MICROBIAL ECOLOGY

Microb Ecol (2002) 44:118–126
DOI: 10.1007/s00248-001-1047-z

Effectiveness of Bioremediation of Crude Oil Contaminated Subantarctic Intertidal Sediment: The Microbial Response

D. Delille,[1] B. Delille,[2] E. Pelletier[3]

[1] Observatoire Océanologique de Banyuls, Université P. et M. Curie UMR-CNRS 7621, Laboratoire Arago 66650 Banyuls sur mer, France
[2] Université de Liège, Unité d'océanographie chimique, Mécanique des fluides géophysiques, Institut de Physique, B5, B-4000 Sart Tilman, Belgique
[3] Institut des Sciences de la Mer de Rimouski (ISMER), Université du Québec à Rimouski, 310 allée des Ursulines, Rimouski, Canada G5L 3A1

Received: 24 July 2001; Accepted: 19 December 2001; Online Publication: 13 June 2002

Abstract

A field study was initiated in February 1996 in a remote sandy beach of The Grande Terre (Kerguelen Archipelago, 69° 42′ E, 49° 19′ S) with the objective of determining the long-term effects of some bioremediation agents on the biodegradation rate and the toxicity of oil residues under severe subantarctic conditions. A series of 10 experimental plots were settled firmly into sediment. Each plot received 2L of Arabian light crude oil and some of them were treated with bioremediation agents: slow release fertilizer Inipol EAP-22 (Elf Atochem) or fish composts. Plots were sampled on a regular basis over a 3-year period. A two-order of magnitude increase of saprophytic and hydrocarbon-utilizing microorganisms occurred during the first month of the experiment in all treated enclosures, but no clear differences appeared between the plots. Very high microbial populations were present during the experiment. Biodegradation within treated spots was faster than within the untreated ones and appeared almost complete after 6 months as indicated by the degradation index of aliphatic hydrocarbons within all plots. The analysis of interstitial water collected below the oily residues presented no toxicity. However, a high toxicity signal, using Microtox solid phase, appeared for all oiled sand samples with a noticeable reduction with time even if the toxicity signal remained present and strong after 311 days of oil exposition. As a conclusion, it is clear that the microbial response was rapid and efficient in spite of the severe weather conditions, and the rate of degradation was improved in presence of bioremediation agents. However, the remaining residues had a relatively high toxicity.

Introduction

Oil pollution of the oceans and coastal environments has been a problem ever since man began to transport and use

Correspondence to: D. Delille; E-mail: dellile@arago.obs-banyuls.tr

fossil fuels. Biological degradation represents one of the major routes through which hydrocarbons can be removed from contaminated environments. As pointed out by Minas and Gunkel [29], since hydrocarbons are natural products, it is not surprising to find organisms that are able to degrade these energy-rich substrates. Bioremediation is a treatment technology for the cleanup of polluted sites that uses a range of methodologies to enhance the natural biodegradation of contaminants. It generally involves the use of indigenous or introduced (allochthonous) microorganisms to detoxify and degrade environmental contaminants.

In recent years, there has been increasing interest in developing cost-effective *in situ* technique for bioremediation of oil-contaminated sites [22, 30, 36]. Biostimulation treatments have been shown to enhance the biodegradation of oil on a number of contaminated shorelines [5, 26, 34]. Following the accidental oil spill of the *Bahia Paraiso* in Antarctica, questions raised about the rate of oil biodegradation under Antarctic weather conditions and about the applicability of bioremediation in cold regions [23]. The Antarctic marine ecosystem is considered one of the last remaining pristine zones and is almost uncontaminated by anthropogenic hydrocarbons [2, 8, 9]. However, small accidental fuel oil spills occurred frequently [1, 27] and little is known about hydrocarbon degradation processes in very cold environments. Several observations have been made in the Arctic region [5, 19, 21], Antarctic ice-free seawater [7, 12, 16], and ice-covered marine areas [6, 10, 11, 15, 32], but relatively few data are available for Antarctic intertidal sediments [13]. In January 1997 a field study was undertaken along a subantarctic sandy beach of Kerguelen Islands in which a limited amount of Arabian light crude oil was intentionally released onto plots to evaluate bioremediation processes. The objective was to determine the effects of adding inorganic and organic nutrients on the removal of crude oil contamination under severe field environments. To our knowledge, this is the first study to examine the long-term effects of petroleum contamination and its bioremediation in Antarctic intertidal sediments.

Materials and Methods

Study Site

A long-term experiment was conducted at Anse sablonneuse (49° 19′ S, 69° 42.5′ E), a remote sandy beach of the main island of the Kerguelen Archipelago (Fig. 1). The beach has a horseshoe shape of about 2-km width, well protected from ocean furies and formed of fine and very fine white-gray sand. This sandy cove is located in a pristine region with very low boat traffic and no human activity. These combined factors lead to a high probability that the sediments had never experienced chronic exposure to either refined or crude hydrocarbons.

Experimental Design

To examine the effects of crude oil pollution on intertidal microbial assemblages a series of 10 enclosures of 1 m^2 (1 m × 1 m × 0.4 m deep) was settled in January 1997 in the intertidal zone at the mid-tide mark. Waterproof wood frames without bottom were settled on the beach, in a row parallel to the shoreline. Each enclosure was separated by 10 m from the others and equipped with a top cover constituted of a 100 μm mesh net and a stainless steel grid allowing the free circulation of tidal seawater but avoiding any contact of the oily sand with sea birds and sea mammals. The protective grids obscured the enclosures and oily sand was not exposed to direct sunlight, most probably preventing photodegradation of hydrocarbons. The enclosures were completely flooded at high tide during each tidal cycle (2 times a day). A sampling site located at 100 m from the western enclosure was used for the entire experiment as a beach reference point.

After 15 days of self-setting, 2.85 L m^{-2} of light Arabian crude oil (BAL) topped at 150°C were spread in each enclosure after emulsification in seawater (25%, v/v). A buffer zone was kept to prevent contact of oil with enclosure frames. Without disturbing the sand surface, the contaminant was added uniformly to each enclosure over a surface of about 0.60 m^2, leaving a 12 cm clean strip between the enclosure wall and the oiled surface. Four different fertilizing treatments were applied in duplicate to enclosures. Two enclosures received only crude oil (BAL) without any fertilizing treatments (Fig. 1). The fertilizers used were three different fish composts (F1, F2, F3) and the liquid slow release Inipol EAP 22 (INIPOL). Each fertilizer was added two times to the surface of oily sand in a 5% proportion to the oil. The first fertilization occurred 10 days after the spill, and the second 21 days later. Sand was sampled on day 0 before the oil spill to determine the initial bacterial population.

Sampling procedure was carried out just after mid-tide. Sand samples were collected using 2 mL sterile plastic cores for microbial counting and prewashed (hexane/acetone) glass vials for chemical analysis and toxicity assays. Samples for microbiology were preserved on ice and treated immediately after their arrival at the biological laboratory of the research ship *La Curieuse*. Samples for chemistry and toxicity assays were frozen at −20°C and sent to Institut des Sciences de la Mer de Rimouski (Canada) for analysis.

Bacteriological Counts

Total bacteria were determined after dilution in sterile artificial seawater by acridine orange direct counts (AODC) on black Nuclepore filters (0.2 μm) using an Olympus BHA epifluores-

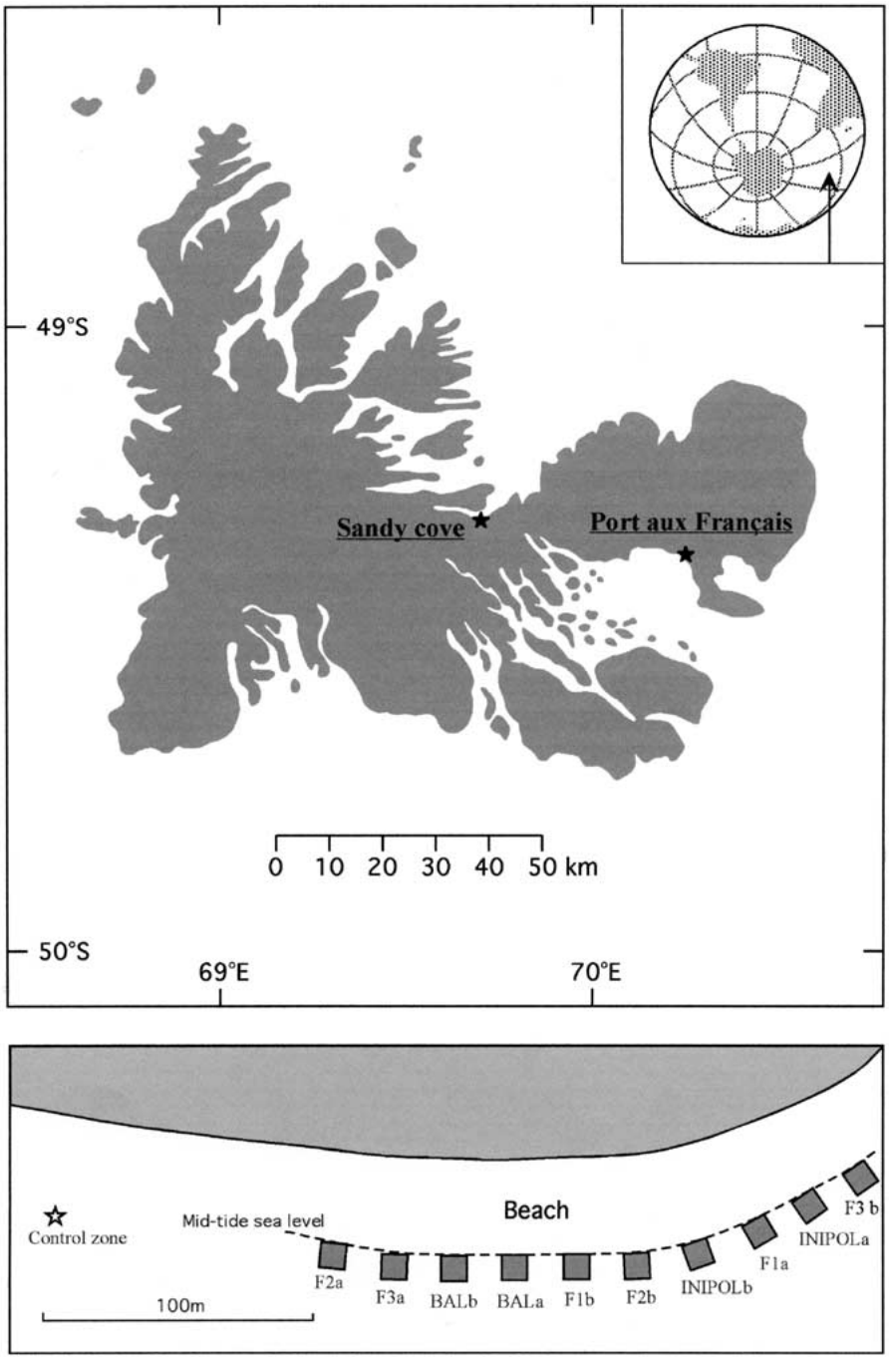

Fig. 1. The Kerguelen archipelago with the location of the study area. The bottom part of the figure shows the relative positions of the 10 contaminated sectors.

cence microscope according to the method of Hobbie et al. [20]. A minimum of 500 fluorescing cells with a clear outline and well-defined cell shape were counted under oil immersion (1000×) in a minimum of 10 randomly chosen fields. Biovolumes were estimated using an ocular micrometer.

The number of viable psychrotrophic aerobic saprophytic microorganisms in each sediment sample was estimated using the most probable number (MPN) technique. Saprophytic microorganisms were defined as those microbes growing on Marine Broth 2216 (Difco). After inoculation (3 tubes per dilution) the tubes were incubated at 12°C for 30 days, A large majority of the microbial strains isolated from Antarctic seawater must be considered psychrotrophic and not true psychrophiles, and there was no significant difference between MPN counts obtained after incubation at 4°C and 20°C [14]. Thus, the relatively high incubation temperature used in the present study had no significant effect on the data and allowed a substantial reduction of the incubation time. Incubation of MPN requires 3 months at 4°C; such a long time is usually not compatible with Antarctic field work. Similarly, hydrocarbon-degrading bacteria were counted using the MPN method with a basal mineral medium without carbon, but supplemented with "Arabian Light" crude oil [28].

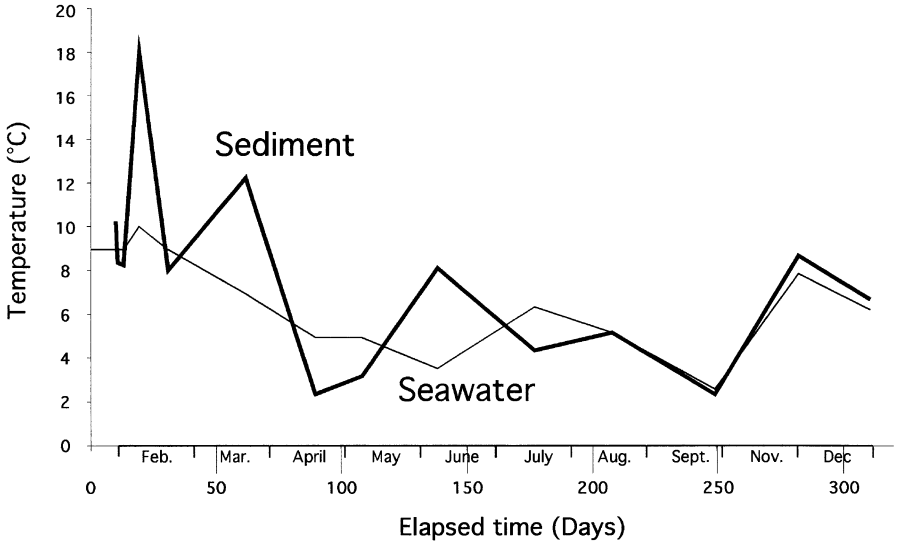

Fig. 2. Changes in seawater and sediment temperatures during the first year of the survey.

Rezasurin was used as a growth indicator [11]. After inoculation (3 tubes per dilution) the tubes were incubated at 12°C for 30 days.

Hydrocarbon Analysis and Toxicity Determination

Sediment samples was analysed for remaining petroleum hydrocarbons. Oiled samples were extracted twice with dichloromethane/hexane (50:50). Extracts were analyzed by gas chromatography/flame ionization detection using a 30-m glass capillary column and GC/MS analyses were carried out to confirm the identity of unknown peaks. The peaks were identified using standard hydrocarbon mixture from Supelco and some individual PAHs when required. The toxicity of oiled sands was evaluated by the response of the luminescent bacteria *Vibrio fischeri* using the Microtox Analyser 500 and solid-phase test kit (AZUR Environmental, Carlsbad, CA). The assays were conducted as described by Microbics Manual incubating suspended sediment concentrations in a thermoregulated bath at 15°C for 20 min. The sand dilution that inhibits 50% (EC50) of the light output relative to oil-free sand collected at the external sampling site was calculated for each oiled sample and expressed as a percent (%) of the pristine sample. The toxicity increases when EC50 decreases. Full details of chemical analysis and toxicity testing are reported elsewhere [31].

Results

During the course of the experiment seawater temperatures ranged from 2°C in winter to 10°C in summer (Fig. 2). The observed yearly average of 5°C is usual in subantarctic conditions. Intertidal sediments experienced larger temperature fluctuations with the temperature reaching 18°C during sunny summer days.

There were only slight changes in total bacterial abundance after contamination. During the course of the experiment, total bacterial abundance ranged from 7.8×10^8 to 1.7×10^9 cells mL^{-1}. The total bacterial biomass did not evolve in a significantly different way between pristine and contaminated zones (data not shown). However, all the results clearly revealed a significant response of more specific microbial communities to hydrocarbon contamination. One order of magnitude increase of saprophytic microbial abundance occurred after 2 weeks of contamination (Fig. 3). After 3 months, differences between a pristine zone and contaminated plots reached 2 orders of magnitude. Within the same interval, differences between treated and untreated contaminated sediments exceeded 1 order of magnitude. In contrast, there were only slight differences between the four treated enclosures.

A substantial enrichment in oil-degrading bacteria was observed in all contaminated plots. With values generally lower than 1% MPN mL^{-1}, hydrocarbon-degrading bacteria (Fig. 4) found in pristine intertidal sediments never represented more than 1% of the saprophytic assemblage. One month after the contamination this proportion exceeded, in some cases, 95% in contaminated plots. Specific microbial abundances were generally 10-fold higher in bioremediated than in untreated contaminated zones. As observed for the saprophytic microbial abundance there were no clear differences between the four bioremediation treatments. Very high concentrations of saprophytic and oil degrading microorganisms remained present after 1 year of contamination (Fig. 5). One and one-half year later (total elapsed time of 2.5 years since the spill), strong differences in specific microbial abundances were still

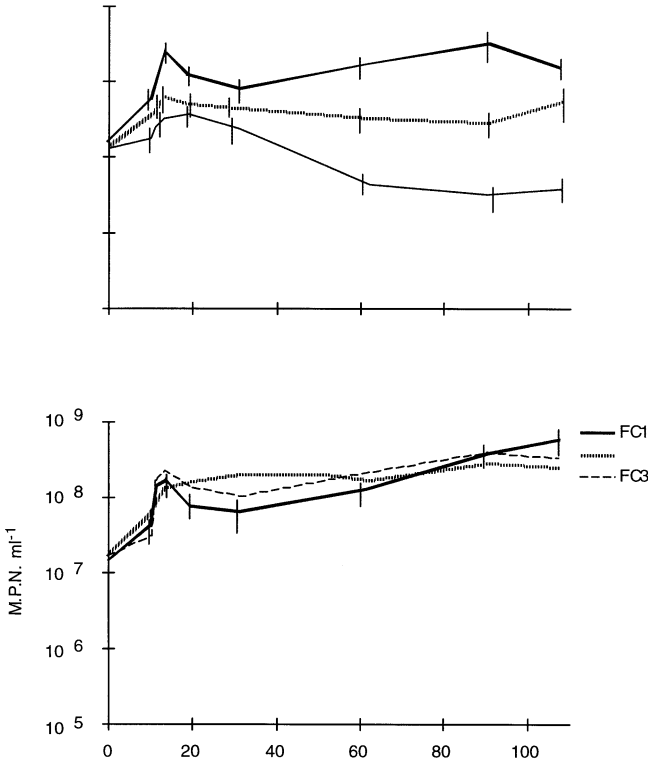

Fig. 3. Changes in abundance of saprophytic microorganisms during the first 100 days of contamination. Bars indicate standard deviations (3 subsamples in each of the 2 replicate sectors). Bars corresponding to treatments FC2 and FC3 have been deleted for clarity reasons.

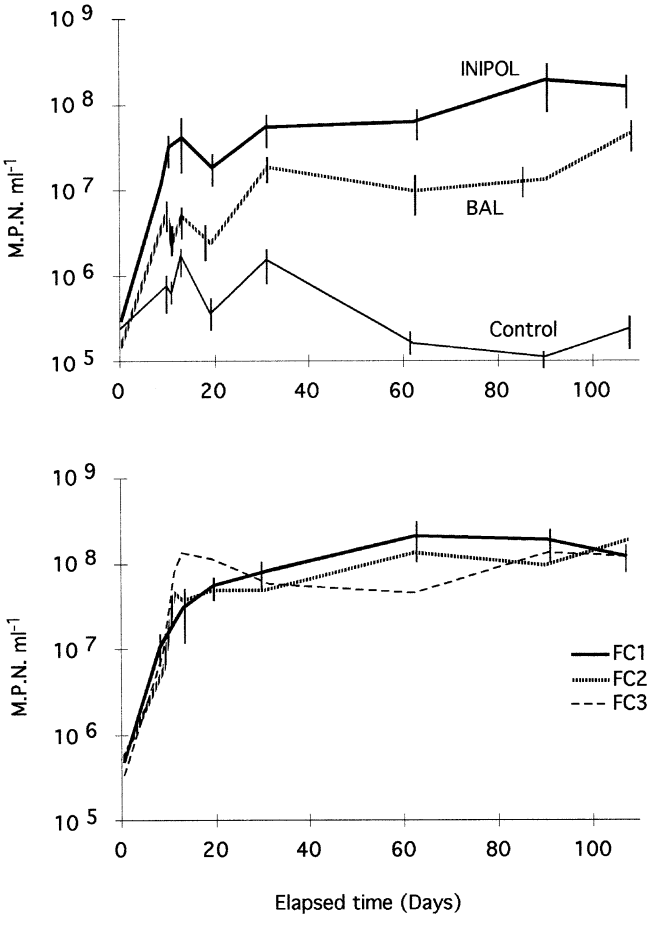

Fig. 4. Changes in abundance of hydrocarbon-degrading bacterial abundance as measured by MPN method during the first 100 days of contamination (3 subsamples in each of the 2 replicate sectors). Bars corresponding to treatments FC2 and FC3 have been deleted for clarity reasons.

present in pristine and contaminated intertidal sediments. It was also interesting to note that, with values closer to the pristine ones, the INIPOL treatments differed from the three other bioremediation treatments.

The simultaneous chemical analysis of oiled sediment in each treatment provided important information on the rate of degradation of saturated and aromatic hydrocarbons during the first year of treatments. Briefly, the C18/hopane ratio followed a constant decrease until day 177, which is representative of a regular oil biodegradation process (Fig. 6). Biodegradation rate within treated plots was faster than within untreated ones. After about 6 months, the C18/hopane ratio gathered to a same point in all enclosures. It was then possible to observe a nearly complete degradation of aliphatic hydrocarbons within all plots including untreated ones.

Analysis of interstitial water showed no toxicity at all as determined by Microtox regardless of the sampling time or treatments. In contrast, the analysis of sediments sampled inside each contaminated zone gave a positive response. A high toxicity signal appeared in all contaminated zones with only a slight reduction observed over time (Fig. 7). After 1 year, the signal of a relatively high toxicity of oiled residues remained present in all treated and untreated plots.

Discussion

The ubiquitous distribution of oil-degrading microorganisms has already been reported [25]. Oil-degrading microbial abundance ranged from 1.0 to 7.0 $\times$ 10^5 bacteria mL^{-1} in pristine sediments of Anse sablonneuse, values which are comparable to those reported by Delille and Delille [13] in various locations of the Kerguelen archipelago and by Venkateswaran and Harayama [35] in Japanese sediments. Before contamination, hydrocarbon-degrading microorganisms comprised less than 2% of the total num-

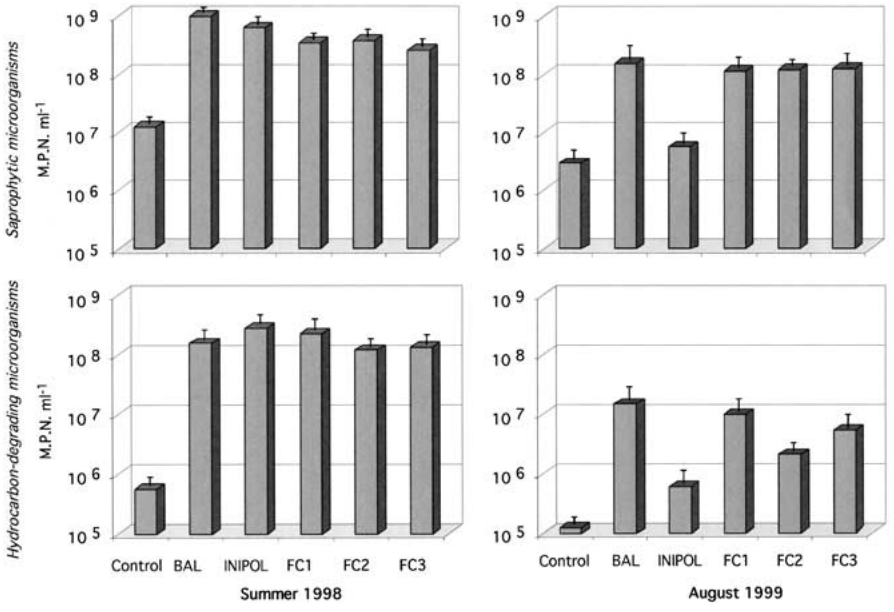

Fig. 5. Saprophytic and hydrocarbon degrading microbial abundance observed in pristine and contaminated zone after 1 and 2.5 years of contamination (summer 1998: mean values of the data collected between November 1997 and February 1998).

ber of saprophytic bacteria. These results are consistent with those of Wright et al. [38], who reported that hydrocarbon-degrading microorganisms ranged from 1 to 10% of the total number of saprophytic microorganisms in marine microbial communities. The presence of oil-degrading microorganisms in a remote location not previously exposed to fossil hydrocarbons has been related to the presence of terrestrial biowaxes derived from vascular plants and other terrestrial carbon sources [8, 31, 33]. The ubiquitous presence of natural biowaxes at the Anse sablonneuse seems to be the same as observed elsewhere even if terrestrial vegetation might be sparse.

In contrast to the situation reported by Kennicutt [24] for seawater in the vicinity of the Palmer station on the Anvers Island (64° 46.8′ S, 64° 06′ W) after the spill of the *Bahia Paraiso,* the introduction of oil into the previously oil-free subantarctic sediments of the Anse sablonneuse resulted in enrichments for hydrocarbon-degrading microorganisms by several orders of magnitude within a few days. Such a very large enhancement of specific microorganisms has been previously reported [13, 37]. Over a 3-year period following the *Exxon Valdez* oil spill in Alaska and under climatic conditions quite comparable to those of observed at Kerguelen Island, Braddock et al. [4] found significantly higher numbers of hydrocarbon-degrading microorganisms at sites with oil slick (from 3.6×10^3 to 5.5×10^3 bacteria mL^{-1}) than at reference sites ($<10^2$ bacteria mL^{-1}).

The application of fertilizers did not have an immediate effect on hydrocarbon-degrading microorganisms. However, stimulating effects became clearly visible after 3 months of contamination. Oil-degrading bacteria then represented 31 ± 7% of total saprophytic microbiota in biostimulated enclosures compared to 9% for control enclosures. The situation evolved slowly in the following weeks and months. The mean proportion of oil-degrading bacteria in fertilised enclosures decreased to 14 ± 9% compared to 29% in controls after 1 year of the experiment. Mean hydrocarbon-degrading microbial abundance observed at day 177 is significantly higher ($P < 0.05$) than means at days 10, 19, 90, and 208. This confirms that hydrocarbon-degrading production peaked about 6 months after the spill, a period corresponding to the subantarctic winter (July–August 1997) with the seawater and sand temperatures recorded at 5 ± 1°C. Microbial metabolism usually increases as the ambient temperature increases [3, 25], but the metabolism of psychrophilic and psychrotrophic bacteria is adapted to work at low temperature [14, 17, 18]. Under more severe Antarctic conditions, ambient temperature close to 0°C did not stop oil biodegradation in seawater and sea ice [11, 12].

As oil-degrading microorganisms are using carbon from hydrocarbons to their development, it should be possible to establish a relationship between specific microbial populations and degradation indicators. The correlation between hydrocarbon-degrading microbial abundance and the biodegradation index, C18/hopane, was generally poor ($r^2 < 0.4$) except at day 90 where the determination coefficient r^2 reached 0.74. Furthermore, when the mean daily growth rate of hydrocarbon-degrading microorganisms (δ abundance per day) and the

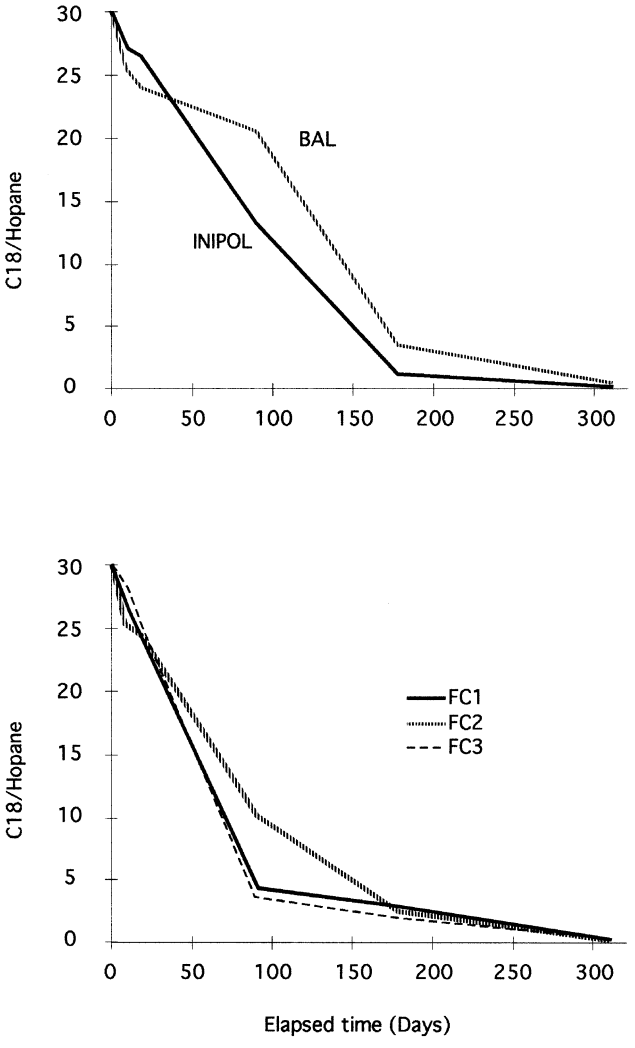

Fig. 6. Changes in the C18/hopane ratio during the first year of contamination.

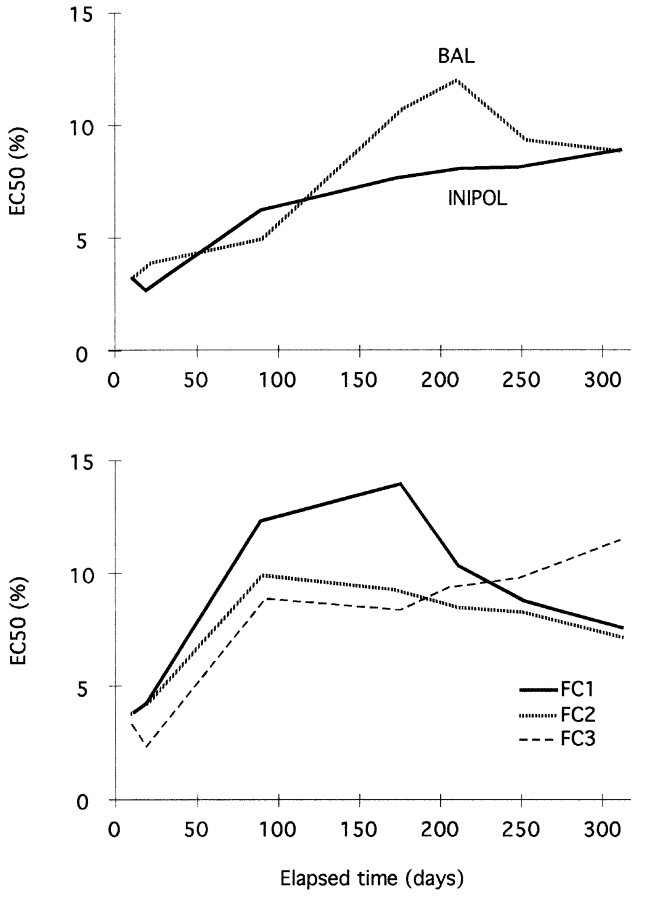

Fig. 7. Changes in sediment toxicity (EC50 calculated from Microtox solid phase biotest) during the first year of contamination.

apparent degradation rate (δ C18/hopane per day) are calculated between sampling days 10 and 90, a clear correlation appears between the microbial production and hydrocarbon consumption [31]. This expected correlation was rarely observed in the field because a number of confounding environmental factors (photooxidation, external carbon sources) can obscure it.

In conclusion, the present data set provides further evidence of the presence of indigenous hydrocarbon-degrading microorganisms in Antarctic sediments and their high potential for rapid biodegradation of hydrocarbons action. However, the toxicity evaluation of sediments in the contaminated zone did not harmonize well with the oil degradation response. Its appears that the less toxic material was degraded first and a large part of the toxic fraction remained for a much longer period. At the present stage of our research, it is impossible to relate this observation with the low-temperature conditions recorded at Anse sablonneuse. The rate of oil degradation was improved by bioremediation treatments but final residues giving a high toxic signal were unchanged, and biotreatments did not induce a complete disappearance of toxicants. The rate of microbial degradation of hydrocarbons in sediments is affected by several physicochemical and biological parameters, including the abundance and diversity of microorganisms present in the pristine environment before the spill; the conditions for microbial activity (e.g., concentration of nutrients, oxygen and temperature); the quality, quantity, and bioavailability of the contaminants; and some sediment characteristics. Surface waves as well as the interaction of tide sand underground water flow are important transport mechanisms for nutrients and bacteria. After the *Exxon Valdez* oil spill on shorelines of Alaska, Sugai et al. [33] examined the mineralization potential of hydrocarbon-degrading

bacteria from three field seasons and confirmed that environmental factors strongly influenced the ability of microbial populations to mineralize polycyclic aromatic and aliphatic compounds. Thus, the quantitative importance of the biodegradation process may differ greatly from one beach to another, and its presence cannot be directly extrapolated to other subantarctic beaches.

Acknowledgments

This research was supported by the "Institut Français pour la Recherche et la Technologic Polaires" the Natural Sciences and Engineering Research Council of Canada, and the Beligian Federal Office for Scientific, Technical and Cultural Affairs through the Belgian Scientific Programme on the Antarctic (contracts A4/DD/B14 and Ev/7/12E).

References

1. Bej AK, Saul D, Aislabie J (2000) Cold-tolerant alkane degrading *Rhodococcus* species from Antarctica. Polar Biol 23:100–105
2. Berkman PA (1992) The Antarctic marine ecosystem and humankind. Rev Aquatic Sci 6:295–333
3. Bossert I, Bartha R (1984) The fate of petroleum in Soil Ecosystems. In: RM Atlas (ed) Petroleum Microbiology, MacMillan Publishing Co., New York, pp 434–476
4. Braddock JF, Lindstrom JE, Brown EJ (1995) Distribution of hydrocarbon-degrading microorganisms in sediments from Prince William Sound, Alaska, following the *Exxon Vajdez* oil spill. Mar Poll Bull 30:125–132
5. Bragg JR, Prince RC, Harner EJ, Atlas RM (1994) Effectiveness of bioremediation for the *Exxon Valdez* oil spill. Nature 368:413–418
6. Cavanagh JE, Nichols PD, Franzmann PD, McMeekin TA (1998) Hydrocarbon degradation by Antarctic coastal bacteria. Antarctic Sci 10:386–397
7. Clarke A, Law RJ (1981) Aliphatic and aromatic hydrocarbons in benthic invertebrates for two sites in Antarctica. Mar Pollut Bull 12:10–14
8. Cripps GC (1990) Hydrocarbons in the seawater and pelagic organisms of the southern ocean. Polar Biol 10:393–402
9. Cripps GC (1992) Baseline levels of hydrocarbons in seawater of the Southern Ocean. Natural variability and regional patterns. Mar Pollut Bull 24:109–114
10. Cripps GC, Priddle J (1991) Hydrocarbons in the Antarctic environment. Antarctic Sci 3:233–250
11. Delille D, Bassère A, Dessomrnes A (1997) Seasonal variation of bacteria in sea ice contaminated by diesel fuel and dispersed crude oil. Microb Ecol 33:97–105
12. Delille D, Bassères A, Dessommes A, Rosiers C. (1998) Influence of daylight on potential biodegradation of diesel and crude oil in Antarctic seawater. Mar Environ Res 45:259–268.
13. Delille D, Delille B (2000) Field observations on the variability of crude oil impact on indigenous hydrocarbon-degrading bacteria from subantarctic intertidal sediments. Mar Environ Res 49:403–417.
14. Delille D, Perret E (1989) Influence of temperature on the growth potential of southern polar bacteria. Microb Ecol 18:117–123
15. Delille D, Siron R (1993) Effect of dispersed oil on heterotrophic bacterial communities in cold marine waters. Microb Ecol 25:263–273
16. Delille D, Vaillant N (1990) The influence of crude oil on the growth of subantarctic marine bacteria. Antarctic Sci 2:655–662
17. Feller G, Narinx E, Arpigny JL, Aittaleb M, Baise E, Genicot S, Gerday Ch (1996) Enzymes from psychrophilic organisms. FEMS Microbiol Rev 18:189–202
18. Gounot AM (1991) Bacterial life at low temperature: physiological aspects and biotechnological implications. J Appl Bacteriol 71:386–397
19. Griffiths RP, MacNamara TM, Cadwell BA, Morita RY (1981) Field observations on the acute effect of crude oil on glucose and glutamate uptake in samples collected from Arctic and subarctic waters. Appl Environ Microbiol 41:1400–1406
20. Hobbie JE, Daley RJ, Jasper S (1977) Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl Environ Microbiol 33:1225–1228
21. Horowitz A, Krichevsky MI, Atlas RM (1983) Characteristics and diversity of subarctic marine oligotrophic, stenoheterotrophic, and euryheterotrophic bacterial populations. Can J Microbiol 29:527–535
22. Joergensen RG, Schmadeke F, Windhorst K, Meyer B (1995) Biomass and activity of microorganisms in a fuel oil contaminated soil. Soil Biol Biochem 27:1137–1143
23. Karl DM (1992) The grounding of the *Bahia Paraiso*: Microbial ecology of the 1989 Antarctic oil spill. Microb Ecol 24:77–89
24. Kennicutt MC II (1990) Oil spill in Antarctica. Environ Sci Technol 24:620–624
25. Leahy JG, Colwell RR (1990) Microbial degradation of hydrocarbons in the environment. Microbiol Rev 54:305–315
26. Lee K, Levy EM (1991) Bioremediation: waxy crude oils stranded on low-energy shorelines. Proc 1991 Inter Oil Spill Conf, Amer Petrol Inst Pub N° 4529, Washington DC, pp 541–547
27. Mac Cormack WP, Fraile ER (1997) Characteristion of a hydrocarbon degrading psychrotrophic Antarctic bacterium. Antarctic Sci 9:150–155
28. Mills AL, Breuil C, Colwell RR (1978) Enumeration of petroleum-degrading marine and estuarine microorganisms by the most probable number method. Can J Microbiol 24:552–557
29. Minas W, Gunkel W (1995) Oil pollution in the North Sea. A microbiological point of view. Helgol Meeres 49:143–158

30. MØller J, Winther P, Lund B, Kirkebjerg K, Westermann P (1996) Bioventing of diesel oil-contaminated soil: comparison of degradation rates in soil based on actual oil concentration and resprometric data. J Industrial Microbiol 16:110–116
31. Pelletier E, Delille D, Delille B Effectiveness of bioremediation of crude oil contaminated subantarctic intertidal sediment: chemistry and toxicity of oiled residues. Submitted
32. Siron R, Pelletier E, Delille D, Roy S (1993) Fate and effects of dispersed crude oil under icy conditions simulated in mesocosms. Mar Environ Res 35:273–302
33. Sugai SF, Lindstrom JE, Braddock JF (1997) Environmental influences on the microbial degradation of *Exxon Valdez* oil on the shorelines of Prince William Sound, Alaska. Environ Sci Technol 31:1564–1572
34. Sveum P, Ladousse A (1989) Biodegradation of the oil in the Arctic: Enhancement of oil-soluble fertilizer application. Proc 1989 Inter Oil Spill Conf, Amer Petrol Inst Pub No 4529, Washington DC, pp 436–446
35. Venkateswaran K, Harayama S (1995) Sequential enrichment of microbial populations exhibiting enhanced biodegradation of crude oil. Can J Microbiol 41:767–775
36. Venosa AD, Suidan MT, Wrenn BA, Strohmeier KL, Haines JR, Eberhart BL, King D, Holder E (1996) Bioremediation of an experimental oil spill on the shoreline of Delaware Bay. Environ Sci Technol 30:1764–1775
37. Wagner-Döbler I, Bennasar A, Vancanneyt M, Strttmpl C, Brammer I, Eichner C, Grammel I, Moore ERB (1998) Microcosm enrichment of biphenyl-degrading microbial communities from soils and sediments. Appl Environ Microbiol 64:3014–3022
38. Wright AL, Weaver RW, Webb JW (1997) Oil bioremediation in salt march mesocosms as influenced by N and P fertilization, flooding and season. Wat Air Soil Pollut 95:179–191