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# Is Crude Oil Bioremediation Affected by Changes in Ambient Ultraviolet Radiation?

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**Biodegradation of Iranian light crude oil using an oleophilic fertilizer (F1) was investigated in two 30-day runs in a mesocosm simulation of a typical Mediterranean shore in the winter and spring periods of 1996 and 1997. At the end of the 30-day periods alkane biodegradation was dramatic (70%) and statistically significant only in the first run (November–December). Given that the fluctuations in all other parameters were minimal, the notable reduction in biodegradation efficiency during the second run is thought to be related to the nearly three-fold increase in total dose of solar UV-B radiation. © 1999 Elsevier Science Ltd. All rights reserved.**

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## Introduction

The application of nitrogen- and phosphorus-containing fertilizers has been established as a method to accelerate the natural self-cleaning process of oil-polluted environments. The nutrients enhance the growth of autochthonous and/or introduced allochthonous oil-degrading microorganisms. The largest bioremediation project to date was undertaken in the *Exxon Valdez* spill, in Prince William Sound, Alaska, March 1989. Approximately 2000 km of rocky shores were contaminated with some 41 million litres of crude oil. Over the summers of 1989–92, about 50 000 kg of nitrogen and 5000 kg of phosphorus were applied to the affected shoreline. Extensive testing in the laboratory and in the field showed that nutrient application has a good potential as a treatment for oil spills on rocky intertidal shores (Bragg *et al.*, 1994).

Several other studies have examined the effects of various factors on petroleum bioremediation (Sugai *et al.*, 1997; Wolter *et al.*, 1997). Laboratory experiments have demonstrated cause-and-effect relations between bioremediation techniques and petroleum hydrocarbon degradation (Atlas and Bartha, 1972; Broholm *et al.*, 1990; Margesin and Schinner, 1997). Promising laboratory findings, however, cannot always be repeated in the field; while it is possible to control each parameter individually in the laboratory, parameter control is usually very limited in the natural environment due to the complex interactions between biotic and abiotic factors. Thus, field biodegradation rates are often slower than expected compared to laboratory trials (Harms and Bosma, 1997). Therefore, laboratory assays cannot always be extrapolated to the field unless they are paralleled by larger-scale outdoor counterparts. Mesocosm experiments are a logical intermediate step between in vitro findings and full-scale applications.

Among the factors likely to affect microbial activity during bioremediation is short-wavelength solar ultraviolet radiation. Although solar UV-B penetrating the atmosphere (286–315 nm) has deleterious effects on most aquatic organisms (Häder, 1993), its possible role in bioremediation has been largely overlooked. In the past, similar negligence has led to serious flaws; for example, global oceanic productivity had been overestimated for many decades due to the use of UV-filtering glass bottles in phytoplankton productivity assays (Smith and Baker, 1980). The growth and survival of bacteria, one of the major categories of hydrocarbon degraders, are affected by UVR (Munakata, 1974, 1981, 1989). Due to their predictable UV action spectrum, *Escherichia coli* K12 and *Bacillus subtilis* have been recommended for use as biological dosimeters (Tyrrell, 1978; Karentz and Lutze, 1990; Puskeppeleit *et al.*, 1992).

This paper describes a 'paradox' observed during mesocosm bioremediation experiments and discusses the role of solar UV-B as a possible explanation.

## Materials and Methods

Alkane biodegradation was studied in a mesocosm simulation of a typical Mediterranean shore under

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different conditions of solar irradiance. Two runs were carried out during the periods 28 November–27 December 1996, and 22 February–23 March 1997. The type of crude oil used in both runs was Iranian light. The mesocosm consisted of a 3-$m^3$ (3×1×1 m) fibreglass tank with a sloping 'beach' and an 'open water' end. The sloping 'beach' was constructed from cinderblocks covered with beach pebbles collected from the intertidal zone of a nearby shore of Saronikos Gulf and graded to form a smooth slope (Santas *et al.*, 1998). The tank was filled with seawater to the topmost layer of the 'beach'. Seawater was added periodically to make up for losses due to evaporation and spray. Salinity ranged from 36 ppt at the beginning to 40 ppt at the end of each experiment. The water was recirculated on a 24-h basis by pumps (5 $m^3$/h) installed at the 'open water' end of the tank. The rapid turnover rate maintained dissolved oxygen levels near saturation at all times.

Polypropylene netting enclosures (2 mm mesh size) were filled with 1 kg portions of beach pebbles and immersed in Iranian light crude after weathering by air bubbling for 1 day. Excess oil was removed by dripping for approximately 30 s. The enclosures were subsequently placed at 10 and 60 cm below water surface, designated as 'shore' and 'deep water' sites. A simulated oil slick was formed by spilling 3 litres of oil on the water surface of the mesocosm. 330 g F1, a modified fish meal oleophilic fertilizer with a C:N:P ratio of 24:18:3.5, were spread on the oil slick. In both runs, the only source of hydrocarbon degraders were the microorganisms indigenous to the seawater and the sediment used.

Two replicate samples per sampling point were collected on Days 0, 1, 3, 7, 15 and 30 from the water surface (designated as 'surface') and from the enclosures at the 'shore' and 'deep water' points. Pebbles were sampled only from the surface of the enclosures. This was achieved by sampling each polypropylene enclosure only once. After sampling, the enclosures were returned to their original place to leave tank conditions undisturbed. At the beginning of each run, 12 enclosures (6 sampling dates × 2 replicates) were placed at the 'shore' sampling point, and another 12 enclosures at the 'deep' sampling point of the mesocosms.

Hydrocarbon extraction and gas chromatographic techniques were as described by Santas *et al.* (1999). Oil biodegradation was assessed by measuring the $n$-$C_{17}$/pristane and $n$-$C_{18}$/phytane ratios (Sveum and Bech, 1994). These ratios are conventionally used as indices of biodegradation (Atlas, 1991), based on the assumption that pristane and phytane are more resistant to biodegradation than $n$-alkanes and naphthalene (Prince *et al.*, 1993; Gundlach *et al.*, 1983; Oudot, 1984; Lee and Levy, 1987). Although the validity of these ratios in assessing biodegradation has been recently questioned (Bregnard *et al.*, 1997; Venosa *et al.*, 1997), they provide reasonably accurate results in short-term assays such as the study at hand.

Solar irradiance was measured using an ELDONET dosimeter equipped with three sharp band sensors (Gröbel, Ettlingen) for PAR, UV-A and UV-B calibrated against an Optronic 752 spectroradiometer (Santas *et al.*, 1997). The signals from the sensors are amplified, digitized and stored in a dedicated computer. The doses for the three bands are in turn calculated by the 'Windose' program (M. Lebert, University of Erlangen, Germany).

Climatological data of atmospheric temperature and precipitation were obtained from the Velo, Korinthos weather station of the Greek National Weather Service, 5 km NW of the experimental site.

Data were analysed by a three-way ANOVA using time, sampling site and season as the main sources of variation. Significant differences are reported at the 0.05 level.

## Results and Discussion

The difference in average water temperature between the two runs was only 1°C (first run: 10.4°C; second run: 11.4°C). Daily water temperature fluctuations were within 3°C in both runs. During the two runs, precipitation was 57.3 and 45.3 mm, with prevailing weak NE–NW winds (Greek National Weather Service, 1998).

The $n$-$C_{17}$/pristane and $n$-$C_{18}$/phytane ratios yielded comparable results throughout the experiments. The results from only one ratio ($n$-$C_{17}$/pristane) are presented for simplicity. Statistical analysis indicated that there is a significant time × season × depth interaction ($F = 7.33$; df = 10 and 36; $p < 0.05$).

Season had a significant effect on biodegradation of Iranian light: at the water surface, alkane biodegradation was dramatic during the first run (70% reduction on day 30, Fig. 1), while no statistically significant differences were observed between days 0 and 30 in the second run. This was a surprising result considering that the variation in most experimental parameters (treatments, precipitation, temperature, etc.) was virtually negligible between the two runs. The presence of a more efficient hydrocarbon degrading strain in the first run is not likely, given the large volume of seawater (3 $m^3$) sampled from the same site within the interim two-month period. Moreover, microorganism activity was expected to be higher in the spring (second run).

Solar radiation incidence differed dramatically between the two runs due to the progressing season (Table 1, Fig. 2). The total UV-B (290–315 nm), UV-A (315–390 nm) and PAR (390–700 nm) doses in the second run were elevated by a factor of 2.77, 1.89 and 2.10, respectively. Of these three bands, short wavelength UV-B radiation is thought to be the most harmful to microorganisms. Harmful UVR effects include inhibition of spore germination and outgrowth (Munakata, 1974), DNA lesions (Murphy, 1975), inactivation of certain transport systems (Sharma and Jagger, 1981) a decrease in the lipid content of membranes and ATPase inacti-

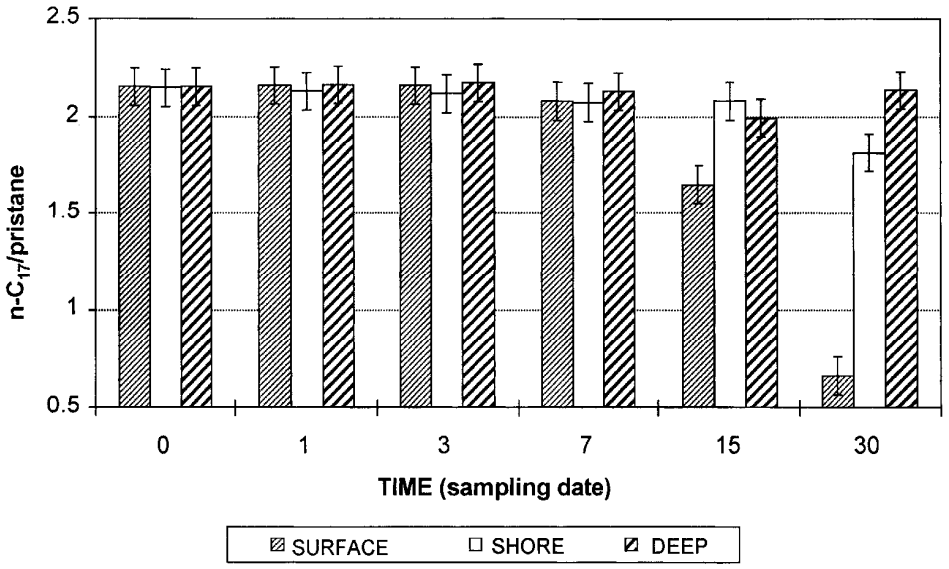


**Fig. 1** Effects of time and depth on biodegradation of Iranian light during run 1 (November–December 1996). Means ± 95% confidence intervals. Overlapping of confidence intervals indicates non-significantly different means.

**TABLE 1**

Total doses (kJ $m^{-2}$) of the three bands of solar radiation for the two runs (Run 2/Run 1 ratios: PAR 2.10; UV-A 1.89; UV-B 2.77; total 2.06).

| | PAR | UV-A | UV-B | Total |
|---|---|---|---|---|
| Run 1 | 57 637 | 12 240 | 57 | 69 934 |
| Run 2 | 121 023 | 23 137 | 159 | 144 320 |

vation (Murphy, 1983), etc. UV-A causes moderate damage, but also has a beneficial role in photorepair mechanisms (Smith, 1977). Therefore, the reduced biodegradation activity of microorganisms in the spring is likely to have been an effect on the large increase in total UV-B (and/or UV-A) dose.

Microbes capable of degrading oil grow on alternative substrates in the absence of oil. If oil spills were a regular phenomenon through evolutionary time, they would probably generate a selective pressure favouring the evolution of oil-specific adaptations. In addition, since oil slicks float on the UV-exposed water surface, the same organisms would also have some UV-adaptations. However, oil spills are only recent and accidental; time has been too short and recurrence too random to allow for the evolution of organisms which are both

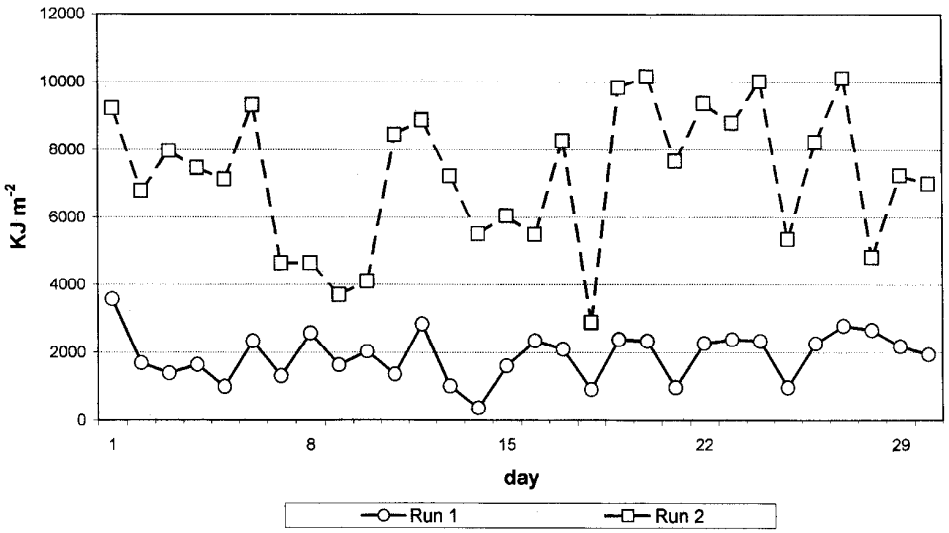


**Fig. 2** Daily UV-B doses during the two runs. The total UV-B dose during run 2 was 2.77 times higher than the corresponding dose in run 1

effective hydrocarbon degraders and UV-tolerant. Therefore, the inhibition of bioremediatory activities by ambient UVR might be a significant factor in environments exposed to solar radiation.

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