

## TOXICITY TRENDS DURING AN OIL SPILL BIOREMEDIATION EXPERIMENT ON A SANDY SHORELINE IN DELAWARE, USA

Alan Mearns<sup>1</sup>, Kenneth Doe<sup>2</sup>, William Fisher<sup>3</sup>, Rebecca Hoff<sup>1</sup>, Kenneth Lee<sup>4</sup>, Robert Siron<sup>5</sup>, Cornelia Mueller<sup>6</sup>, and Albert Venosa<sup>7</sup>

<sup>1</sup>National Oceanic and Atmospheric Administration, Seattle WA, 98115

<sup>2</sup>Environment Canada, Bedford Institute of Oceanography, Dartmouth, NS Canada B2Y 4A2

<sup>3</sup>U.S. Environmental Protection Agency, Gulf Breeze, FL USA 32561

<sup>4</sup>Department of Fisheries and Oceans Canada, Marine Environmental Sciences Division, Institut Maurice-Lamontagne, Mont-Joli, Quebec Canada G5H 3Z4

<sup>5</sup>Institut National de Recherche Scientifique-Océanologie, Rimouski, Quebec Canada G5S 3A1

<sup>6</sup>Science Application International Corp., Narragansett, RI USA 02882

<sup>7</sup>U.S. Environmental Protection Agency, Cincinnati, OH USA 45268

**ABSTRACT.** A 13-week, refereed, inter-agency toxicity testing program involving five bioassay methods was used to document the effectiveness of shoreline bioremediation to accelerate toxicity reduction of an oiled sandy shoreline at Fowler Beach, Delaware, USA. The study was part of an intentional oiling experiment using a randomized complete block design with repeated measures. Bioremediation - treatment with nutrients or nutrients and oil-degrading bacteria - did not accelerate toxicity reduction. Nor did treatment increase toxicity at weeks 0, 6 or 12-13. However, results of one high-frequency test suggested there may have been a substantial delay in toxicity reduction due to treatment during the first few weeks of treatment. All tests provided information but the most sensitive tests were the 10-day sediment amphipod and grass shrimp embryo bioassays. Standardized sediment and water toxicity tests can play a valuable role in evaluating the effectiveness and effects of oil spill shoreline countermeasures.

### INTRODUCTION

The 'effectiveness' of oil spill countermeasures ultimately must be judged by their ability to reduce injury to marine life caused by oil. From a marine biologist's point of view, response methods accomplish nothing if they do not markedly reduce the threat or impacts of oil to marine life. The worst situation is use of a response method that is not effective in reducing exposure and increases injury to marine resources.

In the past, the health, abundance and biodiversity of marine life has rarely been considered in field tests of oil spill countermeasures. Fortunately, this situation is changing. This paper presents preliminary results of the first intentional use of bioassays to document and compare toxicity of sediments among unoiled, oiled, nutrient-treated and bacteria-treated plots on a sandy shoreline. The primary goal of the study was to determine if bioremediation could not only accelerate the degradation of oil but also significantly reduce the toxicity of an oiled shoreline. Further, we wanted proof that treatment was at least innocuous, i.e., that it did not in fact increase sediment toxicity. This report provides guidance for further use of standard toxicity tests to evaluate the effectiveness and effects of oil spill shoreline countermeasures.

## METHODS

### Overall Experiment

Trends in toxicity were monitored as part of a larger long-term (14-week) experiment to document effectiveness of oil spill bioremediation on a sandy shoreline. The U.S. Environmental Protection Agency (EPA) and the Delaware Department of Natural Resources and Environmental Control (DNREC) authorized intentional oiling of fifteen 36 sq m plots in the intertidal zone along a stretch of sandy shoreline at Fowler Beach, Delaware in July, 1994. The overall objectives of the study were to 1) obtain sufficient statistical evidence to determine if bioremediation with inorganic mineral nutrients and/or microbial inoculation enhances the removal of crude oil contaminating a sandy beach, 2) to compute the rate at which such enhancement takes place, 3) to establish engineering guidelines on how to bioremediate an oil-contaminated shoreline and 4) to characterize the impact of bioremediation on toxicity to marine organisms.

**Design and Layout.** The experiment involved a randomized complete block design with repeated measures as described in Venosa et al., 1995. There were four treatments (no oil, oil, oil plus nutrients and oil plus nutrients and oil-degrading bacteria) and five replicate plots per treatment. Plots were arranged parallel to shore in each of five blocks. Blocks were separated by a minimum distance of 10 meters (range 10 to 150). Within each block, plots were separated by 10 m. The four treatment types were randomly assigned to plots in each block. The fifteen oiled plots were fitted with skirted boom attached to vertical steel posts so they could rise and fall with the tide.

**Oiling.** On July 1, 1994 each of the fifteen oiled plots were each treated with 36 gallons of two-day weathered Nigerian Bonny light crude oil (API = 35.3). Oil was applied evenly using a 4-m wide spray diffuser. On application, it was expected the oil would penetrate approximately one foot into the sand. Cores taken several hours following treatment confirmed that the nominal penetration depth and concentration (5000 ppm) were in fact approached.

**Treatment.** Beginning July 5 (four days after oiling) each plot was treated approximately daily during ebbing tide with 200 gallons of either natural seawater (un-oiled and oiled-only plots), seawater with nutrients (nutrient-treated plots) or seawater with nutrients and bacteria. The five nutrient-treated plots received 2.0 kg of sodium nitrate and 0.13 kg of sodium tripolyphosphate dissolved in the seawater. Nutrients were obtained from a local commercial agriculture supply. Bacteria-treated plots received a water-suspended inoculum of alkane and aromatic hydrocarbon-degrading bacteria isolated from the site and cultured at ambient temperature in a 55-gallon drum. Treatment materials were delivered via an impact sprinkler system involving 6 sprinkler heads, one at each corner and two on the sides of each plot. Treatment and chemical, physical and biological monitoring continued for 14 weeks.

### Toxicity Tests

There are literally dozens of methods now available to assay the toxicity of water and sediment samples. After consideration of costs, availability and possible applicability to operational monitoring, we settled on a core of five bioassay methods, two for sediments (solid-phase) and three for pore-water, each yielding one or two endpoints (Table 1). All replicate plots were sampled for toxicity testing during Week 0, Week 6 and Weeks 12 (pore water) and 13 (sediment). In addition, one test (grass shrimp embryo hatch bioassay) was used at a lower replication rate ( $n=2$ , not 5) but at a higher frequency (biweekly) in case important trends were missed by the other tests. Samples were also collected for mutagenicity testing (to be reported later).

Table 1. Summary of data from toxicity tests conducted as part of the Delaware Bay Bioremediation Experiment.

Test organism	Medium	Exposure	Test conditions	Endpoint
Amphipod- <i>Leptocheirus plumulosus</i>	sediment	10 days	static	% survival
Microtox <i>Vibrio fischeri</i> ( <i>Photobacterium phosphoreum</i> )	sediment	15 min	static	EC50
Grass shrimp embryo <i>Palaemonetes pugio</i>	porewater	14 days	static	% larval survival % hatch success
Microtox	porewater	15 min	static	EC50 % light loss
Urchin fertilization <i>Lytechinus pictus</i>	porewater	10 min sperm + 10 min combined	static	% fertilized

**Sediment Sampling.** For sediment sampling, each plot was divided by elevation (tidal height) into four equal portions, landward to seaward. Two core samples (total depth, 14 inches) were taken from pre-selected random points in each elevation and composited into glass I-Chem jars labeled according to plot number and elevation. After mixing, equal amounts of sediment from each of these four "elevation-composites" were, in turn, placed into a fifth glass I-Chem jar and mixed. This sample was the "plot" composite for sediment toxicity testing. Samples of sediment for the 10-day sediment amphipod bioassay were removed from this jar and placed in 2L high-

density polypropylene jars. Samples for the sediment Microtox bioassay were removed from the same can and placed in 250 mL wide-mouth jars.

**Pore Water Sampling.** Pore water was collected biweekly by inserting a 12 in x 1 in d slotted PVC well point pipe approximately 10 in into the sand and extracting collected water using a 50 mL syringe. The first water entering the well-point tube usually contained suspended silt and solids and was removed by syringe and discarded. Clear water seeping into the well point was collected by syringe. At each plot approximately 1 L of pore water was collected during ebbing tide by inserting the well point at a minimum of three haphazardly-located points in the mid-tide elevation. Repeated collections of porewater were released to over-flowing into a clean 1L jar and the jar immediately capped.

**Sample Handling and Shipment.** Sediment and porewater samples were placed on ice packs in sealed ice chests and shipped by overnight courier to participating laboratories. Upon arrival, sediment samples were removed, inventoried and stored at 4C in the dark until testing.

**10-Day sediment Amphipod Bioassay.** The tube-building amphipod, *Leptocheirus plumulosus* (range Cape Cod to northern Florida) was used as the test organism for the 10-day sediment bioassay. Briefly, replicate thawed sediment samples (200 mL) and sea water (800 mL) are placed in containers with 20 randomly-selected amphipods and the animals monitored daily for emergence and death. Standard procedures (ASTM, 1992) were followed with the exception that the number of laboratory replicates was reduced from five to three (SAIC, 1994). Animals were obtained from both a commercial supplier and in-house cultures maintained by SAIC (1994). The performance of animals used for each test series were evaluated using clean sediment collected from a frequently-used Long Island Sound reference station. Tests were done for three sampling events: weeks 1, 6 and 13.

**Sediment Microtox Bioassay.** Testing was done using a modification of the standard Microtox method (Microbics, 1992). Briefly, 7 g of wet sediment were resuspended into 56 mL glass bottle filled with 35 mL of Microtox SPT diluent and stirred for 20 minutes with a vortex about half the volume. Following 15 minutes of settling time a one-mL aliquot of the aqueous phase was transferred into appropriate cuvettes to make an eight-dilution series (from 5.6 to 99% by volume). Each sample was run in the Microtox unit using the 100% test protocol. For each sample the EC50 was calculated after transforming the dose-response curve in a log-log scale for linear regression. The EC 50- was expressed as % volume of dilution. A standard 100g/L phenol solution was used to check the performance of both the operator and analytical system and a coefficient of variation (CV) was maintained below a 10% range throughout the study. Tests were done for three sampling events, weeks 1, 6 and 12, using sub-samples of the composites used in the amphipod tests (above).

**Porewater Sea Urchin Fertilization Test.** Testing was conducted for pore water samples according to protocols in Environment Canada, 1992a. Briefly, sperm from ripe white sea urchins (*Lytichinus pictus*) were mixed for 10 min in sample and then eggs added at a ratio of 1:20,000 with 10 min additional exposure. Subsamples were

then examined for mean percent fertilization computed from counts from three replicate containers. Tests were done for the three sampling events (weeks 0, 6 and 13) using sub-samples of the composite water used for the other pore water tests (below).

**Porewater Microtox Test.** Porewater samples ( aliquots of the same samples used for the fertilization test, above) were subjected to Microtox testing using the methods described in Environment Canada, 1992b. Endpoints examined included both EC 50, from serial dilution, and per cent light level of undiluted sample. Tests were done for three sampling events: weeks 0, 6 and 13 using sub-samples of the same water used for the sea urchin and shrimp embryo tests.

**Porewater Grass Shrimp Embryo Test.** The method used is described in Fisher and Foss (1993). Briefly, male and ovigerous (egg-bearing) female grass shrimp were collected from local estuaries near Gulf Breeze, Florida, transported to the laboratory and held in flow-through water systems prior to testing. Embryos from gravid (fertilized) females were examined with a dissecting microscope and a single female possessing a clutch of embryos at the blastoderm-tissue cap stage (2-4 days old) was selected. Embryos were teased apart and placed individually and randomly in small acid-washed tissue culture tubes containing 6 mL of test pore water. Racks of tubes were placed on a circular rotator and incubated at 27 C and 20 ppt salinity. There were twenty embryos for each sample. Embryos were maintained for 10 days or until hatching at which time numbers surviving and percent hatch was recorded. Only 8 of the 20 plots were monitored for porewater toxicity using this test. However, the test was run seven times at eight sampling intervals: weeks 0 (day 2), 1, 2, 4, 6, 8, 10, 13). Also, on week 13, all 20 plots were sampled and tested by a second, independent, laboratory (data to be reported later).

**Coordination.** A NOAA representative (senior author) served as coordinator and referee, insuring that identity of the treatment types remained unknown until termination of the experiment, October, 1994. NOAA scientists and Scientific Support Coordinators (SSC's) also collected data from participating laboratories, performed preliminary visual and statistical analyses and sought resolution of uncertainties.

**Data Analysis and Interpretation.** Visual plots of treatment means and standard deviations were made to aid in comparing the results. Using SysStat software, repeated measures ANOVA, the appropriate statistical method for this experimental design, was run on data from each assay separately. The ANOVA were conducted on means of laboratory replicates where they occurred. Missing values were entered as ".". SysStat software automatically leaves out any missing values from the analysis. The two factors of interest were between subjects: treatment (un-oiled, oiled, nutrient, and nutrient+bacteria) and within subjects: week (1, 6, 12). Endpoints used in the ANOVA are specified in Table 1. Except for the Microtox tests, all are direct observations (% survival, % fertilized, etc.). Since the standard reporting measure in the Microtox test is the EC50 (Environment Canada, 1993b), we used this value as a relative representative of toxicity, even though it is not a direct observation per se. All EC50 data with values greater than 100 per cent were recoded as 100. Those listed as "no toxicity" were also coded as 100.



## RESULTS

Upon oiling, sediment oil concentrations (not shown) averaged about 4,700 mg/kg in all 15 oiled plots. Due primarily to physical wash-out, concentrations in all plots decreased by 75 per cent during the first six weeks of the experiment over 90% the next six weeks. These data will be described in more detail in subsequent papers.

Regardless of treatment, pore water and sediments from all oiled plots were toxic to all test organisms during the first sampling event (Week 0, Figures 1a, b and c, 2a and b and 3a and b). Results of the toxicity tests varied by test (see Table 2) but all assays showed differences in toxicity over time. In general, assays using sediment samples were more sensitive and detected more differences than assays using porewater samples.

### Visual Comparisons

**Pore Water Toxicity.** The sea urchin fertilization test was least sensitive (Figure a), the grass shrimp embryo hatching success assay the most sensitive (Figure 1c) and the Microtox assay intermediate in sensitivity (Figure 1b; for this comparison, Microtox light output in undiluted sample as percent of control is shown Figure 1b). By Week 6, the sea urchin fertilization and Microtox test results suggested that there was little or no toxicity left in pore water from either treated or untreated oiled plots (Figure 1a and b); however, although not statistically significant (see below), there appeared to be some residual pore water toxicity remaining as indicated by the grass shrimp embryo hatching success (Figure 1c). By Week 13, there was apparently no remaining toxicity to all three test organisms.

Pore water from unoiled plots may have been slightly toxic at the onset of the experiment; there was a slight tendency for all three endpoints to show decreasing toxicity (i.e., increasing survival, light output and hatching success) over time.

**Sediment Toxicity.** In contrast to pore water, sediments from all treated and untreated plots were toxic to the Microtox test organism and amphipods during both of the first two sampling events (Weeks 0 and 6, Figure 2a and b). Amphipod toxicity continued to occur through Week 13 (Figure 2b). In addition, unoiled sediment appeared to be toxic to the amphipods throughout the course of the experiment (relative to a performance control sediment from Long Island Sound (LIS in Figure 2b).

**High Frequency Sampling Trend.** Based on limited, but high frequency, grass shrimp embryo hatching success trends, pore water lost most of its toxicity in two untreated but oiled plots by Week 2 (Figure 3a and b). However, treated plots appeared to remain toxic through at least Week 6; the mean toxicity of nutrient treated plots recovered to oiled and unoiled plot toxicity by Week 8 whereas at least one bacteria treated plot apparently remained toxic through Week 10. Combined nutrient and bacteria treatment toxicity data ( $n=4$  per sampling event) suggests these plots experienced a delay in toxicity reduction relative to oiled (only) and unoiled plots (Figure 3b).

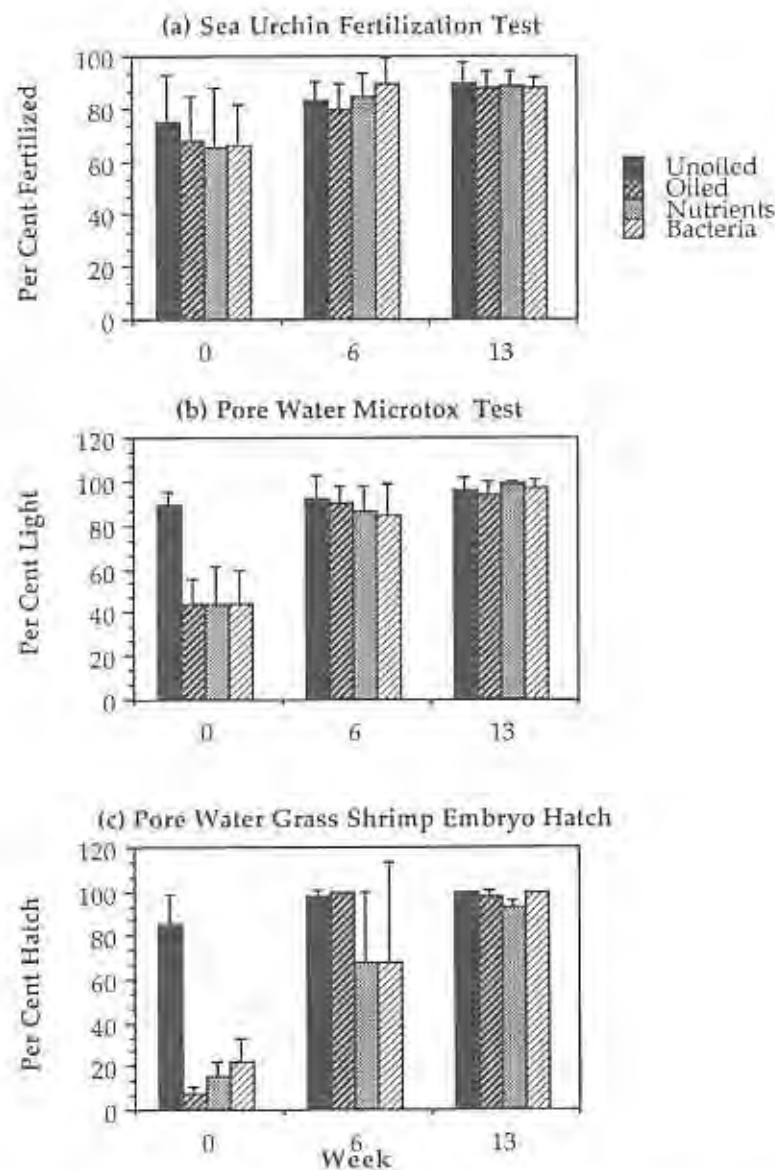


Figure 1. Toxicity of pore-water composite samples from unooled, oiled and treated intertidal sediments at Weeks 0, 6 and 13 to (a) sea urchin fertilization, (b) Microtox percent light output and (c) percent hatch of grass shrimp embryos. Data are mean and one SD. Unless noted otherwise,  $N=5$  samples for sea urchin fertilization and Microtox tests and  $N=2$  for the grass shrimp embryo test.

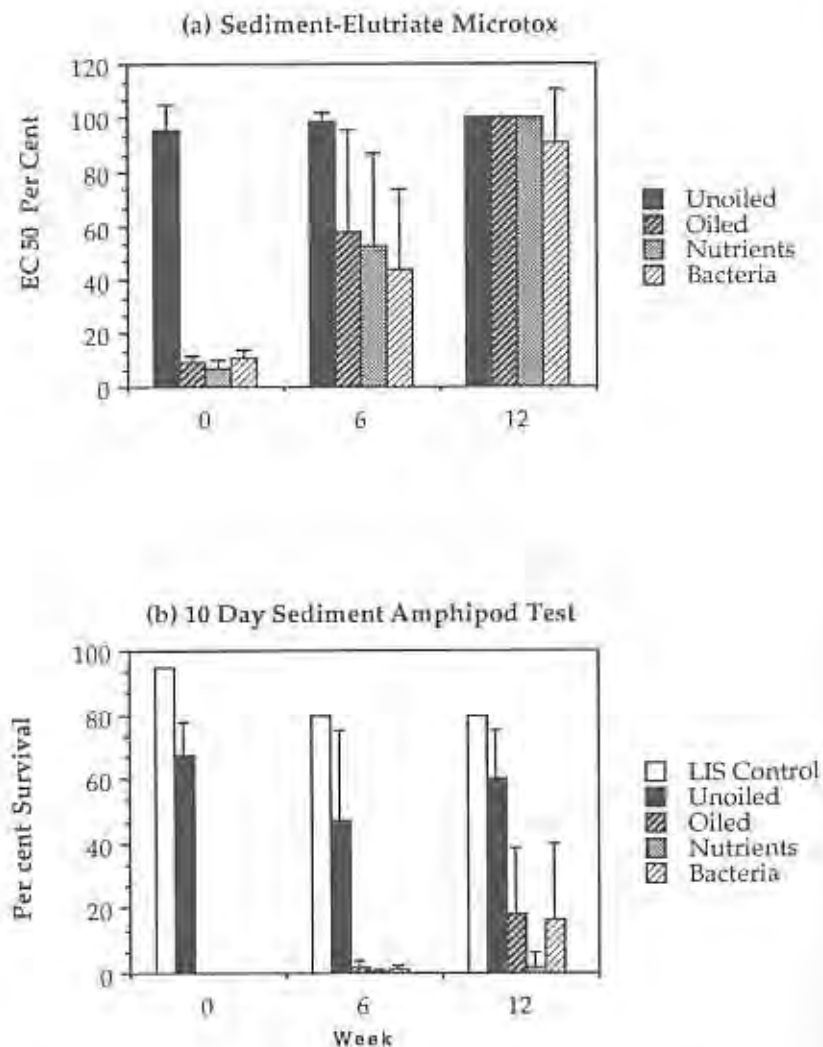


Figure 2. Toxicity of unoiled, oiled and treated sediments to (a) marine amphipod, *Leptochirus plumulosus* and (b) Microtox EC50. Data are mean and one SD and  $n=5$  unless noted. LIS = Long Island Sound clean performance control sediment for comparison (amphipod only). (Label Y % Survival.)

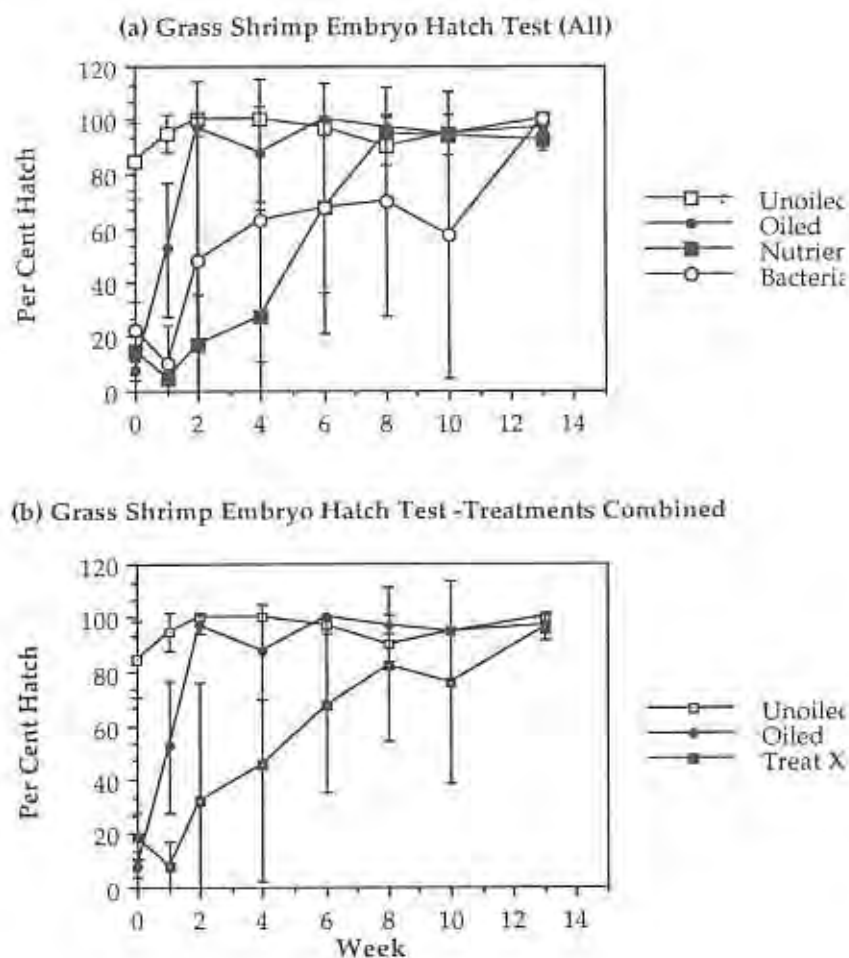


Figure 3. Trends in grass shrimp embryo hatching success in pore water composites from eight sampling events from. Data are mean and one SD. (a)  $N=2$  samples per event and treatment type for samples per event for unoiled, oiled, nutrient-treated and nutrient+bacteria treated plots and (b) same plot as (a) with all four treated plots combined.

*Significance of Trends.* Though significant treatment effects were found in the amphipod and Microtox assays and near-significant effects in the grass shrimp embryo assay, the differences were primarily due to the oiled/unoled effect rather than the bioremediation treatments (Table 2). To distinguish this, a second repeated measures ANOVA was run, using only the oiled plots in the treatment group (nutrient, no nutrient, and nutrient+bacteria).

Table 2. Summary of statistical analysis of toxicity data from Delaware Bay Bioremediation Experiment, using repeated measures ANOVA and all data.

Test organism	Probability of effects			Endpoint
	Treatment	Week	Treatment times week interaction	
Amphipod-sediment	0.00 *	0.02 *	0.14	% survival
Microtox-sediment	0.00 *	0.00 *	0.00 *	EC50
Microtox-porewater <sup>1</sup>	0.30			EC50
Grass shrimp embryo-porewater	0.58 <sup>1</sup> 0.07	0.00 *	0.09	% larval survival % hatch success
Urchin fertilization-porewater	0.92	0.00 *	0.89	% fertilized

\* $p \leq 0.05$   
<sup>1</sup>: analysis is from one-way ANOVA on week 1, since results were identical for all treatments for weeks 6 and 12 (sediment) or 13 (pore water).

Removing the oiled/unoled difference resulted in non-significant treatment and interaction effects, with the only significant differences remaining due to changes over time (Table 3). This confirmed that the dominant effect on toxicity throughout the experiment was the oil itself and the time of sampling. Effects that the different treatments may have had on toxicity were overwhelmed by these two factors.

In several cases (Microtox porewater and grass shrimp embryo-percent larval survival) the data showed no variation past week one. In these cases, a one-way ANOVA was run on treatments for week one only.

Table 3. Summary of statistical analysis of toxicity data from Delaware Bay Bioremediation Experiment, using repeated measures ANOVA and data from oiled plots only (treatments include oiled only, nutrient, and nutrient+bacteria).

Test organism	Probability of effects			Endpoint
	treatment	week	treatment times week interaction	
Amphipod-sediment	0.37	0.01 *	0.45	% survival
Microtox-sediment	0.53	0.00 *	0.80	EC50
grass shrimp embryo-porewater	0.68	0.00 *	0.51	% hatch success

\*  $p \leq 0.05$

## DISCUSSION

Treatment (with nutrients or nutrients plus oil-degrading bacteria) did not accelerate reduction of the toxicity of the oiled shoreline plots. Graphically (Figures 1, 2 and 3) there appeared to be small differences among treated and untreated oiled plots with respect to toxicity; however, none of these differences were statistically significant at Weeks 0, 6 or 12 and 13. Thus, with this oil on this type of shoreline, treatment with nutrients or oil-degrading microbes offers no benefits in terms of speeding the loss of toxicity of the oil.

It also appears, at least statistically, that treatment did not increase toxicity at Weeks 0, 6 or 12 and 13. However, based on the high-frequency/low replication grass shrimp embryo bioassay, several treated plots were more toxic than the two untreated oiled plots, which basically lost their pore-water toxicity by Week 2 (Figure 3). Thus, we cannot rule out the possibility that treatment increased the toxicity of the beach pore water during the first several weeks of treatment and that this event was missed by the majority of the tests. The hypothesis needs to be tested that treatment increased toxicity during the first few weeks.

This study involved a range of organisms, endpoints and exposure conditions. Of the sediment bioassays, the 10-day amphipod test was clearly more sensitive than the modified sediment Microtox test. Likewise, for pore-water, the grass shrimp embryo bioassay was clearly more sensitive than either the Microtox or sea urchin fertilization tests. Logistics during a spill response would favor use of the small-volume, short-term tests such as Microtox. However, we do not know the extent to which sensitivity is

enhanced or not by factors such as exposure time. Work is underway to answer this question and to develop toxicity testing tools useful for operational monitoring.

Until recently, oil spill response science has not availed itself of tools available to convincingly document the effects as well as the effectiveness of oil spill countermeasures such as bioremediation. That situation is changing rapidly as biologists and toxicologists bring a variety of standardized testing tools into the realm of oil spill research (Lee et al., 1995). One such set of tools are bioassays or toxicity tests where organisms are exposed to contaminated water or sediment and observed for effects such as mortality, disease, reproductive success, enzyme inhibition, abnormal development and a host of other "endpoints" indicative of the presence of toxic chemicals. Sediment and sediment elutriate bioassays have now been used to diagnose effects at a few oil spills such as the Exxon Valdez (Wolfe et al., 1993). Native organisms were also used to assess the efficacy of using a shoreline cleaner at a recent oil spill in Puerto Rico (Shigenaka et al., 1995). The work reported here is the first comprehensive study we know of that explicitly examines the toxicity of an intentionally oiled and treated shoreline using standardized bioassay methods. Hopefully, it will not be the last.

#### ACKNOWLEDGEMENTS

We especially thank Ben Anderson, Delaware Department of Natural Resources and Environmental Control, for undying support for this work. We also thank for their assistance Chris Shlekat, SAIC and Dr. Glen Thursby, formerly SAJC, Steve Foss, EPA Gulf Breeze, Tim Reilly of the Marine Spill Response Corporation and the many individuals who assisted in the field. This work was sponsored, in part, by contracts from the Marine Spill Response Corporation (MSRC) and Texas General Land Office (through the Geochemical and Environmental Research Group, Texas A&M University). We also appreciate support from the Hazardous Materials Response and Assessment Division, National Oceanic and Atmospheric Administration, Seattle, WA, and the Environmental Protection Agency Gulf Breeze Laboratory.

#### REFERENCES CITED

- ASTM. *Standard guide for conducting 10-day static sediment toxicity tests with marine and estuarine amphipods. Annual Book of ASTM Standards. Volume 13.A, E1367-92.* Philadelphia, PA, 1992.
- DNREC. Delaware Department of Natural Resources and Environmental Control. Field bioremediation study of spilled crude oil on a sandy and slightly gravelly beach in South Slaughter Beach, Delaware (1993).
- Environment Canada. Biological test method: fertilization assay using echinoids (Sea urchins and sand dollars). Report EPS 1/RM/27, Environmental Protection Series, Environment Canada, Ottawa, Ont, 97 pp. December, 1992 (1992a).

Environment Canada. Biological test method: Toxicity test using luminescent bacteria (*Photobacterium phosphoreum*). Report EPS 1/RM/24, Environmental Protection Series, Environment Canada, Ottawa, Ont. 97 pp. November, 1992. (1992b).

Fisher, W.S. and S. S. Foss. A simple test for the toxicity of Number 2 fuel oil and oil dispersants to embryos of grass shrimp, *Palaemonetes pugio*. *Marine Pollution Bulletin* 26(6): 385-391 (1993).

Lee, K., R. Siron and G.J. Tremblay. Effectiveness of bioremediation in reducing toxicity in oiled intertidal sediments. Proceedings, Third International In Situ and On-Site Bioreclamation Symposium, April, 1995, San Diego, CA. In press.

Microbics. *Microtox Manual*. Microbics Corporation, Carlsbad, CA, January, 1992 (1992).

SAIC (Science Application International Corporation). Sediment toxicity results for shoreline oil spill bioremediation experiment in Delaware Bay. Final report, November 30, 1994. Submitted to Texas A&M University, Geochemical and Environmental Research Group, College Station, Texas (1994).

Shigenaka, G., M. Angela McGehee, Vance P. Vicente and Charles B. Henry, Jr. Biological effects monitoring during an operational application of Corexit 9780. pp 177-184 In 1995 International Oil Spill Conference, Long Beach, CA. American Petroleum Institute Publ. 4620, API, Washington D.C. (1995).

Venosa, A. D., J. R. Haines, B.A. Wrenn, K.L. Strohmeier, B. Loye Eberhart, M. Kadkhodayan, E. Holder, M. T. Suidan, D. King and B. Anderson. Bioremediation study of spilled crude oil on Fowler Beach, Delaware. 1995 International Oil Spill Conference, Long Beach, CA. American petroleum Institute, Washington D.C. pp 888-889 (1995).

Wolfe, D.A., M.M. Krahn, E. Casillas, K.J. Scott, J.R. Clayton, Jr., J. Lunz, J.R. Payne and T.T. Thompson. Toxicity of intertidal and subtidal sediments contaminated by the Exxon Valdez oil spill, 48-51 In Exxon Valdez Oil Spill Symposium, Abstract Book. February 2-5, 1993, Anchorage, Alaska. Oil Spill Public Information center, Anchorage, AK (1993).