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Mesocosm Assays of Oil Spill Bioremediation with Oleophilic Fertilizers: Inipol, F1 or Both?

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The biodegradation of Iranian light crude in seawater environments was examined in three mesocosms, simulating a wild Mediterranean ecosystem. Two oleophilic fertilizers, Inipol EAP-22 and F1 (modified fish meal), were compared with regard to the biodegradation enhancement achieved by them. Hydrocarbon degradation proceeded faster at the water surface than at the sediment, as assessed by the *n*-C17/pristane and *n*-C18/phytane indicator ratios. Alkane biodegradation was higher in the presence of F1 (70% in 30 days). However, treatment with Inipol produced another desirable effect, the quick disappearance of the oil slick. The data led to the formulation of the hypothesis that the combined use of both fertilizers may be the treatment of choice. © **1999 Elsevier Science Ltd. All rights reserved**

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An estimated 100000000 tons (Solberg and Theophilopoulos, 1997) — about one-third of the global petroleum production annually loaded to tankers — is transported through the Mediterranean (Clark, 1989). Of these, an estimated 330000 tons are deliberately dumped into this closed sea, whereas accidents account for an additional pollution of 1000000 tons per year (Solberg and Theophilopoulos, 1996). The resulting oil slicks are washed ashore by the winds onto the densely populated Mediterranean coasts. Despite the high susceptibility of the Mediterranean to oil spill accidents, literature on oil spill decontamination attempts from this area of the world is scarce.

Bioremediation is a developing technology that enhances natural microbial activity to reduce the concentration and/or toxicity of various chemical substances such as petroleum products (Atlas, 1981), aliphatic and aromatic hydrocarbons (including polyaromatic hydrocarbons and polychlorinated biphenyls), industrial solvents (phenols, benzene, acetone, etc.), batter liquids, pesticides, and metals (arsenic, chromium, selenium, etc.; (US EPA, 1991)). Significant technological advances have been made since oil spill bioremediation first appeared in 1967 (reviewed by Santas and Santas, 1994; Korda *et al.*, 1997). Among them, bioaugmentation involves the direct application of microorganisms isolated from the contaminated site or from an off-site vendor adapted to the specific contaminant and site conditions, cultured, and enhanced. By contrast, pure cultures of bacteria introduced into natural environments, often do not persist for various reasons including competition, environmental toxicity, nutrient depletion, etc. (Venosa *et al.*, 1991).

Another widely used bioremediation approach is biostimulation — the acceleration of microbial reproduction and metabolic activity through the addition of oxygen, water, and nutrient media (combinations of nitrogen, phosphorus, and sometimes surfactants and trace metals). Numerous studies on the effects of fertilizers on oil biodegradation exist (Atlas and Bartha, 1973; Rivet *et al.*, 1993; Lacotte *et al.*, 1995; Marty and Martin, 1996; Delille *et al.*, 1997). These studies offer proof of bioremediation enhancement with the application of both oleophilic and water-soluble fertilizers. Oleophilic products are considered more suitable for surface bioremediation, since nutrients remain in the oil phase and not washed away to the open water. By contrast, slow-release, water-soluble formulations are recommended for subsurface hydrocarbon biodegradation (Prince, 1992). The effectiveness of the addition of an oleophilic (Inipol EAP-22) and a slow-release, water-soluble nitrogen–phosphorus fertilizer (Customblen) was tested during the bioremediation of the *Exxon Valdez* oil spill (Lindstrom *et al.*, 1991). Laboratory and field tests showed that Inipol enhanced biodegradation in a safe and effective way: laboratory studies elucidated the biocleansing action of Inipol, while winter monitoring showed a higher number of hydrocarbon degrading organisms in treated beaches as opposed to untreated ones. In a previous experiment

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conducted in mesoscale beaches, in basins with simulated tide and continuous exchange of seawater, with mesophilic mixed cultures of bacteria, fish meal appeared to result in an increased reduction of the n-C_{17}/pristane ratio, compared to non-treated crude oil or crude oil treated with Inipol (Sveum *et al.*, 1994).

The results obtained by isolating a single factor in laboratory experiments cannot be extrapolated to provide realistic assessments of cause-and-effect relationships in the wild. Field attempts, on the other hand, are often inconclusive due to the complexity of the open systems used and the intricacy of the biological processes involved. To address such issues, this study employed a realistic mid-scale simulation of typical local average conditions. Mesocosm experiments provide reasonable control and at the same time allow adequate interaction between the independent variables. In addition to their economic and technical manageability, such attempts are free of the many legal and social issues stemming from full-scale trials. This approach was chosen to compare the effectiveness of Inipol and F1, a new, modified fish meal fertilizer, in petroleum hydrocarbon breakdown under Mediterranean climatic conditions. The interaction of these fertilizers with habitat type (supra-littoral zone vs intertidal zone), and depth within the water column was also investigated.

Materials and Methods

The experiments were carried out under typical, mild Mediterranean winter conditions. Ambient temperature ranged from 1.6 to 19°C, with an average of 11.55°C. Daytime average mesocosm water temperature was 14°C, with daily water temperature fluctuations not exceeding 5°C. Precipitation was 15 mm, while weak NE–NW winds prevailed during the experimental period. The fertilizers used were Inipol EAP-22 (oleic acid, lauryl phosphate, 2-butoxy-1-ethanol, urea, and water; Sveum *et al.*, 1994; C:N:P = 62:5:1; Button *et al.*, 1992), and F1 (modified fish meal, C:N:P = 24:18:3.5).

The experiments were performed in three 3-m^3 ($3 \times 1 \times 1$ m) fiberglass tanks. The sloping 'beach' of each mesocosm was constructed by placing cinderblocks in a four-tier arrangement (Fig. 1). Beach pebbles collected from the intertidal zone of a nearby shore of Saronikos Gulf were transported to the mesocosm, placed on the cinderblocks and graded to form a smooth slope.

The tanks were filled with seawater from the same site to the topmost cinderblock layer. Seawater was added periodically to counter-balance losses due to evaporation, spray etc. Salinity ranged from 36 ppt at the beginning to 40 ppt at the end of the experiment. The water was recirculated on a 24-h basis by pumps (5 $m^3\ h^{-1}$) installed at the 'open water' end of each tank. Water recirculation rate was arbitrarily set at this high level to compensate for the lack of water renewal and to achieve sufficient oxygenation in the mesocosms.

Polypropylene netting enclosures (mesh size: 2 mm) were filled with 1 kg portions of beach pebbles. Two petroleum–fertilizer mixtures were prepared as follows: 3 l of Iranian light were mixed with (a) 330 ml Inipol, and (b) 330 g F1. Each enclosure was immersed in its

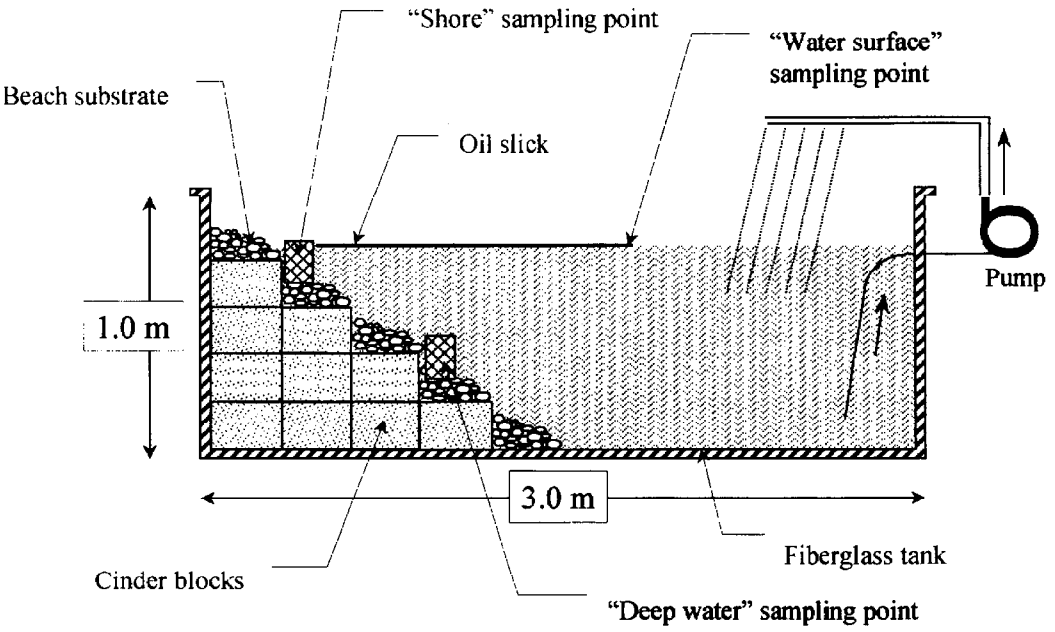

Fig. 1 Mesocosm setup. Three mesocosms were constructed from inert materials (fiberglass, plywood, cinderblocks, etc.). Sediment was transported from a nearby natural ecosystem.

assigned petroleum–fertilizer mixture. Enclosures designated as 'control' were immersed in crude oil without any fertilizer added. Excess crude was removed by dripping for approximately 30 s. The enclosures were subsequently placed at two different depths designated as 'shore' (10 cm below water surface) and 'deep water' (60 cm) in each tank. Subsequently, artificial oil slicks were created by spreading the oil–fertilizer mixtures (no fertilizer for the control) on the water surface of each tank.

'Surface' oil samples were obtained by skimming, while sediment samples were obtained from the enclosures at the 'shore' and 'deep water' points. Samples were prepared for gas chromatography as follows: (a) 20 ml surface water samples and, (b) 10 g sediment samples were placed in 100 ml Erlenmeyer flasks. 10 ml *n*-hexane (Merck, *proanalysi*, > 99%) were added in each flask, and mixed by swirling to extract hydrocarbons in the solvent. Water remaining in the *n*-hexane phase was removed by adding excess sodium sulfate. The supernatant was filtered through a silica gel column (particle size: 2–25 μm) and the saturated hydrocarbon fraction was eluted with 1 ml *n*-hexane. Hydrocarbons in the elutriate were analyzed with a Hewlett Packard 5890 Series II GC, equipped with a flame ionization detector (FID) and a splitless injector. Oil biodegradation was assessed by measuring the n-C_{17}/pristane and n-C_{18}/phytane ratios as biodegradation indices (Sveum and Bech, 1994; Atlas, 1991).

Two replicate samples were collected on days 0, 1, 3, 7, 15 and 30 from the three sampling sites in each mesocosm. The data for the n-C_{17}/pristane and n-C_{18}/phytane ratios were subject to a three-way ANOVA. The sources of variation were fertilizer type (F1, Inipol, no fertilizer), depth ('surface', 'shore', and 'deep water') and sampling date.

Results and Discussion

The two index ratios yielded almost identical results. The results from only one ratio (n-C_{17}/pristane) are plotted here for simplicity. The interaction between fertilizer type, depth and sampling date was significant ($F = 10.68$; $df = 20$ and 54; $p < 0.05$). The lowest index ratio (0.65) was observed in the F1-treated mesocosm on day 30 at the water surface (Fig. 2). This value was significantly lower than any other day or depth and represented a 70% reduction in the n-C_{17}/pristane ratio compared to the initial value (2.15 on day 0, Fig. 2).

On the same day the index ratio for the shore samples was significantly lower than any other respective value for the same depth on any other date (Fig. 2). For the deep-water samples, no significant changes were observed throughout the project (Fig. 2).

No statistically significant differences were observed within the Inipol and the control mesocosm throughout the experiment.

The assessment of biodegradation by monitoring the n-C_{17}/pristane and n-C_{18}/phytane indicator ratios was based on the assumption that biodegradation of pristane and phytane was negligible during the 30-day experimental period. For longer periods, and under controlled conditions of aeration and nutrient additions, a 30% pristane biodegradation has been reported (Samson *et al.*, 1994). Therefore, the results presented herein may be a conservative estimate of the biodegradation efficiency of the two fertilizers, since pristane and phytane are at the denominator of the two

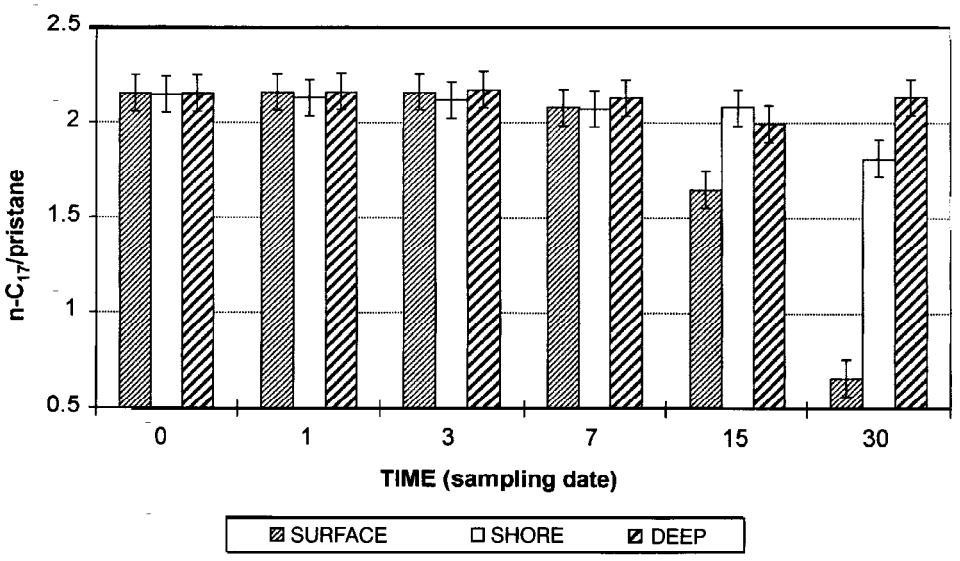

Fig. 2 Effects of depth and time on n-C_{17}/pristane ratio in the F1-treated mesocosm. The most dramatic differences are observed in the surface samples on days 15 and 30. Overlapping of confidence intervals indicates no significant differences at the $p = 0.05$ level. Each mean is the average of $n = 2$ values.

indicator ratios. Analytical procedures using more stable biomarkers, such as hopanoids (Venosa *et al.*, 1997), would have likely shown a higher biodegradation in the two treatments.

The results obtained show that, in terms of petroleum hydrocarbon biodegradation, F1 was the most effective and fastest-acting treatment. This fertilizer with a C:N:P ratio of 24:18:3.5 is a newly developed product currently under extensive evaluation and it is not yet commercially available. F1 contains considerably more nitrogen and phosphorus per unit mass than other fish meal fertilizers, such as the one used by Sveum *et al.* (1994) with a C:N:P ratio of 46.8:10.5:2.0. Such fertilizers contain a very large number of compounds of biological origin including proteins, lipids and carbohydrates.

On the other hand, alkane biodegradation by Inipol EAP-22, a synthetic fertilizer with only four ingredients and a C:N:P ratio of 62:5:1, was not dramatic. Since Inipol does not contain protein, a lower nutritional value and thus a reduced utilization by microbes may be expected. The absence of significant hydrocarbon reduction by Inipol in our mesocosm experiments implies that laboratory attempts probably overestimate hydrocarbon breakdown enhancement by this product. Similar trends were observed by Sveum *et al.* (1994) who attributed the better performance of a fish meal fertilizer to increased nutrient availability. The authors speculated that nutrients supplied by this fertilizer are initially immobilized in microbial cells. The nutrients are slowly released after the death of the microbes and used by subsequent generations. Based on these findings, one could conclude that if the only concern is petroleum hydrocarbon degradation using environmentally acceptable methods, the application of fish meal fertilizers is undoubtedly the treatment of choice.

In terms of appearance, however, Inipol yielded far superior results than F1. The oil slick in the Inipol mesocosm disappeared completely after day 15. The water surface was clear with only a few irridescent patches. Inipol application results in solubilization of the oil slick in the water. The emulsion is in turn washed away by currents and spread by waves, presumably without the harmful effects of dispersants on aquatic life. By contrast, in the F1 mesocosm there were free-floating patches of a dark, thick oil residue surrounded by a thin, irridescent film throughout the duration of the trial. Even if such a condition represented a minor disturbance for the aquatic environment, the establishment of such a treatment as a valid decontamination technology would be prevented by the lack of public acceptance for aesthetic pollution.

New products combining the nutritional value of F1 and the dispersant-like properties of Inipol may be an upcoming technological advancement in petroleum hydrocarbon bioremediation. This assumption awaits validation in future experiments.

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