respectively, whole and seawater-extracted sewage sludge. The response of adult mussels was measured as change in feeding and respiration rate after four weeks' continuous exposure, while embryo response was assessed as the percentage of normal, D-shaped larvae after 24 hours' exposure. Adult feeding rate was significantly depressed, and respiration rate significantly increased, at the lowest sludge concentration used (0.02%), and the effect appeared to be attributable to the dissolved rather than to the particulate phase of the test medium. The developmental EC50 for embryos was estimated to be 0.15% sewage sludge. At dilutions of 1:600 to 1:5000, therefore, the sewage sludge investigated contained unidentified desorbable components toxic to planktonic and benthic life-history stages of a common marine invertebrate.

KEY WORDS Mussel, sewage sludge, toxicity

INTRODUCTION

There is great concern within Europe regarding the environmental effects of the disposal of sewage sludge to sea—a practice used by many UK water undertakings, and licenced under the Food and Environment Protection Act (FEPA) 1985. In order to assess the impact of this practice on the marine environment, it is necessary to develop and apply routine toxicity-testing procedures which are sufficiently sensitive to respond at environmentally-relevant concentrations.

Whilst, ideally, testing procedures should mimic temporal patterns of disposal, there remains a basic and initial need to determine whether or not a particular sludge contains toxic contaminants, and whether or not these contaminants are released to the water column during disposal and dispersal.

The common mussel, *Mytilus edulis*, has been used widely for toxicity testing (e.g. adult mussels, Bayne *et al.* 1985; embryos and larvae, Woelke 1972, Johnson 1988). As part of a collaborative programme with the Department of Agriculture and Fisheries for Scotland, Aberdeen, and Napier Polytechnic, Edinburgh, *Mytilus* adults and embryos were exposed to a mixed industrial/domestic sewage sludge from a Glasgow treatment works. The aim was to examine the sublethal physiological effects of chronic exposure to both the dissolved and particulate phases in adult mussels, and the acute effects of short-term exposure to the

dissolved phase on embryos and early larvae. Although not indigenous to sludge disposal grounds, *Mytilus* is a cosmopolitan species of considerable economic and ecological importance, and one of the few marine species for which well-developed test procedures for different life-history stages are available.

MATERIALS AND METHODS

Chronic exposure of adult mussels

This work was conducted at the DAFS Marine Research Unit, Wester Ross, Scotland. Mussels were obtained from culture rafts in a nearby loch, having been relaid from the east coast some four months earlier.

Sufficient sewage sludge (3–5% dry solids content) for the duration of the experiment was obtained from Shieldhall treatment works in Glasgow, and held at 4–15°C prior to use. The sludge was supplied to the experimental tanks via peristaltic pumps and banjo filters from either of two pre-diluted stocks. These stock solutions were pre-screened through 2 mm nylon mesh to remove large solids which might clog the pumps, and each stock tank contained a submersible pump to maintain the particulate phase in suspension. Each experimental tank also contained a submersible pump, and any accumulated material was regularly removed by siphoning. Fresh stock dilutions were made up at intervals of a few days.

Groups of 40–50 mussels were exposed to each of three concentrations of sludge and a sea water control for 5 weeks:

Control (0% sludge)

Low (0.02% nominal)

Medium (0.084% nominal)

High (0.42% nominal)

Mussels were maintained in plastic-covered wire-mesh cages in 400 l tanks, each with a flow rate of 150 l/h. Sea water was supplied, unfiltered but settled, from an offshore pump via a ten ton header tank.

Dissolved oxygen levels and temperature were monitored daily in each tank. Suspended solids levels in each tank were estimated gravimetrically every third day.

Feeding and respiration rates were measured to estimate sublethal physiological stress. Measurements were made on a random sample of 15 51–53 mm length mussels prior to exposure, and on a sample of 15 mussels in the same size class from each tank after 5 weeks' exposure. Control animals received no food supplement.

Feeding rate was measured as diatom clearance rate (l/h), in a flow-through apparatus. Sea water containing a suspension of the diatom *Phaeodactylum tricornutum* was supplied to 500 ml vessels holding individual mussels. Cell concentrations in the vessels' inflow and outflows were monitored using a Coulter Counter model ZM set to count all particles greater than 4 μm spherical equivalent diameter, and clearance rate estimated from the proportional decrease in cell counts and the flow rate through the vessels.

Respiration rate (ml oxygen/h) was measured in sealed 350 ml respirometer vessels; the rate of decline in oxygen tension was monitored over a 30–40 minute

period using microcathode electrodes connected via amplifiers to a multichannel chart recorder.

Before measurement of clearance and respiration rates, mussels were allowed to acclimate to experimental conditions for at least 30 minutes. Following these measurements, mussels were sacrificed, and tissue dry weight determined. Mussel flesh was dissected from the shell, blotted dry, and placed in foil boats at 60°C for 24 h.

Both feeding and respiration rate were allometrically corrected to a standard 1.0 g dry flesh weight, using exponents of 0.4 for clearance rate and 0.7 for respiration rate. These values were chosen to represent the mid-range of exponents from a wide range of studies by Bayne (1976).

To permit an integrated assessment of the effects of sludge on clearance and respiration rates, both rates were expressed in common energy terms, and a nominal net energy budget calculated on an individual basis

"assimilation" = Clearance rate * 16 * 0.6 J/g-h

"respiration" = Respiration rate * 20.33 (J/g-h)

"nominal budget" = "Assimilation"–"Respiration" (J/g-h)

where

16 is an estimate of the energy available (J/litre of sea water)

0.6 is an estimate of absorption efficiency

20.33 is an oxycalorific conversion factor based on mixed substrate metabolism.

It should be noted that this nominal net energy budget simply reflects differences between rates within treatments, and does not estimate actual energy budgets.

From each treatment, twenty mussels were dissected from their shells and held frozen for chemical analysis. Ten mussels were defrosted and acid digested in pairs (i.e. 5×2) by boiling in concentrated nitric acid, and analysed by Inductively Coupled Plasma Optical Emission Spectrophotometry (ICPOES) for Cu, Cd, Ni, Zn, Pb and Cr. The remaining 10 animals were thawed, acid digested in pairs by an HNO3/H2SO4/Vn2O5/ mixture, and analysed by Cold Vapour Atomic Absorption Spectrometry (CVAAS) for mercury.

Acute exposure of mussel embryos

The acute toxicity of sea water extracts of sewage sludge was examined using a modified version of an embryo-larval bioassay originally developed by Woelke (1972). The test was conducted on gametes obtained from ripe *Mytilus* from Whitsand Bay, Cornwall, southern England, and used as an endpoint the development of embryos to the D-shaped larval stage. Prior to this stage, the larva has no shell and is very sensitive to dissolved toxicants.

At the end of the adult mussel exposure period, a 5 litre sample of the sludge used was retained, and stored at 4°C until used with *Mytilus* embryos. An aqueous extract was prepared from a 1:9 dilution of this sludge in artificial sea water (Tropic Marin). The mixture was shaken (100 rpm) for 16 h, settled for 2 h, and the supernatant decanted and filtered to 0.45 μm.

Concentrations of 0, 0.1, 0.32, 1.0, 3.2 and 10% sludge extract were prepared in artificial sea water. Controls for the effect of salinity reduction at higher concentrations were included in the experimental design; 10, 20 and 50% distilled-water-in-sea water treatments were prepared.

Mussels were induced to spawn by thermal shock, and then held individually until spawning was complete. Eggs and sperm from two individuals were selected to give a single "pairing", to minimise genetic variability in the test embryos. Gametes were filtered through 100 μm mesh prior to mixing in the ratio 1000 sperm: 1 egg.

When embryos had reached the 16–32 cell stage (after approximately 90 minutes), a subsample of the suspension was placed in a Sedgewick-Rafter cell and examined microscopically to assess quality and quantity.

Three 30 ml aliquots of each test concentration were poured into 30 ml glass jars, and inoculated with 0.5 ml of embryo suspension to give a density of approximately 20 embryos per ml. Test vessels were incubated at 20°C for 48 h in darkness, following which 0.5 ml of buffered formaldehyde was added to each vessel to preserve embryos prior to counting.

Single 5 ml subsamples were withdrawn from each (well-mixed) replicate vessel, and the numbers of normal and abnormal larvae counted in a sedimentation tube with the aid of an inverted microscope.

RESULTS

Chronic toxicity to adult mussels

Dissolved oxygen (DO) levels were maintained above 70% saturation in all tanks throughout the exposure period, and only dropped below 80% on infrequent occasions in the intermediate and high exposure tanks. This is not likely to have constituted an additional stress upon the test organisms. Aeration was installed in the high exposure chambers to maintain adequate oxygen levels following a decline in DO over the first 6 days of exposure.

Water temperature fluctuated between 13 and 16°C over the exposure period, and is not likely to have posed an additional stress upon the experimental mussels.

Suspended solid levels were an order of magnitude lower than intended in all three treatment tanks (Figure 1) due to losses of particulate material from a combination of the effects of pre-screening process (accounting for a loss of approximately 50% of sludge dry weight) and settlement in stock B (the high concentration stock). Suspended solids levels in ambient Loch Ewe sea water stayed below 1 mg/l throughout the exposure period.

Nominal estimates of energy assimilated, energy respired, and net energy balance are summarised in Figure 2. All variables measured were subjected to one way ANOVA, followed by Tukey's test for Honestly Significant Differences between means. Unless otherwise indicated, statistical significance has been tested at the 5% probability level.

Energy assimilated (directly proportional to feeding rate) showed a logarithmic decline with increasing sludge concentration (Figure 2, Table 1). All treatment means differed significantly (Table 1), but the control mean was not significantly different from the "initial" mean.

Energy respired differed less markedly between treatments, but exhibited a significant trend of increase with increasing sludge concentration (Figure 2).

The nominal net energy budget displayed a pattern very similar to that of energy assimilation; again, all treatment means differed significantly (Table 1, Figure 2).

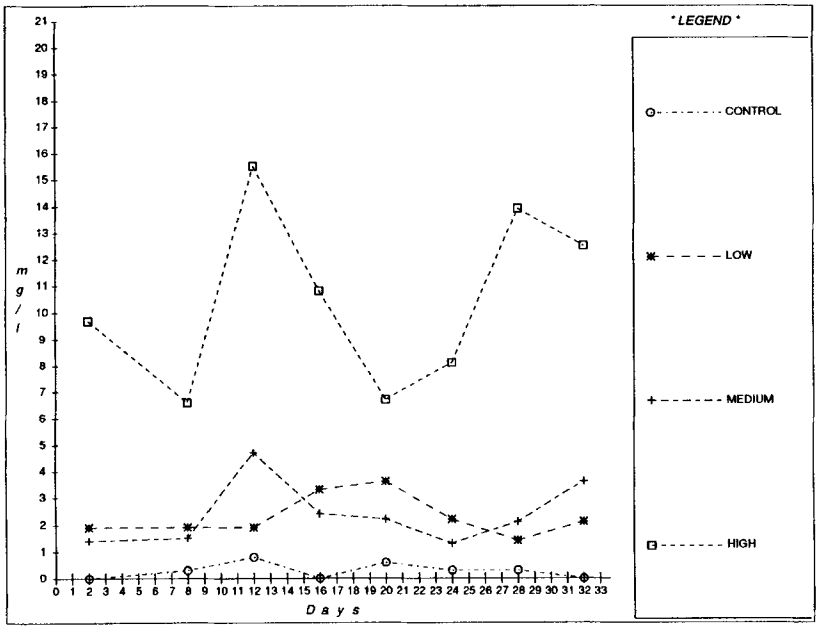

Figure 1 Suspended solids concentrations (mg/l) in control, low (0.02%), medium (0.1%) and high (0.45%) sludge treatments.

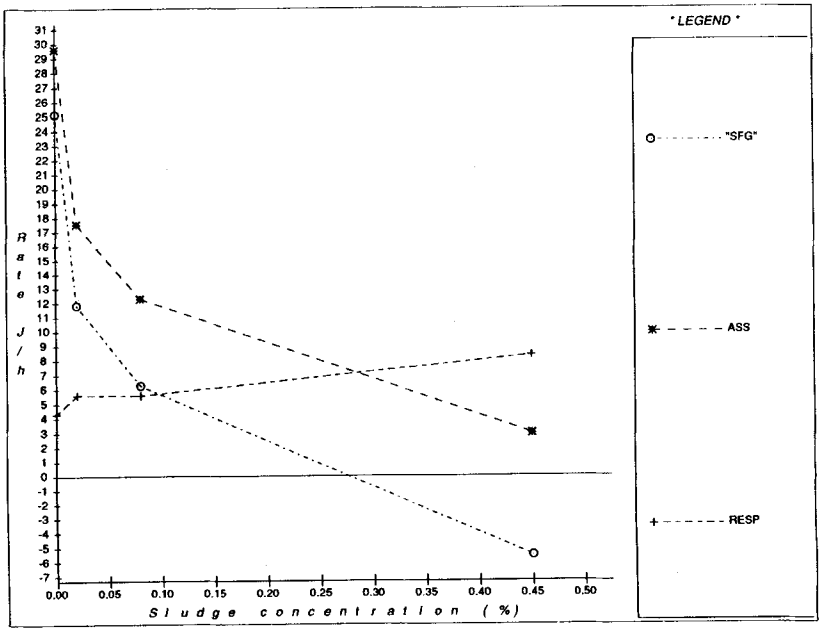

Figure 2 Nominal net energy budget ("SFG"), energy assimilated (ASS) and energy respired (RESP) in *Mytilus* exposed to sewage sludge for 33 days.

Table 1 Physiological responses of *Mytilus* to exposure to three dilutions of sewage sludge. (Horizontal bars connect values not significantly different from each other at $P < 0.05$, using Tukey's Honestly Significant Difference—HSD)

Energy Assimilated (Nominal, J/g-h)		*HSD* = 4.35		
Control	Initial	Low dose	Medium dose	High dose
29.60	28.33	17.55	12.28	2.95
Energy respired (Nominal, J/g-h)		*HSD* = 0.98		
High dose	Initial	Medium dose	Low dose	Control
8.33	6.06	5.88	5.61	4.32
Net energy budget (Nominal, J/g-h)		*HSD* = 4.74		
Control	Initial	Low dose	Medium dose	High dose
25.16	22.16	11.82	6.26	−5.52
Dry weight (g)		*HSD* = 0.177		
Initial	Low dose	Control	Medium dose	High dose
1.188	1.609	1.062	0.915	0.901

Dry flesh weight was significantly lower in all treatment groups after 5 weeks compared to the initial weight, but did not differ significantly between treatment groups.

Tissue concentrations of cadmium, copper and mercury (Table 2) changed little in any treatment during exposure to sewage sludge and concentrations of zinc increased only slightly. Concentrations of chromium, lead and nickel increased in mussels during exposure, but increased also to a lesser extent in the control animals. The highest concentrations of these three metals were observed in mussels exposed at the lowest sludge concentration.

Table 2 Tissue concentrations of heavy metals in *Mytilus* exposed to three dilutions of sewage sludge. (Values are means of 5 pairs of animals in μg/g wet wt, with 95% confidence limits in brackets below each mean)

Dose	*Cd*	*Cr*	*Cu*	*Pb*	*Ni*	*Zn*	*Hg*
Initial	0.204	0.087	1.44	0.223	0.124	17.8	0.015
(95% CI)	(0.027)	(0.012)	(0.07)	(0.062)	(0.024)	(2.6)	(0.002)
Control	0.209	0.180	1.37	0.295	0.387	26.2	0.014
(95% CI)	(0.053)	(0.027)	(0.12)	(0.064)	(0.534)	(6.3)	(0.001)
Low dose	0.164	0.238	1.38	0.614	0.821	21.3	0.015
(95% CI)	(0.021)	(0.047)	(0.10)	(0.056)	(0.195)	(3.0)	(0.002)
Medium dose	0.183	0.176	1.32	0.418	0.557	22.9	0.012
(95% CI)	(0.041)	(0.040)	(0.07)	(0.117)	(0.070)	(6.8)	(0.002)
High dose	0.209	0.201	1.17	0.487	0.719	25.3	0.014
(95% CI)	(0.024)	(0.027)	(0.12)	(0.140)	(0.230)	(4.8)	(0.005)

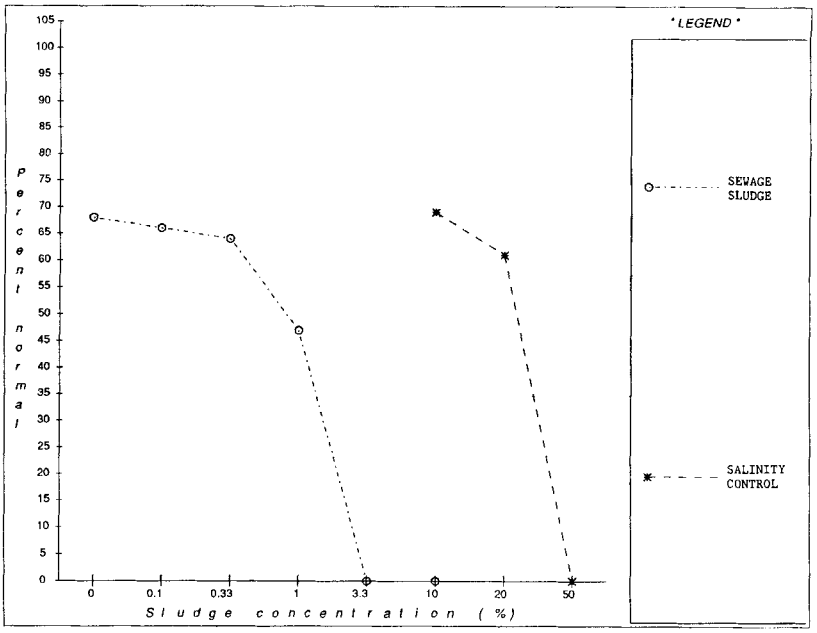

Figure 3 Percentage normal development of *Mytilus* embryos exposed to dilutions of sea water extracts of sewage sludge.

Acute toxicity of sludge to mussel embryos

The number of normal D-larvae per 5 ml of treatment sample was significantly reduced with respect to controls at sludge extract concentrations of greater than 0.32% (Figure 3). Salinity controls demonstrated that a reduction in salinity of up to 20% had no effect on embryonic development, and that consequently effects observed in sludge extract treatments could be attributed to desorbed sludge contaminants.

The 48 h EC50 for the 10% sludge extract was estimated by graphical interpolation to be 1.5%, equivalent to an EC50 of 0.15% for whole sludge in sea water.

DISCUSSION

All mussels lost weight during the experiment, but mean weight after 5 weeks did not differ significantly between treatments. The population from which the experimental animals were obtained spawned during the study period, and spawning (with associated weight loss) may also have occurred in the experimental tanks.

No-effect concentrations were not established for assimilation rate and nominal net energy budget in this study, although suspended solids concentrations in the tanks were an order of magnitude lower than intended. Some solids were lost due to the pre-screening treatment, and some may have been lost due to settlement in

the stock and experimental tanks despite recirculation. Fine particulates, to which contaminants would be preferentially adsorbed, are less likely to have been lost than coarser material. However, toxicity appeared to be more systematically related to nominal dilution than to actual suspended solids levels, and this suggests that effects were mainly attributable to the dissolved, rather than the particulate, phase.

No clear relationship existed between tissue concentrations of heavy metals and measured physiological stress, although there was an indication that some metal levels might be a positive function of feeding rate. The metal concentrations measured could not account for the observed sublethal toxic responses.

The EC50 of 0.15% for mussel embryos was similar to values we have obtained using the same test with other mixed industrial/domestic sewage sludges.

The methods for both sublethal toxicity assessment in adult mussels and acute toxicity measurement in embryos are well-documented, and there are clear advantages in the ability to estimate effects (a) on the potential of adults for growth and/or reproduction (as indicated by reduced feeding rate and/or increased respiration rate); and (b) on embryos in terms of survival and therefore potential for recruitment.

With adult mussels, feeding rate appears to respond more sensitively to toxicant impact than other measures of physiological fitness. In some 24 other field and laboratory exercises using the same methods, we have also found this to be the case. Where the aim is simply to detect effects of pollutants, feeding rate will generally be a sufficient measure of response. Donkin *et al.* (1989), for instance, used feeding rate effects in *Mytilus* to evaluate QSARs for hydrophobic organic contaminants, while Widdows and Johnson (1988) found, in a study of the effect of oil and copper on Scope for Growth in *Mytilus*, that changes in feeding rate were more marked than those in respiration or excretion rate.

Both tests proved to be sensitive to (presumed) sewage sludge contaminants. The toxicity of sewage sludge to marine organisms is thoroughly reviewed in a paper presented elsewhere at this Symposium (Dr M. J. Costello), and will therefore not be considered in detail here. The mussel tests described here, however, rank among the most sensitive to be reported; only a few tests with mysids, copepods and brown shrimp larvae have detected effects at lower concentrations.

The lowest concentration used in this study was 0.02%, or a dilution of 1:5000. This dilution would be achieved within, at most, a few hours of sludge disposal at sea. Animals in the receiving environment would not therefore be exposed continuously to this level of impact. Further, it is not clear that the present experimental system could mimic the pattern of desorption and resorption of sludge contaminants which would occur under disposal conditions. Nevertheless, both experiments have demonstrated the toxic potential of sewage sludge to marine organisms via processes directly affecting growth, reproduction and recruitment.

SUMMARY

Sewage sludge may be disposed to the marine environment by coastal dumping or pipeline discharge, and may thus impact both planktonic, and coastal benthic,

communities. Whilst exposure in the field may be episodic, as a first approximation it is useful to know whether sludge components are toxic when presented at constant concentrations and in a steady state of partitioning between the dissolved and particulate phases.

Adult mussels (*Mytilus edulis*) were exposed to a mixed industrial/domestic sewage sludge at concentrations ranging from 0.02% to 0.42%. The sludge was dosed continuously for four weeks; at the end of this period, feeding and respiration rates, physical condition indices, and body burdens of trace metals were measured. Feeding and respiration rates were converted to common energy terms, and the difference expressed as a nominal net energy balance (broadly equivalent to Scope for Growth). Net energy surplus was reduced by 50% at the lowest concentration tested; this was a consequence of elevated respiration rate and reduced feeding rate. Response was related to the dissolved, rather than the particulate, phase of the test medium, and was not related to tissue burdens of trace metals.

At the end of the four-week experiment, early mussel embryos were exposed to serial dilutions of sea water extracts of the same batch of sludge. Embryo development was followed to the D-larval stage (c. 48 h), and an EC50 of 0.15% sewage sludge estimated for the inhibition of normal development. Both studies demonstrated the presence and availability in a batch of sewage sludge of components toxic to both planktonic and benthic life-history stages of molluscs.

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