

Influence of pollution along a natural gradient and in a mesocosm experiment on sediment microbial numbers and biomass

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ABSTRACT: For the GEEP Workshop, microbial communities in sediments from 6 locations in Frierfjord/Langesundfjord, Norway, and from an experimental mesocosm facility at Solbergstrand on the Oslofjord, were sampled and analysed to determine effects of hydrocarbon and metal contamination. The fjord sites showed clear trends of increasing microbial abundance, especially in microflagellates, with increasing distance from known pollution sources. Concentrations of Pb and Mn in the sediments were negatively correlated with microflagellate biomass, and Zn was negatively correlated with bacterial biomass. Results from the mesocosm experiment were less clear.

INTRODUCTION

Static state descriptions of bacterial populations have long been used as indicators of pollution in marine and freshwater environments, less so in sediments than in the water column. A common approach has been to test for certain indicator bacteria that demonstrate the presence of contaminants, for example high faecal coliform counts or the presence of human pathogens where contamination by domestic sewage is known or suspected (American Public Health Association 1981). In more ecologically oriented studies, or where pollution is less easily traced by bacteria associated with human waste, the microbiological approach has been to analyse the 'species' compositions of laboratory cultures derived from natural microbial communities, in a manner similar to ecological studies of other types of communities of organisms (e.g. Hauxhurst et al. 1980, 1981, Bell et al. 1982, Fukami et al. 1985, Sohler & Bianchi 1985). Trousselier & Legendre (1981) have adopted an ecologically and practically more suitable approach where, instead of classifying communities by species composition, they used a classification based on metabolic types of bacteria. This avoids the problems of bacterial species concepts and taxonomy in nature and provides ecologically useful information on the variety and dominance structure of various bacterial metabolic capabilities represented in the sediment.

Although the method of Trousselier & Legendre (1981) provides useful information, especially on the consequences of pollution on the turnover and utilization of materials by bacteria, this method is, like all laboratory culture methods, very time consuming, exacting and costly. In addition, bacterial community analyses require a high level of technical expertise and their interpretation depends on complex multivariate statistical techniques. For many applications in monitoring biological effects of environmental pollution, simpler methods may suffice. In any case, it is desirable to base conclusions on natural sediment conditions rather than on the responses of a highly select assemblage which can be successfully cultured in laboratory media.

The samples collected from the postulated pollution gradient in Frierfjord/Langesundfjord and from the mesocosm experiment at Solbergstrand provided an opportunity to assess the utility of very simple microbial community studies, under sampling conditions and analysis time constraints typical of many pollution studies. Analysis of the samples was by acridine orange direct counts (AODC) and size distributions of total sediment microbial communities, including microbial eukaryotes as well as bacteria. The only taxonomy involved was based on a very few shape and fluorescence characteristics to separate the organisms into major taxa. Statistical analysis consisted of simple ANOVA and *t*-tests, suitable for log-transformed

counts and size distribution data. The skills involved are therefore simple enough to be acquired in a short training course.

METHODS

The 6 sampling locations in Frierfjord and Lange-sundfjord, Norway, and the contaminant inputs to this fjord system, are discussed in Follum & Moe (1988). Sites B to F were expected to encompass a range of contaminant loadings, with A being a less-contaminated reference site. Samples for sediment meiofauna and microbiota were collected on 24 April 1986 using a Craib corer. Meiofauna cores were taken to 8 cm depth; each sampled an area of 24.6 cm². Prior to subsampling 5 of the replicate cores from each site for microbiota, the contents of each core were thoroughly mixed; 2 cm³ of sediment was removed from each mixed sample for bacterial analysis. It was mixed with 8 cm³ of filtered 4 % glutaraldehyde in seawater and stored, refrigerated, in glass vials. Samples were shipped to Bedford Institute, Canada, where they were held in a freezer (-20 °C) until analysis.

In addition to the field pollution gradient, a mesocosm experiment was set up at Solbergstrand (Bakke et al. 1988). Briefly, 3 mesocosm basins were dosed with a diesel oil and copper mixture added to the seawater intake, with a 4th basin acting as control. Five box cores (0.25 m²) of undisturbed natural sediment from the local Bjørnehodet Bay were placed in each mesocosm. Mesocosm dosing started on 24 April 1986 with contaminant levels designated as high dose (H), medium dose (M), low dose (L) and control (C) (Bakke et al. 1988). Microbial samples were collected on 2 occasions, 29 May and 7 July 1986. Core samples similar to those taken in the field for meiofauna and microbiota were taken from 4 of the boxes of sediment in each of the 4 experimental basins. For microbiota, duplicate cores were taken from each box. Preservation and storage methods were similar to those used for the field samples.

The procedure used for staining, enumerating and measuring bacteria, other prokaryotes and small eukaryotes was similar for all samples. It is a modification of the commonly used acridine orange epifluorescence method as outlined, for example, by Zimmermann & Meyer-Reil (1974) and Hobbie et al. (1977). Preserved sediment (100 µl) was withdrawn from each sample vial while being agitated on a vortex mixer; it was diluted to 20 ml with filtered 1 % glutaraldehyde in seawater so 1 ml of the slurry was equivalent to 1 µl of sediment or 10⁻⁴ cm² of sediment surface area. Duplicate dilutions were analysed for several samples to determine the error introduced by this subsampling procedure. Duplicates were always within 5 to 10 % of

the mean value. Nucleopore polycarbonate membrane filters (25 mm diameter, 0.2 µm pore size) were soaked for a minimum of 1 d in 0.2 µm filtered Sudan Black B solution (1:15000 w/v powdered stain in 50 % reagent alcohol). This solution was prepared by first dissolving the stain in 100 % alcohol, then diluting to 50 % with distilled water. The solution was kept refrigerated and was filtered before use. The stained filters were rinsed well with filtered distilled water immediately prior to use.

An all-glass vacuum filtration funnel and support for 25 mm filters (e.g. Kontes # 953700-0000) and a low vacuum pump were used for the acridine orange staining procedure. A glass fibre filter pad (e.g. Whatman GF/A) was placed on the filter support to prevent deformation of the membrane surface during vacuum filtration. A rinsed, Sudan Black-stained filter was placed on the glass fibre filter and the funnel was clamped on; 1 ml of diluted sediment (equivalent to 1 µl of natural sediment) was withdrawn from the slurry while being mixed on a vortex mixer to ensure a representative sample from the suspension. It was placed on the filter and then a low vacuum (ca 100 mm Hg) was applied until the meniscus disappeared. The vacuum was turned off and then 1 to 2 ml of prepared acridine orange solution (Becton-Dickinson #4940) was placed on the filter. The funnel and filter support were covered with foil to keep out light; sediment on the filter was then allowed to stain for 2 min. The stain was filtered off and the filter rinsed with distilled water (immediately prefiltered) sufficient to remove excess stain (1 to 2 ml). With the vacuum still on, the funnel was removed and the filter gently lifted off. The filter was placed sediment-side up on a drop of low fluorescence immersion oil (e.g. Cargille type A) on a glass microscope slide. Another drop of oil was placed on the surface of the filter and a cover slip placed on top. The slides were stored flat in the dark until analysis.

The procedure outlined above resulted in mostly green and yellow fluorescing organisms on a background of dull orange and red fluorescing sediment. Use of plastic manifold filtration apparatus always resulted in a mixture of green, yellow and orange organisms which was difficult to count on the sediment background. Sudan Black B was used as it resulted in a flatter black background with less fluorescence than the more commonly used Irgalan Black. Filters were not rinsed with alcohol after staining as this would rinse out the Sudan Black and also somewhat quench the fluorescence of the organisms.

A Zeiss ICM405 inverted microscope equipped for epifluorescence (HB050 lamp, 100× Neofluar objective, filter combination 9I, focal plane 20×20 counting grid) was used to enumerate the organisms. For measuring their size, a drawing tube was used to over-

lay the microscope image on that of a digitizing board and light-diode cursor placed next to the microscope. A Kontron MOP-40 Videoplan image analysis system was used for data acquisition and analysis. Bacteria and small eukaryotes were enumerated using the 100× objective in grid fields to a total count of at least 600 cells in 6 or more fields on each filter. According to Cassell (1965), this is sufficient for a counting precision of within 10 % of the mean for an individual filter. Counts were converted to numbers per ml and per unit surface of sediment. Organisms were classified as bacterial rods, cocci and filaments, as microflagellates, diatoms (mesocosm only) and other protists (mesocosm only). The term 'microflagellate' is used to indicate pigmented or unpigmented, flagellated, eukaryotic cells of less than 8 µm, and typically between 1 and 2 µm diameter. Measurements were made on all organisms, classed in a similar way, in randomly chosen fields during counting, up to ca 100 individuals in each group.

RESULTS

Field samples

There are differences in sediment metal concentrations among the sampling sites, with the reference site having significantly lower values of lead, zinc and copper than the others (Appendix 1, Fig. 3). However, none of the samples has excessively high metal concentrations (Appendix 1, Table 12) and there is not a simple pollution gradient, uncomplicated by other environmental factors (Gray et al. 1988). Yet there are some significant differences apparent among the meio- and macro-benthic communities (Gray et al. 1988, Heip et al. 1988); these differences are also apparent in the microbial component of the benthos.

Table 1 lists the abundance of bacterial rods, cocci and filaments, and of microflagellates per ml of sedi-

Table 1. Abundances of bacterial rods and cocci, bacterial filaments, and eukaryotic microflagellates per ml in the surface 8 cm of sediments from 6 locations in Frierfjord and Langesundfjord, Norway. Means (\pm SD) of 5 replicate cores taken at each site

Site	Rods and cocci ($\times 10^9$)	Bacterial filaments ($\times 10^7$)	Microflagellates ($\times 10^7$)
A	2.50 \pm 0.92	6.80 \pm 2.75	9.06 \pm 4.34
B	1.72 \pm 0.90	4.80 \pm 2.48	3.42 \pm 1.04
C	2.18 \pm 0.42	6.86 \pm 1.98	4.06 \pm 1.63
D	1.08 \pm 0.34	3.24 \pm 1.07	2.58 \pm 1.33
E	1.46 \pm 0.43	5.30 \pm 1.96	2.26 \pm 0.55
F	1.19 \pm 0.51	3.74 \pm 1.36	1.86 \pm 0.85

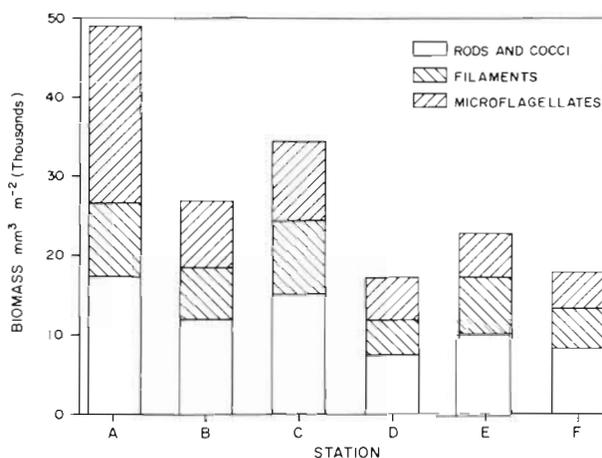


Fig. 1. Biomass ($\text{cm}^3 \text{m}^{-2}$) of bacteria and microflagellates in the surface 8 cm of sediment at 6 locations in Frierfjord and Langesundfjord, Norway

ment averaged to 8 cm depth. Diatoms and other protists were not common in the size range up to 8 µm in the field samples, although they were in the mesocosm samples. Larger diatoms were present in the fjord sediments but were not analysed due to time constraints. The data indicate a trend to increased abundance of the 3 groups as one progresses from the inner Frierfjord (E) to Langesund Bay (A). A 1-way analysis of variance indicated significant differences among sites in log-transformed abundances of rods and cocci ($p < 0.05$) and of microflagellates ($p < 0.01$), though bacterial filaments did not differ significantly. Significance of pairwise differences are tabulated in Table 2. They indicate that Site A and, to a lesser degree, C have generally higher abundances of micro-organisms than the other sites. The 3 most northerly samples (D,E,F), when combined, have significantly lower abundances (t -test) than the combined Langesundfjord samples (A,B,C), in bacterial rods and cocci ($p < 0.01$), bacterial filaments ($p < 0.05$) and microflagellates ($p < 0.001$).

Table 2. Significance levels (p) from t -tests of pairwise differences between microbial abundances (log transformed), at 6 locations in Frierfjord and Langesundfjord, Norway. Upper triangle: bacterial rods and cocci, lower triangle: microflagellates

Site	A	B	C	D	E	F
A	–	ns	ns	0.012	ns	0.026
B	0.018	–	ns	ns	ns	ns
C	0.038	ns	–	0.008	0.036	0.032
D	0.006	ns	ns	–	ns	ns
E	0.004	ns	0.050	ns	–	ns
F	0.002	ns	0.038	ns	ns	–

ns: $p > 0.05$

Size measurements were not done on all field samples due to time constraints; where they were done (1 sample in each of A, D, and E), size distributions within groupings were similar to those in the mesocosm samples. As in the mesocosm study, only the relative proportions of groups changed markedly among samples. Biomasses of rods + cocci, of bacterial filaments and of microflagellates were calculated from the abundance values (Table 1) and from average size distributions for each of the groups. Fig. 1 demonstrates that there is a general trend of increasing biomass along the inner to outer axis of the fjord system, with Sites B, C and especially A having greater microbial biomass in all 3 groups than the inner sites. Lead and manganese concentrations in the sediments are negatively correlated with the biomass of microflagellates ($r^2 = 0.47$, $p < 0.01$) and zinc concentrations are negatively correlated with bacterial biomass ($r^2 = 0.35$, $p < 0.01$).

Mesocosm samples

Analysis of the microbial communities in the mesocosm sediments included bacteria and eukaryotes up to approximately 40 μm in diameter. Abundances of microbial organisms, in numbers per ml of sediment, are given in Table 3. Analysis of variance of the various microbial groups indicates that only the benthic diatoms showed significant differences in abundance among experimental treatments. A 2-way analysis of variance reveals a significant ($p < 0.01$) increase in diatom abundance in all basins between 29 May and 7 July and also significant ($p < 0.01$) differences among treatments. However, the pattern of abundance levels across treatments did not reflect nominal dosing levels nor was it consistent on both sampling dates. The biomasses of microbial organisms in mesocosm sediments on July 7 (Fig. 2) illustrate the lack of clear biological effects of contaminant exposures on any of the microbial groups.

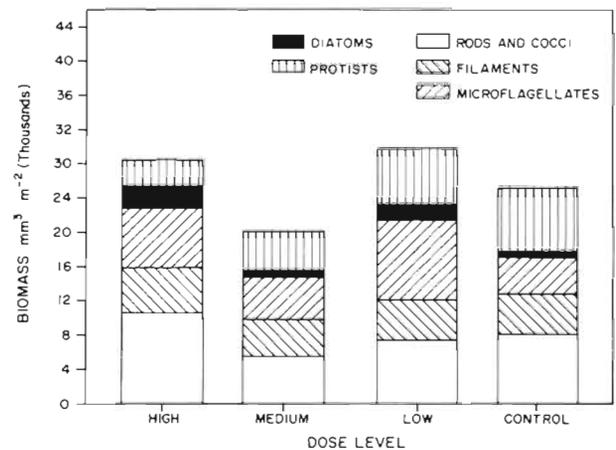


Fig. 2. Biomass ($\text{cm}^3 \text{m}^{-2}$) of microbial groups in sediments from the mesocosm experiment, subject to 3 dosing levels and control

DISCUSSION

Throughout the mesocosm experiment, dosed contaminants did not appear to penetrate the sediments in any of the treatments (Gray et al. 1988). The microbial community analysis therefore reflected chemical evidence: differences among treatments were either of no statistical significance or, if significant, did not correspond with dosing levels in the water. Even benthic diatoms, most of which are concentrated at the sediment surface, showed little evidence of response to the hydrocarbon and copper contamination. The core samples were unnecessarily deep for microbial studies, so that changes in surface abundances for the other groups of organisms could have been averaged out; however this is not thought likely here, in view of the low surface concentrations of contaminants.

For the field samples, microbial abundance and biomass clearly differ between the reference site and sites closer to the known pollution sources. It may at first seem unreasonable that 'unpolluted' sites have higher

Table 3. Abundances of components of the microbenthos at control and 3 dosing levels in the Solbergstrand mesocosm experiment, on 2 sampling dates. Means (\pm SD) of numbers per ml of sediment to 8 cm depth, over 4 replicate boxes in each basin

Basin	Sampling date	Rods and cocci ($\times 10^9$)	Filaments ($\times 10^9$)	Microflagellates ($\times 10^7$)	Diatoms ($\times 10^3$)	Protists ($\times 10^5$)
C	29 May	1.19 \pm 0.23	3.41 \pm 0.72	6.22 \pm 3.31	1.35 \pm 0.97	3.64 \pm 0.00
L	29 May	1.26 \pm 0.20	4.06 \pm 1.40	6.73 \pm 2.92	3.81 \pm 1.07	5.53 \pm 0.84
M	29 May	1.81 \pm 0.32	7.04 \pm 2.59	5.79 \pm 2.20	6.04 \pm 1.39	6.02 \pm 2.29
H	29 May	1.45 \pm 0.10	7.55 \pm 2.26	5.22 \pm 1.41	1.89 \pm 1.53	7.21 \pm 2.60
C	7 Jul	1.25 \pm 0.24	5.65 \pm 0.80	4.35 \pm 0.74	11.00 \pm 6.00	5.11 \pm 0.04
L	7 Jul	1.10 \pm 0.28	5.24 \pm 1.90	5.13 \pm 1.30	29.70 \pm 15.70	5.15 \pm 0.74
M	7 Jul	1.19 \pm 0.29	4.64 \pm 1.04	5.06 \pm 1.16	17.10 \pm 5.38	7.83 \pm 1.42
H	7 Jul	1.24 \pm 0.28	4.91 \pm 1.71	4.76 \pm 1.59	21.90 \pm 4.91	5.03 \pm 2.03

microbial biomass than 'polluted' sites but we are not dealing with sewage organisms or other indicator types but with the whole natural assemblage. Though it would be unwise, on these data alone, to draw causal inferences from the significant correlations between microflagellate biomass and certain trace metal levels, the results indicate that more attention should be given to this group of organisms in marine benthic studies. Microflagellates can exert considerable effects on bacterial populations and thus on nutrient regeneration and carbon flux, both in the water column (Haas & Webb 1979, Goldman & Caron 1985, Sherr et al. 1986) and in sediments (Grant & Schwinghamer 1987). Other community interactions also affect microbial abundance: Schwinghamer (1983) documented a positive relation between macrobenthic biomass and microbial biomass in continental shelf sediments off the east coast of Canada, and many experimental studies have demonstrated the stimulating effect of benthic grazing on bacterial growth. This also appears to be the case for the current field study (Gray et al. 1988, Schwinghamer 1988), where the reference site (A) has by far the highest macrofaunal and microbial biomass.

In summary, the methods required for microbial analysis are simple and relatively inexpensive, yet they yield information that may be useful in detecting pollution-induced changes in the benthic community. The methods presented here are preliminary study techniques which should be followed, where possible, by more definitive analyses such as those of Trousselier & Legendre (1981). In the absence of more sophisticated analyses, however, they offer some potential as a monitoring tool.

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