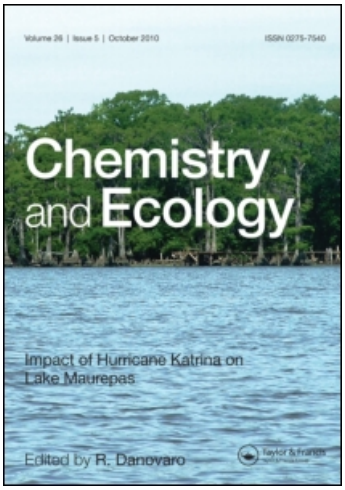

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MICROBIAL RESPONSE TO OIL DISTURBANCE IN THE COASTAL SEDIMENTS OF THE LIGURIAN SEA (NW MEDITERRANEAN)

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The effects of the oil contamination (10 April 1991) on microbial assemblages of the Ligurian Sea (northwestern Mediterranean) were investigated on samples collected from January 1991 to January 1992. High hydrocarbon concentrations (up to 214.3 $\mu g\ g^{-1}$ DW) occurred during a phytoplankton bloom and had a significant impact on phytoplankton biomass at the sediment-water interface. Chlorophyll-a concentrations showed an abrupt decline (from 2.49 $\mu g\ l^{-1}$ in pre-pollution conditions on April 8, to 0.75 $\mu g\ l^{-1}$ just after the oil contamination on April 22). A concomitant increase of phaeopigment concentration was observed (from 0.37 $\mu g l^{-1}$ to 2.24 $\mu g l^{-1}$). Oil contamination apparently had a limited effect on microphytobenthic communities. Sedimentary chlorophyll-a increased after oil contamination together with a major peak of phaeopigments. By contrast, increased oil concentrations stimulated a significant bacterial response. Benthic bacteria showed a significant increase in density of all the size classes (small, medium and large bacteria) as well as an enhanced bacterial activity. Consistently with meiofaunal response to hydrocarbons and with functional changes in organic matter composition (RNA:DNA ratios), oiled sediments had only a temporary effect on microbial assemblages. Phytoplankton growth started again immediately after oil decontamination whilst benthic bacterial biomass and activity reached the pre-pollution values after few weeks.

KEY WORDS: Benthic bacteria, microphytobenthos, phytoplankton, chlorophyll-a, oil spill.

INTRODUCTION

Oil spills are still considered one of the major sources of organic contamination of the marine environment. Recent major oil spills have emphasized the need for a better understanding of the effects of naturally or chemically dispersed oil on marine ecosystems (Siron *et al.*, 1993). Bacteria are generally considered among the most resistant organisms to xenobiotics but little is known about the response of natural bacterial assemblages to these compounds, including oil. The consistent and repeated result of experimental investigations is that, in most cases, microbial heterotrophic assemblages respond to the input of crude oil by increasing in density (Delille and Vaillant, 1990). Previous studies have demonstrated that natural bacterial communities show a highly opportunistic behaviour and are able to exploit hydrocarbons (Tagger *et al.*, 1983).

Other field investigations have indicated that natural microbial assemblages can be affected by heavy metals (Sunda and Ferguson, 1983; Fabiano *et al.*, 1994) and occasionally by oil contamination (Jensen *et al.*, 1986; Delille and Siron, 1993).

Although the toxicity of petroleum products for microorganisms, especially phytoplankton, is quite well documented, most of the information available is based on laboratory and mesocosm experiments (Griffiths *et al.*, 1981; Pengerud *et al.*, 1984; Cahet *et al.*, 1986; Delille and Vaillant, 1990). The information dealing with bacterial response to oil contamination should be widened with field investigations on natural microbial assemblages and considered in various environmental constraints that may play a key role in determining the specific microbial response to the oil disturbance.

Relatively few investigations have been carried out in the field and most of them intensified after the *Amoco Cadiz* oil spill (on microphytobenthos: Bodin and Boucher, 1981; and on benthic bacteria: Atlas and Bronner, 1981; Traxler and Vandermeulen, 1981). Field studies dealing with the oil spill effects on benthic microbial communities in the Mediterranean are practically non-existent.

A more general investigation on a subtidal pilot station of the Ligurian Sea, water and sediment samples were collected from January 1991 to January 1992. On 21 April 1991, the Golfo Marconi (Zoagli) was contaminated by crude oil that affected significantly the metabolic state of suspended and sedimentary organic matter (Danovaro *et al.*, 1995a) as well as the structure of the meiofaunal communities (Danovaro *et al.*, 1995b). Such release of crude oil provided also an unique opportunity to study the changes of the microbial assemblages in relation to oil contamination.

The aim of this study was to follow the structural and functional changes of the natural microbial assemblages (benthic bacteria, microphytobenthos and phytoplankton at the sediment-water interface) in a subtidal coastal station in relation to a hydrocarbon disturbance.

MATERIALS AND METHODS

Study Area

The study site (Zoagli), located at 10 m depth in the Golfo Marconi (Ligurian Sea) is exposed to wave action (Fig. 1). This area has been investigated intensively over the past 10 years for the dynamics of benthic bacteria, macrofauna, phytoplankton, particulate matter composition, seasonal cycles of sedimentation and primary production (Fabiano *et al.*, 1984; Albertelli and Fabiano, 1990; Danovaro, 1993).

Sampling

This study was conducted from January 1991 to January 1992. Sediment samples were collected on a fortnightly basis from March to June, and on a monthly basis for the rest of the year by SCUBA divers.

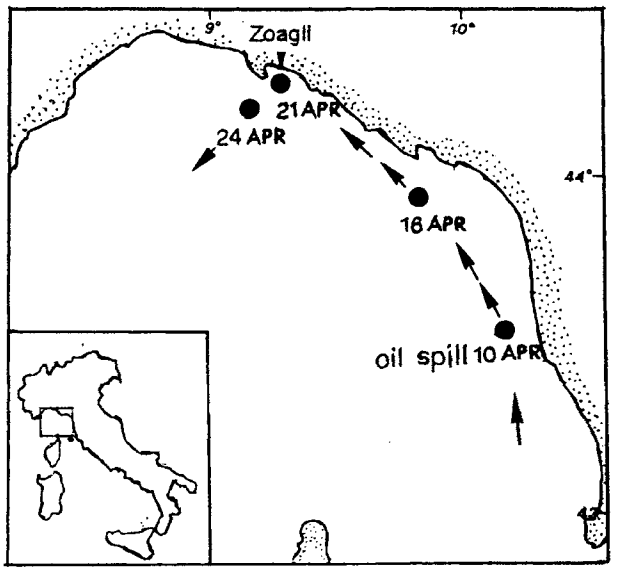

Figure 1 The sampling station (Zoagli) and the location of the *Agip Abruzzo* accident. Illustrated are the position of the oil slicks in the two weeks following the oil spill in April 1991 (according to satellite observations).

For bacterial analyses, 3–5 replicate micro-cores (surface area 0.64 cm^2, top 0–1 cm) were collected using sterile syringes and processed within two hours from collection. Three additional cores (surface area 10.7 cm^2) were sectioned into sediment layers (0–2, 2–4, 4–6, 6–8 and 8–12 cm) and frozen at -20 °C for the analysis of photosynthetic pigments. Only the top 2 cm layer of the sediments is considered in this study.

Water samples were collected at the sediment-water interface using two 5 l Niskin bottles which were placed horizontally at 10 cm above the sediment interface and closed manually by SCUBA. For photosynthetic pigment analyses (chlorophyll-a and phaeopigments), water samples (1 l) were filtered through Whatmann glass fibre filters (0.4 μm pore size) and stored at -20 °C. Environmental data have been reported previously in Danovaro *et al.* (1995a).

Chlorophyll Analysis

Chlorophyll-a analysis at the sediment-water interface ($n = 1$) and in the sediments ($n = 3$) was carried out according to Lorenzen and Jeffrey (1980). Pigments were extracted with 90% acetone. After centrifugation, the supernatant was used to determine the chlorophyll-a concentration using a Varian spectrophotometer (mod. DMS90).

Bacterial Analysis

Bacterial analysis was carried out using epifluorescence microscopy as described by Danovaro *et al.* (1994a). The frequency of dividing cells (FDC) was estimated

(Newell and Christian, 1981; Fry, 1990). Subsamples were stained with Acridine Orange (final concentration 5 mg l^{-1} for 3 min) and filtered on black Nuclepore (polycarbonate, 0.2 μm filters, 25 mm diam). The contribution by different size classes of bacteria (small, medium and large size bacteria) to the total biomass was determined according to Palumbo *et al.* (1984). Bacterial biovolume was converted to carbon content assuming 310 fgC μm^{-3} (Fry, 1990). The efficiency of bacterial extraction in these sediments has been previously tested by Danovaro *et al.* (1994 b).

Data Analysis

Differences in sediment and microbial parameters among seasons were tested using one-way analysis of variance (ANOVA). Microbial data were Log$(x+1)$ transformed when homogeneity of variance was rejected by using F_{max} test. Only the surface layer (0–2 cm) was considered for testing correlation, dealing with bacterial parameters and sediment chemistry. A correlation analysis has been carried out to examine relationships among organic chemistry, bacteria and other environmental factors (Sokal and Rohlf, 1969).

RESULTS

Changes in hydrocarbon concentrations are shown in Figure 2 (Danovaro *et al.*, 1995a). Hydrocarbon concentrations on the sea bed of the Golfo Marconi showed a most evident peak on 22 April (214.3 μg g^{-1} DW hereafter referred as μg g^{-1}). As indicated by RPD values, increased oil levels did not determine any hypoxia or anoxia. Hydrocarbon content decreased to pre-pollution levels after a few weeks.

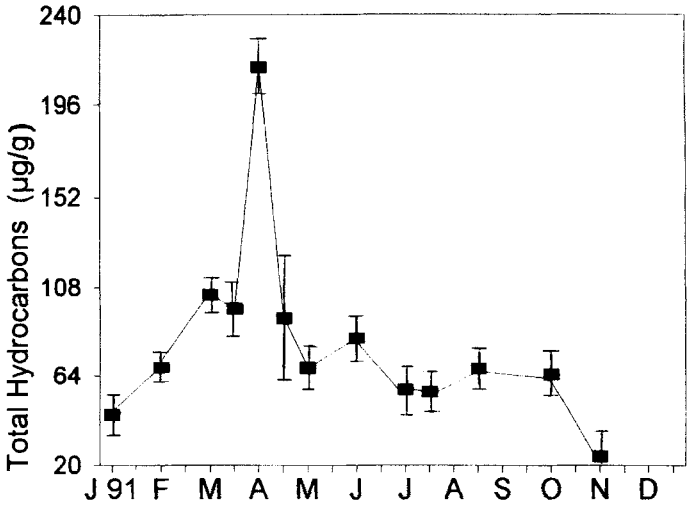

Figure 2 Seasonal variations of hydrocarbon concentrations in the study area (from Danovaro *et al.* 1995a).

Phytoplankton

Phytoplankton biomass at the sediment-water interface (as chlorophyll-a concentrations) showed wide variations during the sampling period (Fig. 3a). Chlorophyll-a concentrations ranged from 0.21 μg l^{-1} in December to 2.67 μg l^{-1} on May 29. Sampling was carried out on April 8 and 22, with collision taking place on April 10. An abrupt decrease of chlorophyll-a concentration was observed after the oil spill (from 2.24 μg l^{-1} in pre-pollution conditions, 8 April, to 0.75 μg l^{-1} after hydrocarbon disturbance, April 22). Phaeopigment concentrations (as measure of degraded phytoplankton biomass, Fig. 3b) ranged from 0 μg l^{-1} in September to 2.24 μg l^{-1} on April 22. Highest phaeopigment concentration was found to be coincident to the decrease of chlorophyll-a concentrations on April 22.

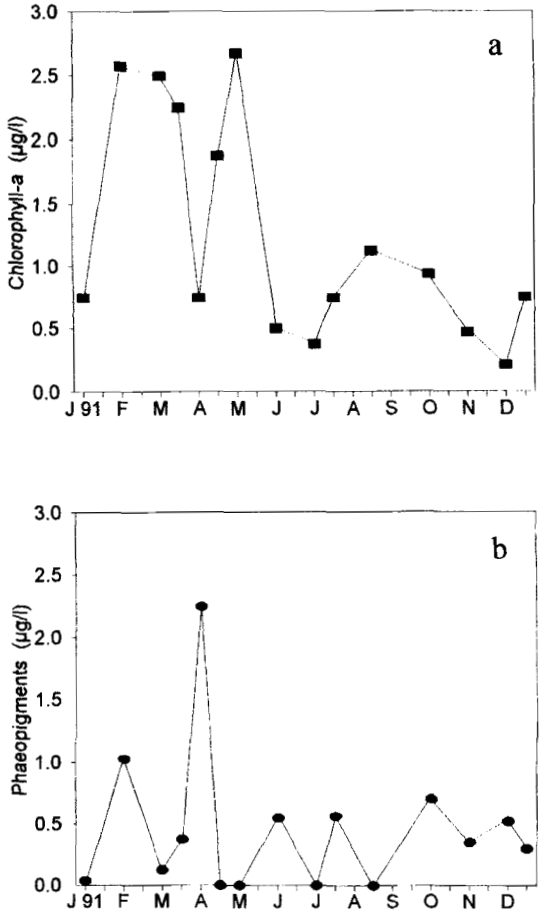

Figure 3 Changes in photosynthetic pigment concentrations in suspended particulate matter at the sediment-water interface: a) Chlorophyll-a concentrations; b) Phaeopigment concentrations.

Microphytobenthos

Microphytobenthic biomass (as sedimentary chlorophyll-a concentrations, Fig. 4a) in the top 2 cm of the sediments ranged from 2.14 μg g^{-1} in February to 3.84 μg g^{-1} in December 1991. Chlorophyll-a concentrations increased, from April 8 to April 22, from 2.41 to 3.83 μg g^{-1}. Phaeopigment concentrations ranged from 2.28 μg g^{-1} in February to 3.94 μg g^{-1} on April 22 (Fig. 4b). Highest phaeopigment concentrations were thus reported immediately after the oil contamination.

Benthic Bacteria

Seasonal changes of total bacterial number (TBN), biomass (BBM) and frequency of dividing cells (FDC) are shown in Figure 5a, b and c (respectively). Bacterial density varied significantly during the study year and ranged from $0.25 \cdot 10^8$ cells g^{-1} in March to $26.70 \cdot 10^8$ cells g^{-1} in October 1991. Similar pattern was shown by

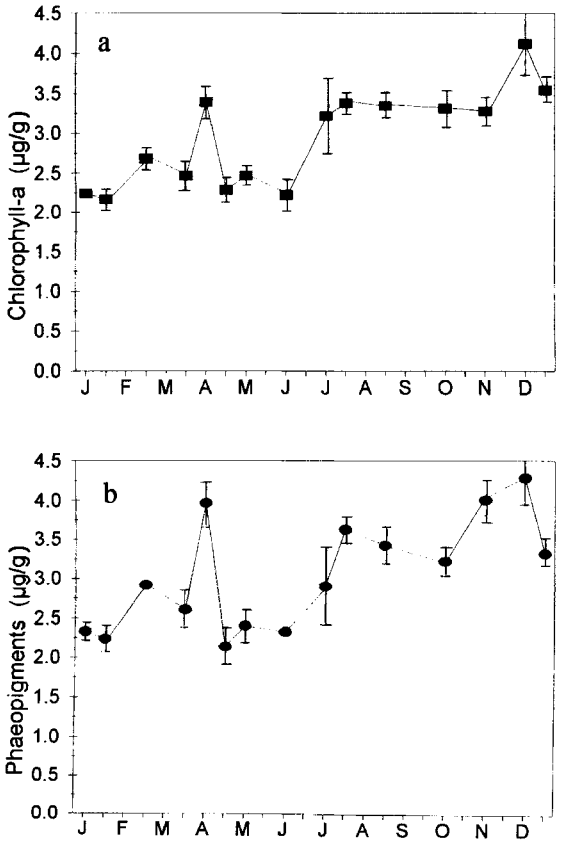

Figure 4 Seasonal variations of sedimentary photosynthetic pigments (as measure of the microphytobenthic biomass) in the uppermost 2 cm sediment layer: a) Chlorophyll-a concentrations; b) Phaeopigment concentrations.

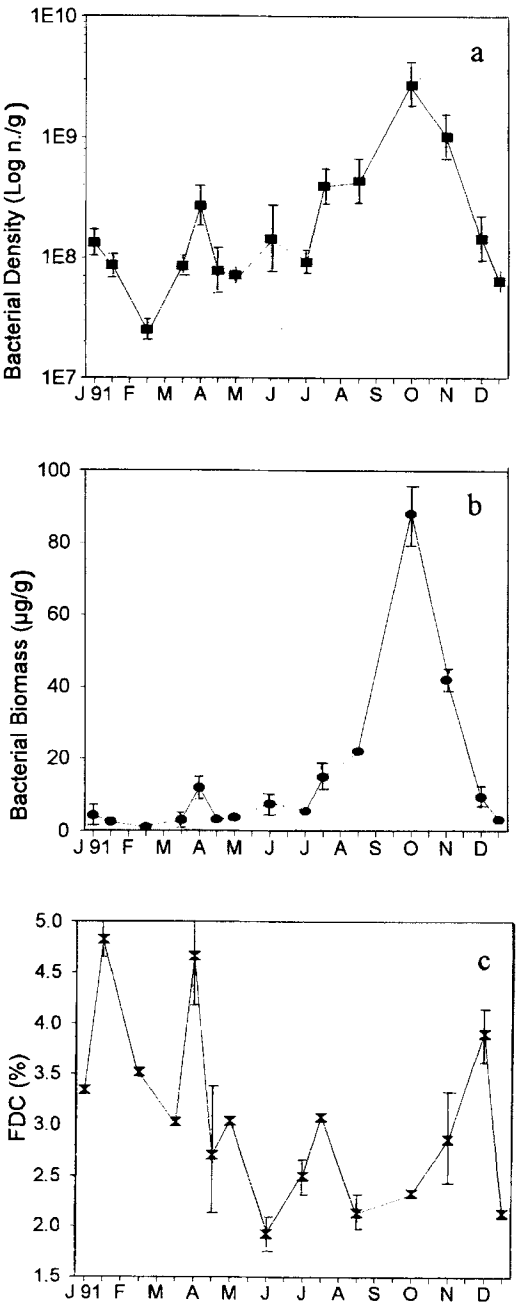

Figure 5 Temporal changes in bacteria parameters. Illustrated are: a) total bacterial number (TBN); b) bacterial biomass (BBM); c) Frequency of dividing cells (FDC), in the uppermost 2 cm sediment layer.

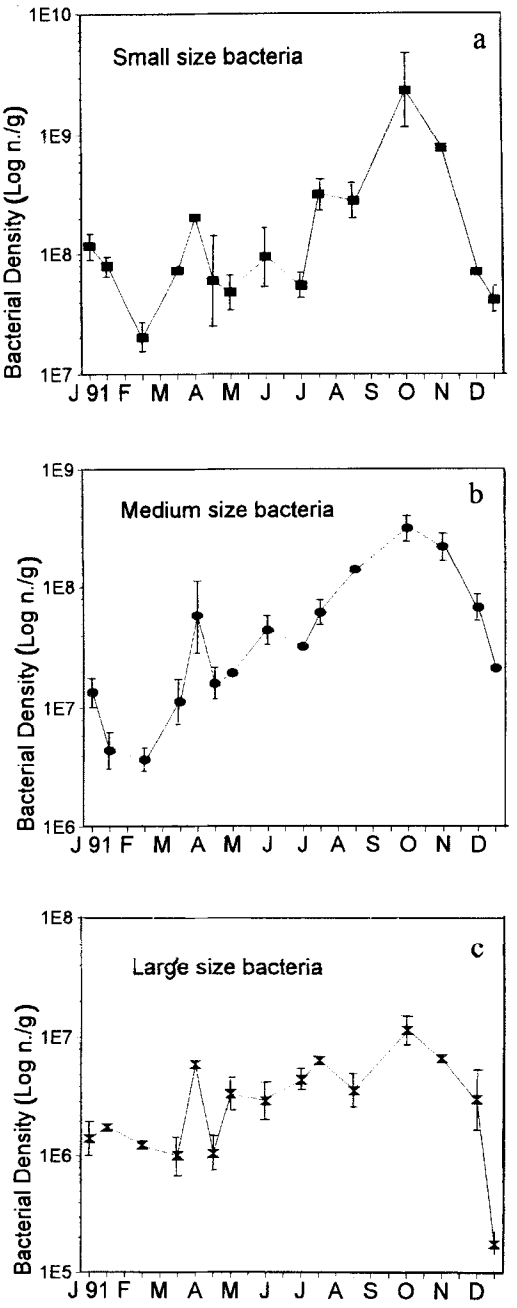

Figure 6 Changes in density of the different size classes of benthic bacteria: a) small size bacteria (TBN1); b) medium size bacteria (TBN2); c) large size bacteria (TBN3).

bacterial biomass which showed two main peaks: the first one in correspondence of the oiled sediments (11.94 $\mu g C\ g^{-1}$ on April 22) and a second in October (88.00 $\mu g C\ g^{-1}$). FDC values presented a less defined pattern. However, highest values were reported in February (4.83%) and on April 22 (4.66%) in oiled sediments.

Seasonal changes in the bacterial density per size class are reported in Figure 6 a, b and c (for small, medium and large bacteria, respectively). All the bacterial size classes showed a second peak in oiled sediments. Small-size bacteria ranged between $0.20 \cdot 10^8$ cells g^{-1} in March and $23.40 \cdot 10^8$ cells g^{-1} in October. They were dominant throughout the year and were followed by medium-size bacteria which ranged between $0.37 \cdot 10^7$ cells g^{-1} in March and $31.5 \cdot 10^7$ cells g^{-1} in October. Finally the large-size bacteria ranged between $0.18 \cdot 10^6$ cells g^{-1} (in March) and $11.60 \cdot 10^6$ cells g^{-1} (in January 1992).

DISCUSSION

Seasonal changes of chlorophyll-a concentrations at the sediment-water interface follow a pattern characteristic of the NW Mediterranean with 2 major peaks: the first in February and the second, generally, in September-October (Fabiano, 1984, Fabiano *et al.*, 1984). In the present study, chlorophyll-a concentrations in late winter-spring were significantly higher than in other periods of the year (ANOVA, $p = 0.01$). By contrast, microphytobenthic biomass showed a less defined seasonality (Fig. 4). However, in the Golfo Marconi, similar patterns have been reported during a three year study by Fabiano *et al.* (1995). Sedimentary chlorophyll-a concentrations showed little interannual changes with values generally higher in spring-summer. Finally, bacterial dynamics appear to be quite predictable. Total bacterial density was significantly higher in spring and autumn ($p < 0.05$) than in other sampling periods. Similar seasonal patterns were observed in the year February 92–February 1993 with peaks in April and December (5.07 and $5.87 \cdot 10^8$ cells g^{-1}, Danovaro unpublished data). Therefore, the patterns described for the main biological parameters (phytoplankton, microphytobenthos and bacteria) are consistent with the results of other investigations carried out in the same area.

However, major differences arise when we focus our attention on the changes occurred immediately after oil contamination. The data reported in this study suggest that hydrocarbon disturbance had a significant impact on phytoplankton biomass at the water-sediment interface. Seasonal changes in chlorophyll-a indicate the beginning of a phytoplankton bloom on February, evident until early April. A strong decrease of the chlorophyll-a concentrations was observed with the arrival of the oil spill in the area. The loss of phytoplankton biomass, corresponding to an equivalent increase in phaeopigments (as degradation products of the functional chlorophylls), suggests that hydrocarbon stress had both inhibitory and lethal effects on these microbial communities. Phytoplankton assemblages have been shown to be particularly sensitive to oil disturbance, experimentally (Siron *et al.*, 1993), as well as in the field, where studies on natural spring blooms reported a significant growth inhibition only in presence of high (about 10 mg l^{-1}) hydrocarbon concentrations (Roy *et al.*, 1991).

In the present study, the impact of hydrocarbons on phytoplankton was likely related to the high oil concentrations (comparable to those reported in the Bay of Morlaix and Lannion about 40 days after the *Amoco Cadiz* oil spill). The temporal effects of disturbance were, however, limited. Fifteen days after oil contamination, chlorophyll-a concentrations were comparable to pre-pollution values and further increased until the end of May (up to 2.67 $\mu g\ l^{-1}$, Fig. 3). Immediately after the oil disturbance, a fast decrease of the contaminant was observed (Fig. 2). This was due to the high hydrodynamics of the area, exposed to the wave action. The lower oil levels allowed the phytoplankton assemblages, especially for oil sensitive groups such a diatoms (Ostagaard *et al.*, 1984), to recover rapidly.

The effects of oil contamination on microphytobenthic communities in the field are difficult to identify. Our results showed a significant increase of sedimentary chlorophyll-a concentrations during April suggesting the presence of a microphytobenthic bloom which typically occurs in this period (Fabiano *et al.*, 1995). The sudden increase in phaeopigment concentrations in oiled sediments may be related to the sedimentation of dead phytoplankton, but may also indicate a concomitant oil induced stress on the microphytobenthic community. These results are consistent with those reported by Bodin and Boucher (1981) on the effects of the *Amoco Cadiz* oil spill on microphytobenthos (measured as chlorophyll-a content of the sediments). This seasonal study proved that microphytobenthic community was not very sensitive to oil pollution and was more dependent on other environmental parameters.

As far as bacterial communities are concerned, the oil contamination had apparently a stimulating effect. Bacterial abundance and biomass increased significantly with respect to pre-pollution conditions. This could be due to the increased hydrocarbon concentration which determined an increase of organic carbon potentially available as food source thus allowing the development of the opportunistic bacterial communities. It is also possible that oil contamination had an indirect effect on benthic bacteria. In fact, since increased oil levels determined a stress in the autotrophic microbial communities, an enhanced concentration of algal detritus (as phaeopigments) was available in the sediments to benthic bacteria immediately after the oil spill.

Such an increase in bacterial density was evident for all the bacterial size classes and was consistent with a significant enhancement of the bacterial activity (expressed as FDC). Similar response of the bacterial communities (direct counts) to increased oil concentrations has been reported in laboratory and field studies (Cahet *et al.*, 1986; Parsons *et al.*, 1984; Delille *et al.*, 1988).

Data reported in the present investigation indicate, as expected, that bacterial response to increased hydrocarbon concentrations was rapid. This result is consistent with the increased FDC values we found in oiled sediments. Delille and Vaillant (1990) suggested that bacterial response to oiled conditions within a short time (hours-days) could be explained by the presence of extremely small and active bacteria. However, the results of the present study indicate that all the size classes of bacteria responded similarly to oil contamination, thus revealing a highly opportunistic behaviour.

Direct counts of bacteria reflect the actual bacterial abundance but their variations over time may be less pronounced than changes observed with heterotrophic

bacteria (Delille and Siron, 1993). Therefore our direct counts, lacking in the identification of the specific strains, do not allow us to understand if the enhanced bacterial density and activity are due to the natural populations or to the growth of specifically hydrocarbon utilizing bacteria.

The knowledge of the factors controlling these bacterial enhancements in oiled sediments is still limited. However, in agreement with previous results (Delille and Siron, 1993), two possible complementary explanations may be proposed: 1) direct or indirect stimulating effect of oil on bacterial growth (as suggested by enhanced FDC values); 2) reduced bacterial predation by higher trophic levels (such as meiofauna) impacted by oil pollution. Meiofauna, and in particular nematodes, showed evident decrease in N_1 diversity, species richness and changes in k-dominance curves (Danovaro *et al.*, 1995b). Moreover, among nematodes, the density of microbial feeders declined significantly after the oil spill. These results further support the hypothesis of a reduced meiofaunal grazing on bacteria in oiled sediments.

These data suggest that microbial assemblages of the Golfo Marconi were highly sensitive to the increased hydrocarbon concentrations. The oil spill had different effects on the different microbial assemblages studied. Opposite response was reported for phytoplankton assemblages (stressed by oil contamination) and benthic bacteria (stimulated by increased oil levels), whilst microphytobenthos was probably affected but to a lesser extent. In agreement with data previously reported by Danovaro *et al.* (1995a and b), all assemblages studied recovered rapidly to pre-pollution conditions. In fact, a rapid response to the oil impact was shown by RNA:DNA ratios of the suspended and sedimentary particles and meiofaunal density recovered to pre-pollution conditions few weeks after the oil spill.

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