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# A SCIENTIFIC STUDY TO DEVELOP A PRACTICAL METHOD FOR ASSESSING THE CLEANUP OF "SOUR" (HIGH SULFUR) CRUDE OIL SPILLS IN LITTORAL SANDS USING BENTHIC MICROORGANISMS



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Avery Point, Groton, Connecticut 06340



FINAL REPORT



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**U.S. DEPARTMENT OF TRANSPORTATION**

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# N O T I C E



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16. Abstract

The objective of this study was to determine if a practical method could be developed for assessing the cleanup of a single spill of Arabian Light Stabilized crude oil in littoral sands and gravels by using microorganisms as indicators. Crude oil was spilled on beaches at three localities along the Connecticut shoreline where different environmental conditions are present and the microbiota and sediments in oiled plots were compared with those in unoiled plots for up to 13 months. The study showed that microorganisms cannot be used as indicators of the effectiveness of cleanup operations in these sediments. Species diversity in the oiled and unoiled plots at each study locality was about the same throughout the study and, in general, only slight differences in the number of individuals were observed for most species.

The spilled oil was rapidly removed from these sediments by natural processes. Retention was longest in the sediments that had relatively large amounts of organism produced mucilaginous materials. However, even in these sediments, the crude oil was not detected approximately three months after it was spilled.

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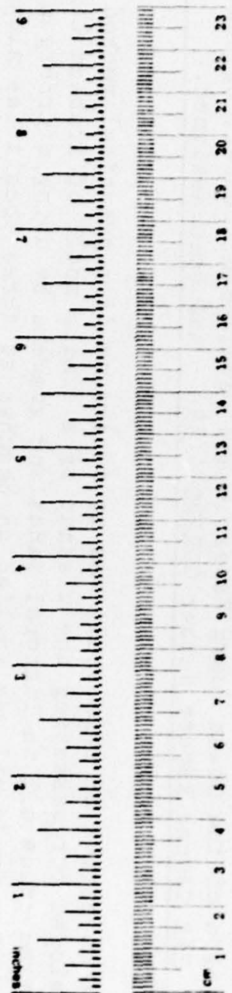
## METRIC CONVERSION FACTORS

### Approximate Conversions to Metric Measures

| Symbol | When You Know | Multiply by | To Find | Symbol |
|---|---|---|---|---|
| | | **LENGTH** | | |
| in | inches | *2.5 | centimeters | cm |
| ft | feet | 30 | centimeters | cm |
| yd | yards | 0.9 | meters | m |
| mi | miles | 1.6 | kilometers | km |
| | | **AREA** | | |
| $in^2$ | square inches | 6.5 | square centimeters | $cm^2$ |
| $ft^2$ | square feet | 0.09 | square meters | $m^2$ |
| $yd^2$ | square yards | 0.8 | square meters | $m^2$ |
| $mi^2$ | square miles | 2.6 | square kilometers | $km^2$ |
| | acres | 0.4 | hectares | ha |
| | | **MASS (weight)** | | |
| oz | ounces | 28 | grams | g |
| lb | pounds | 0.45 | kilograms | kg |
| | short tons (2000 lb) | 0.9 | tonnes | t |
| | | **VOLUME** | | |
| tsp | teaspoons | 5 | milliliters | ml |
| Tbsp | tablespoons | 15 | milliliters | ml |
| fl oz | fluid ounces | 30 | milliliters | ml |
| c | cups | 0.24 | liters | l |
| pt | pints | 0.47 | liters | l |
| qt | quarts | 0.95 | liters | l |
| gal | gallons | 3.8 | liters | l |
| $ft^3$ | cubic feet | 0.03 | cubic meters | $m^3$ |
| $yd^3$ | cubic yards | 0.76 | cubic meters | $m^3$ |
| | | **TEMPERATURE (exact)** | | |
| °F | Fahrenheit temperature | 5/9 (after subtracting 32) | Celsius temperature | °C |

*1 in = 2.54 (exactly). For other exact conversions and more detailed tables, see NBS Misc. Publ. 286, Units of Weights and Measures, Price $2.25, SD Catalog No. C13.10:286.




### Approximate Conversions from Metric Measures

| Symbol | When You Know | Multiply by | To Find | Symbol |
|---|---|---|---|---|
| | | **LENGTH** | | |
| mm | millimeters | 0.04 | inches | in |
| cm | centimeters | 0.4 | inches | in |
| m | meters | 3.3 | feet | ft |
| m | meters | 1.1 | yards | yd |
| km | kilometers | 0.6 | miles | mi |
| | | **AREA** | | |
| $cm^2$ | square centimeters | 0.16 | square inches | $in^2$ |
| $m^2$ | square meters | 1.2 | square yards | $yd^2$ |
| $km^2$ | square kilometers | 0.4 | square miles | $mi^2$ |
| ha | hectares (10,000 $m^2$) | 2.5 | acres | |
| | | **MASS (weight)** | | |
| g | grams | 0.035 | ounces | oz |
| kg | kilograms | 2.2 | pounds | lb |
| t | tonnes (1000 kg) | 1.1 | short tons | |
| | | **VOLUME** | | |
| ml | milliliters | 0.03 | fluid ounces | fl oz |
| l | liters | 2.1 | pints | pt |
| l | liters | 1.06 | quarts | qt |
| l | liters | 0.26 | gallons | gal |
| $m^3$ | cubic meters | 35 | cubic feet | $ft^3$ |
| $m^3$ | cubic meters | 1.3 | cubic yards | $yd^3$ |
| | | **TEMPERATURE (exact)** | | |
| °C | Celsius temperature | 9/5 (then add 32) | Fahrenheit temperature | °F |

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# INTRODUCTION

The objective of this study was to determine if a practical method could be developed for assessing the cleanup of a single spill of Arabian Light Stabilized crude oil in littoral sands and gravels by using benthic microorganisms as indicators. By "practical" is meant that trained Coast Guard personnel, such as Marine Science Technicians, using a microscope, stains and dyes, and a manual of techniques and indicator conditions, would be able to determine if and to what degree contamination by petroleum still existed after cleanup without conducting an extensive biological and/or a chemical survey of the area.

Most post-oil spill investigations in the littoral zone have concentrated on rocky habitats. Only a few sand beaches have been studied; hence, the effects of oil on the sand dwelling organisms, particularly the microorganisms, still remains somewhat a mystery. This lack of study is probably due to the difficulties of sampling beaches caused by the complex textural changes and the tremendous natural variability in the populations of sand beach organisms. These difficulties have often caused investigators to abandon their studies or to conclude that no effects were discernible. However, the recent work of Wormald[1] showed that some oil spills can have severe effects on these sheltered intertidal organisms.

Oil that is stranded on beaches at low tide tends to percolate into the sediments and thus it can come into direct contact with the sand dwelling biota. The entrapped oil may persist in the sediments for

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[1]Wormald, A. P., (1976), Effects of a spill of marine diesel oil on the meiofauna of a sandy beach at Picnic Bay, Hong Kong. Environmental Pollution, v. 11, p. 117-130.

extended periods of time (e.g., Smith[1], Blumer and Sass[2], Hayes *et al*[3]). Ottway[4] reported that different crude oils vary widely in their toxic effects on intertidal organisms and that the toxicity of different crude oils varies greatly with temperature.

To complicate matters further, intertidal sands are not all alike. They may vary greatly in their mineralogies, and Bader[5] has shown that minerals have different capacities for removing organic substances from solutions. Also, the autolytic and extracellular products of the

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[1] Smith, J., (ed.), (1968), *Torrey Canyon-pollution and marine life*. Report by the Plymouth Laboratory of the Marine Biological Association of the United Kingdom. Cambridge University Press, London, 196 p.

[2] Blumer, M., and Sass, J., (1972), The West Falmouth oil spill, data available in November, 1971. II. Chemistry. Technical Report of the Woods Hole Oceanographic Institution, no. 72-19.

[3] Hayes, M. O., Grundlach, E. R., and Perhac, R. M., (1976), Great Patagonian oil spill. Abstracts, Ann. Meeting, Amer. Assoc. Petrol. Geolog. -Soc. Econ. Paleont. & Mineralog., May 23-26, 1976, New Orleans La., P. 71.

[4] Ottway, S. (1973), The comparative toxicities of crude oils, p. 172-180 *in* E. B. Cowell (ed.), *The ecological effects of oil pollution on littoral communities*. Applied Science Publishers, Ltd., Essex, England, 250 p.

[5] Bader, R., (1962), Some experimental studies with organic compounds and minerals, p. 42-47 *in* N. Marshall (ed.), The environmental chemistry of marine sediments. Naragansett Marine Lab., Univ. Rhode Island, Occas. Publ. no. 1, 85 p.

members of the microorganism communities (e.g., Fogg[1], O'Colla[2]) can modify both the chemical and physical characteristics of the sedimentary microenvironments in which they dwell (e.g., Webb[3]) and these conditions, in turn, could effect both the penetration of a pollutant and an organism's susceptibility to it. In some sands, an extensive mucilaginous matrix has developed from these autolytic and extracellular products (Frankel and Mead[4]). Thus it is possible that the effects of the same type and quantity of spilled oil could be different even if climatological and hydrographic factors are similar. Hence, although there have been some laboratory studies of the uptake and the effects of oil on a few of the microorganisms that are known to dwell in sands (e.g., Nuzzi[5], Pulich et al[6]), it is not yet known how these data can be applied to actual pollution situations.

To determine the effects of crude oil pollution in natural situations, study plots were established in the littoral zone at three locations along the Connecticut coast. Crude oil was spilled on the sands at low tide and the study plots were monitored at approximately monthly intervals for up to 15 months.

---

[1] Fogg, G. E., (1962), Extracellular products, p. 475-489, in R. A. Lewin (ed.), Physiology and biochemistry of algae. Academic Press, New York and London, 929 p.

[2] O'Colla, P. S., (1962), Mucilages; p. 337-356, in R. A. Lewin (ed.), Physiology and biochemistry of algae. Academic Press, New York and London, 929 p.

[3] Webb, J. E., (1969), Biologically significant properties of submerged marine sands. Roy. Soc. London, Proc., ser. B, v. 174, p. 355-402.

[4] Frankel, L., and Mead, D. J., (1973), Mucilaginous matrix of some estuarine sands in Connecticut. Jour. Sed. Petrology, v. 43, p. 1090-1095.

[5] Nuzzi, R., (1973), Effects of water soluble extracts of oil on phytoplankton, p. 809-813, in Proceedings, Joint Conference on Prevention and Control of Oil Spills. American Petroleum Institute, Wash D.C.

[6] Pulich, W. M., Winters, K., and Van Baalen, C., (1974), The effects of a no. 2 fuel oil on the growth and photosynthesis of microalgae. Marine Biology, v. 28, p. 87-94.

## STUDY LOCATIONS

### Location of the Study Sites

The three sites used in this study were in properties owned by the State of Connecticut and administered by the Connecticut Department of Environmental Protection. They were in a) Harkness Memorial State Park - on the shore of Long Island Sound; b) Bluff Point Coastal Reserve - on the shore of the Poquonock River estuary; and c) Barn Island Wildlife Management Area - on the shore of Little Narragansett Bay (see Figure 1 for locations). The sediments at these localities are either sandy gravels or gravelly sands. Permission to utilize these areas for the study was obtained from Mr. Theodore B. Bampton, Deputy Commissioner for Conservation and Preservation.

These locations were chosen for two reasons. First, because they are areas that were investigated by the writer in earlier studies and, hence, there is a background of information on the natural seasonal variations in biota and environment. And second, because there are differences in factors such as mineralogy, the intensity of wave and current action, the extent to which the interstitial fluids drain from the sediments at low tide, the rate of ground water flow, and sediment stability. Overall, the sites chosen for the study are moderately representative of conditions that exist throughout New England and elsewhere in places where the sediments in the littoral zone are coarse grained.

At each site two study plots 1 x 2 meters were established. One plot was to be treated with crude oil and the other would be untreated and it would be the control with which any changes in biota or sediment character could be verified. These study plots were located in the lower part of the littoral zone in each locality. By "littoral" is meant the zone embraced between the average of the highest flood and the average of the lowest ebb tides of the month. They were positioned so that each plot would be completely exposed during each low tide cycle throughout the year, except for periods when adverse weather conditions produced higher than normal low tides.

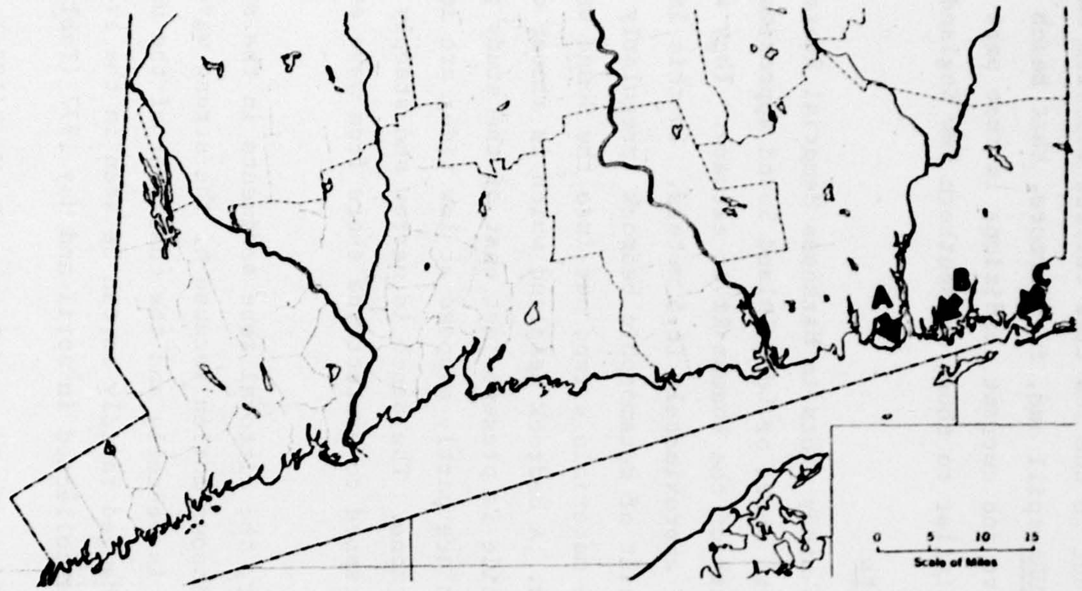


Figure 1. Study locations along the Connecticut shoreline: A-Harkness Memorial State Park; B-Bluff Point Coastal Reserve; C-Barn Island Wildlife Management Area.

This choice of position for the study plots was based on two considerations. First, previous studies in these locations by the writer indicated that they had a large and varied biota. And second, oil may remain in the lower part of the littoral zone longer than in other parts. Hayes et al[1] reported that oil was present in the low tide terrace deposits one year after the August, 1974 METULA spill and, furthermore, that beach morphology, sediment type and wave and current conditions in that part of Patagonia are strikingly similar to those in southern New England.

Harkness Memorial State Park

The oiled and unoiled study plots in Harkness Memorial State Park (Figure 2) were on the north shore of Long Island Sound approximately 2 kilometers west of the mouth of the Thames River estuary. They were separated by a distance of approximately 16.5 meters. In this locality the onshore materials consist of metamorphic bedrock irregularly mantled by bouldery till and these materials extend out into the Sound under a veneer of recent sediments. A bedrock headland which is almost completely covered by water at high tide is present just east of the study plots and many large boulders, which are partly exposed at low tide, are in the shallow water sublittoral zone. The beach is narrow and steeply sloping and its upper part is riprapped to protect the shore from wave erosion. (See figure 3.).

When covered by water, the littoral zone sediments in the study plots were in almost continuous motion because of the strong wave and current action. Beach drift is westerly and the character of the upper 6 centimeters of sediment changed rapidly as can be seen in the average size analyses[2] for samples collected in April and May 1977 (Table 1).

---

[1]Hayes, M. O., Grundlach, E. R., and Perhac, R. M., (1976), Great Patagonian oil spill. Abstracts, Ann. Meeting, Amer. Assoc, Petrol. Geolog. - Soc. Econ. Paleont. & Mineralog., May 23-26, 1976, New Orleans, La., p. 71.

[2]The Phi grade scale was used in these analyses. It is a logarithmic transformation of the Wentworth grade scale based on the negative logarithm to the base 2 of the particle diameter (Phi = $\log_2$ (m.m.)).

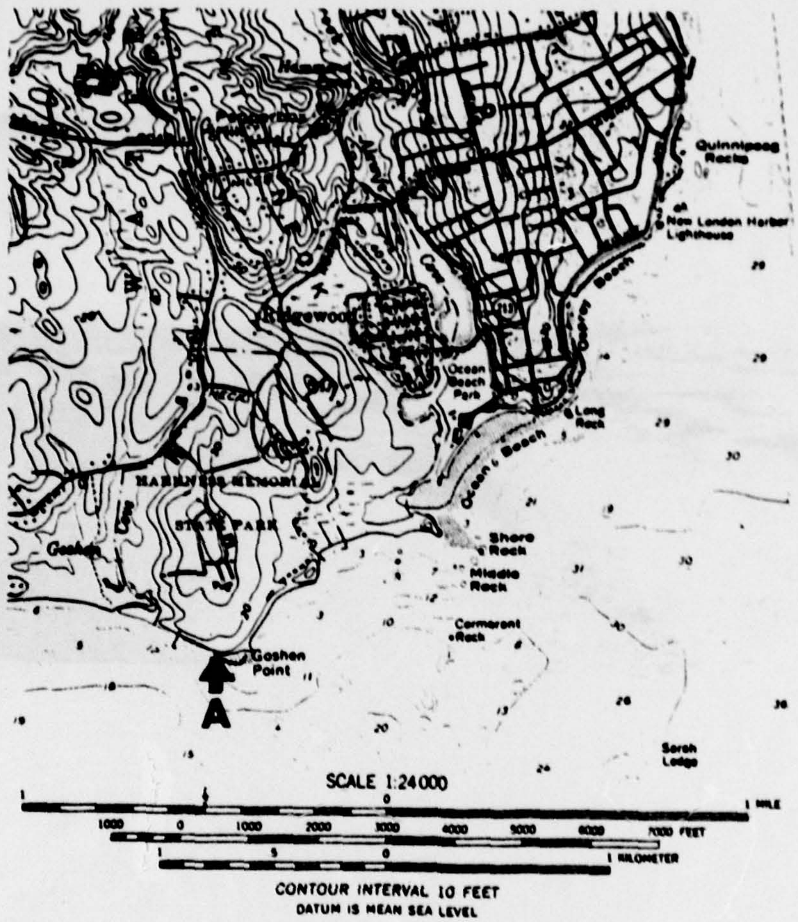


**Figure 2.** Location of the study plots in Harkness Memorial State Park (A).

Figure 3. Part of the beach in Harkness Memorial State Park (looking east). A bedrock headland is in the background. The oiled and unoiled study plots were located between the headland and the large boulder in the midground.

Table 1. Grain size distributions at Harkness Memorial State Park.

GRAIN SIZE DISTRIBUTIONS

PHI PERCENT[1]

| | $\phi$5% | $\phi$10% | $\phi$16% | $\phi$25% | $\phi$50% | $\phi$75% | $\phi$84% | $\phi$90% | $\phi$95% |
|---|---|---|---|---|---|---|---|---|---|
| April, 1977 | -3.30 | -2.65 | -2.10 | -1.40 | .10 | 1.40 | 1.70 | 1.90 | 2.15 |
| May, 1977 | -2.60 | -2.05 | -1.50 | - .90 | .55 | 1.50 | 1.75 | 2.00 | 2.25 |

[1] Phi percent is the calculated particle size at the stated weight percentage of the total sample.

Using Folk's[1] terminology, these sediments vary from gravelly sands or pebbly coarse sands to sandy gravels or sandy pebble gravels.

When the study began, the bouldery subbase in the littoral zone was covered by approximately 60 centimeters of sand and gravel and at low tide the interstitial water drained from these sediments to a depth of at least 25 centimeters. During a storm in mid-October 1977, almost all the sand and gravel on this beach was removed exposing the bouldery substratum it covered. The study of this area was terminated at that time.

Bluff Point Coastal Reserve

The study plots in the Bluff Point Coastal Reserve (Figure 4) were situated on a narrow and moderately steeply sloping beach on the east side of the Poquonock River estuary approximately 2.2 kilometers (via the river) north of Long Island Sound. (See figure 5.) The oiled and unoiled plots were separated by a distance of approximately 46.5 meters. In these localities the estuary is bordered by a low and relatively flat area of glacio-fluvial sands and gravels about 80 meters wide; beyond this is a till covered bedrock area that rises steeply to an elevation of approximately 38 meters.

During periods of normal high tides the study plots were covered by approximately 0.5 meters water. At low tide interstitial water usually drained from only the upper 10-12 centimeters of sediment despite the beach slope. The limited drainage is caused by the flow of large quantities of ground water from the east. During the winter, when the estuary was frozen, holes in the ice north and south of the study plots marked places where very large quantities of ground water issued from the sediments of the littoral zone.

---

[1] Folk, R. L., (1974), Petrology of sedimentary rocks. Hemphill Publishing Co., Austin, Texas, 182 p.

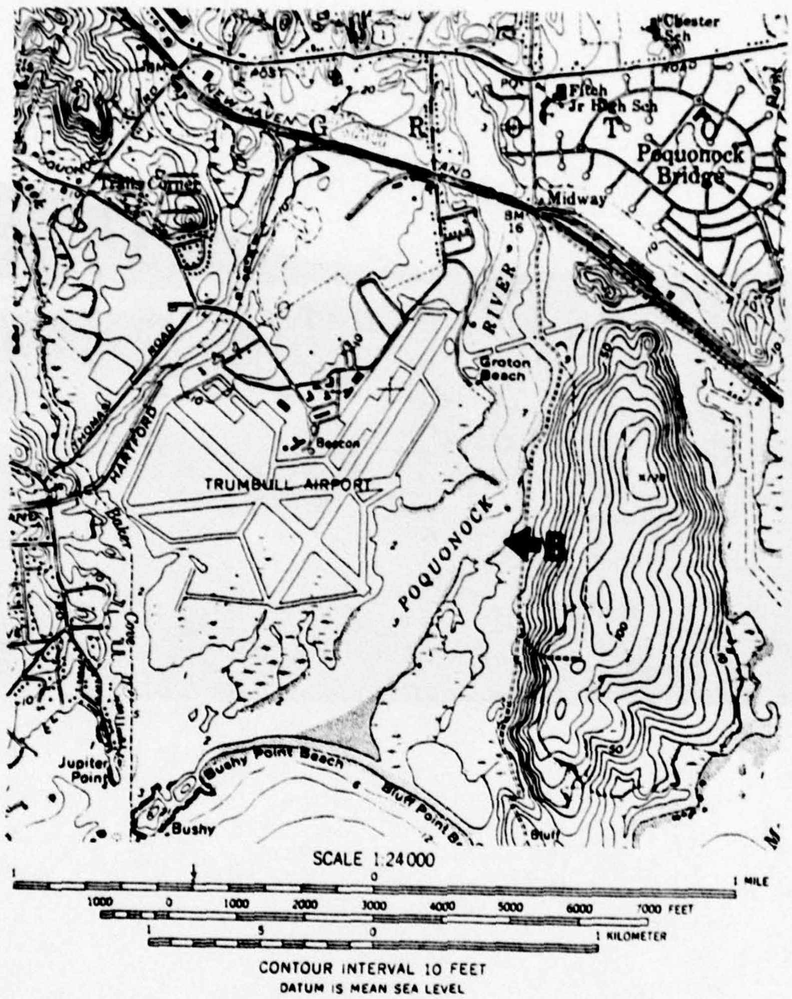


Figure 4. Location of the study plots in Bluff Point Coastal Reserve (B).

Figure 5. Part of the beach along the Poquonock River estuary in the Bluff Point Coastal Reserve (looking north). The oiled study plot was located in the foreground. The unoiled study plot was located in a similar setting approximately 46.5 meters south of this locality.

Table 2. Average grain size distributions at Bluff Point Coastal Reserve and Barn Island Wildlife Management Area.

| | AVERAGE GRAIN SIZE DISTRIBUTIONS | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | PHI PERCENT | | | | | | | | |
| | $\phi$5% | $\phi$10% | $\phi$16% | $\phi$25% | $\phi$50% | $\phi$75% | $\phi$84% | $\phi$90% | $\phi$95% |
| BLUFF POINT | -2.85 | -2.50 | -2.20 | -1.80 | - .50 | 1.60 | 1.90 | 2.20 | 2.50 |
| BARN ISLAND | -1.90 | -1.50 | -1.10 | - .65 | .90 | 2.10 | 2.30 | 2.50 | 2.80 |

Scattered pebbles over 2 centimeters in diameter were present in places on the surface of the oiled and unoiled study plots. These were removed before sediments for grain size analyses were obtained. The average grain size distribution of the sediments in the study plots are shown in Table 2. Using Folk's terminology, these sediments are sandy gravels or sandy pebble gravels.

From April to December, 1977, very little erosion or deposition was seen at the study plots. From January to March, 1978, the study plots were covered by ice. Between April and early May 1978, about 2.5 centimeters of sediment was deposited in places on the study plots. Significant subsequent erosion or deposition of sediment was not observed. Filamentous algae were present on parts of the sediment surface for the entire study period and from September through November, 1977, the algae developed into a mat that covered the entire surface of the study plots.

Barn Island Wildlife Management Area

In the Barn Island Wildlife Management Area (Figure 6) the oiled and unoiled study plots were separated by a distance of approximately 10.2 meters. Both plots were adjacent to a grass covered wave cut peat bank and wave eroded peat underlies the littoral sands. (See figure 7.) At normal high tides the study plots were covered by approximately 0.7 meter of water and the upper part of the wave cut edge of the peat bank was exposed; but during periods of extremely high tides the outer part of the peat bank was submerged.

At low tide the interstitial water usually drained from only the upper 7 to 10 centimeters of sediment. This happens because the littoral zone is narrow and it has an extremely low slope in the area of the study plots. However, when the tides were extremely low and there was a strong offshore wind up to 20 centimeters of drainage occurred.

The average grain size distribution (in phi sizes) of the sediments where the study plots were located are shown in Table 2. Using Folk's terminology, these sediments are gravelly sands or pebbly coarse sands. From April to November 1977 very little erosion or deposition of sediment was seen at the study plots. However, in December 1977 approximately 3 centimeters of sediment was deposited on both the oiled and unoiled plots. From January through March 1978 the study plots were covered by ice. In April and early May 1978 approximately 10 centimeters of sediment were deposited on both plots. Significant subsequent erosion or deposition of sediment was not observed.

Mineralogies at the Study Sites

The experimental work of R. G. Bader[1] showed that minerals have different capacities for removing from solutions organic compounds that are present in marine and estuarine waters. Because the Arabian Light Stabilized crude oil used in this study is a mixture of many different organic compounds, it is possible that some of them could have been preferentially retained by the sediments; and, if the mineralogies at the study sites were significantly different, it is also possible that retention of these organic compounds would not be the same and this could in part explain any observed differences in environmental effects due to the application of the crude oil to the sediments. For these reasons it was necessary to determine the mineralogies of the sediments. The methods used (described below) are basically those of Gabriel and Cox[2], and Hayes and Klugman[3].

---

[1]Bader, R., (1962), Some experimental studies with organic compounds and minerals, p. 42-47 in N. Marshall (ed.), The environmental chemistry of marine sediments. Naragansett Marine Lab., Univ. Rhode Island, Occas. Publ. no. 1, 85 p.

[2]Gabriel, A., and Cox, E. P., (1929), A staining method for the quantitative determination of certain rock minerals. Am Mineralogist, v. 14, p. 290-292.

[3]Hayes, J. R., and Klugman, M. A. (1959), Feldspar staining methods. Jour. Sedimentary Petrology, v. 29, p. 227-232.

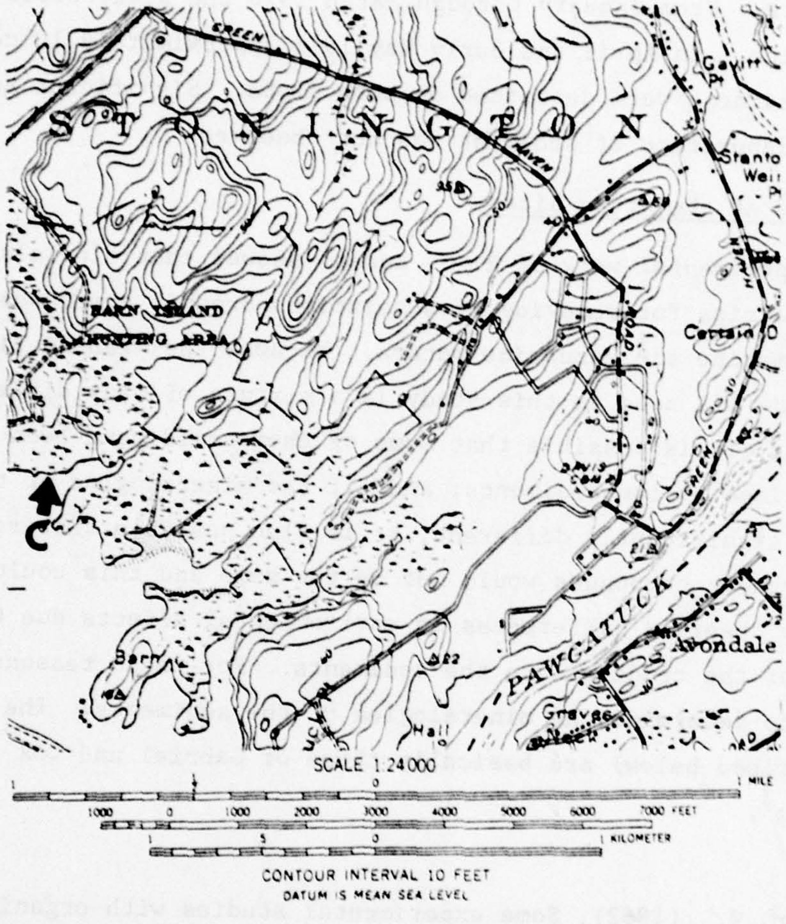


Figure 6. Location of the study plots in Barn Island Wildlife Management Area (C).

Figure 7. Site of the oiled study plot in the Barn Island Wildlife Management Area (looking west). The carrier used to transport the glass collecting tubes is on the grass-covered peat bank. The unoiled study plot is on the west side of the lobate prong of peat in the background.

To determine mineralogies, the sediment samples were first dried in an oven for 12 hours at 100 degrees centigrade. The dried samples were then shaken through a bank of sieves to separate the 1$\phi$ and .5$\phi$ grains from the others in the samples. These grains were gently boiled in a 30 percent hydrogen peroxide solution for 30 minutes to remove organic materials adhering to their surfaces. The samples were then washed with distilled water to remove the hydrogen peroxide and dried. The dried grains were sprinkled on glass slides that had one surface coated with a thin layer of Lakeside #70C Thermoplastic Cement. The slides were heated until the thermoplastic melted and then they were allowed to cool. These slides with mineral grains adhering to their surfaces were suspended in a plastic box that contained hydrofluoric acid and they were exposed to the hydrofluoric acid fumes for 15 minutes. They were then placed in a concentrated sodium cobaltinitrate solution (5 grams of sodium cobaltinitrate in 10 milliliters distilled water) for 3 minutes. The slides were washed with running tap water to remove the excess sodium cobaltinitrate and then they were placed in a .5 percent aqueous solution of eosine B for 5 minutes. The excess eosine B was washed off by running tap water and the slides were air dried.

As a result of these procedures, the dominant minerals in the samples, quartz and feldspars, could be easily distinguished: potash feldspars became orange-yellow, soda-lime feldspars became pink, and quartz remained clear.

The other minerals on the slides were identified by optical methods. The most abundant of these were muscovite, biotite, garnet, hornblende, and tourmaline. Because these and the other identified minerals constitute such a small percentage of the grains in the samples and because each sample contained about the same percentage of each mineral species they were grouped and recorded as "other minerals" in the following tabulations.

Multiple analysis of 1$\phi$ and .5$\phi$ grains from the three study localities show the following average mineral abundances.

A) Harkness Memorial State Park

Quartz 60%

Potash Feldspars 19%

Soda-lime Feldspars 17%

Other Minerals 4%

B) Bluff Point Coastal Reserve

Quartz 61%

Potash Feldspars 20%

Soda-Lime Feldspars 16%

Other Minerals 3%

C) Barn Island Wildlife Management Area

Quartz 58%

Potash Feldspars 29%

Soda-lime Feldspars 10%

Other Minerals 3%

These analyses indicate that the mineralogies of the sediments in the Harkness Memorial State Park and the Bluff Point Coastal Reserve study plots are very similar. For this reason it is postulated that retention of organic compounds from the crude oil by the minerals at these localities would be basically the same. However, because there are more potash feldspars and less soda-lime feldspars in the Barn Island Wildlife Management Area plots than those at the other study locations, it is possible that retention of organic compounds from the crude oil by the minerals was different here.

## OILING THE SEDIMENTS

The sample of Arabian Light Stabilized crude oil used in this study was shipped to the University of Connecticut via air freight from Dhahran, Saudi Arabia by ARAMCO. ARAMCO also provided the following data on its characteristics: API gravity 33.4; Conradson carbon residue 4.2% (wt.); Reid vapor pressure 3.8 psi; sediment and water 0%; sulfur 1.82% (wt.); hydrogen sulfide 40 ppm; vanadium, as $V_2O_5$, 20 ppm.

Approximately one week before the study plots were to be oiled, measured quantities of crude oil were mixed with an equal volume of filtered sea water. This mixture was placed in glass reagent bottles which were agitated by hand daily and were stored in the dark until they were used. Hence, the crude oil used in the study was essentially unweathered.

Measured amounts of the crude oil-sea water mixture were applied to the sediments when they were exposed at low tide by using a galvanized steel garden watering can equipped with a sprinkler head. A 1 x 1/2 meter steel stainless steel frame with 13 centimeter sides was used to mark the areas to be treated and to contain the mixture. As much of the mixture as could be absorbed by a 1 x 2 meter study plot, up to a maximum of 4500 milliliters (equivalent to .1125 ml crude oil/$cm^2$ of sediment surface), was applied. (See figure 8.)

The study plot in Harkness Memorial State Park was treated on 10 May 1977; 4500 milliliters of the crude oil-sea water mixture were absorbed by the sediment. The Barn Island Wildlife Management Area study plot was treated on 11 May 1977; approximately 3000 milliliters of the crude oil-sea water mixture was absorbed by the sediment (equivalent to .075 ml crude oil/$cm^2$ of sediment surface). The study plot in the Bluff Point Coastal Reserve was treated on 12 May 1977; approximately 3600 milliliters of the crude oil-sea water mixture were absorbed by the sediment (equivalent to .09 ml crude oil/$cm^2$ of sediment surface).

The presence of this oil in the sediments was determined by using the following methods on all samples tested. 1) Bausch & Lomb Spectronic 20 spectrophotometer; comparison of pure hexane with a 10 minute hexane-sediment extract at 340 and 350 mu. 2) Thin-layer chromatographic technique developed for the U. S. Coast Guard under contract by Mississippi State University.[1]

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[1] Brown, L. R., (1977), Development of a simple, rapid field technique for estimating oil concentrations in the sediments. Department of Transportation, U. S. Coast Guard, Report No. CG-D-27-77, 22 p.

Figure 8. The oiled study plot in the Bluff Point Coastal Reserve just after the application of the crude oil - seawater mixture.

## COLLECTING SAMPLES AND ENVIRONMENTAL DATA

Sediment core samples were collected from the oiled and unoiled plots in each study locality at approximately monthly intervals and processed into thin-sections which were examined to determine what, if any, changes in biota and sediment character occurred. To make the thin-sections the procedures described below were utilized. It is a modified version of the technique developed by Frankel.[1]

A 35 millimeter (inside diameter) glass tube approximately 20 centimeters long was pushed approximately 10 centimeters into the sediment. The upper end of the tube was tightly plugged with a rubber stopper and the tube was carefully withdrawn from the sediment. A glass wool plug was held at the bottom of the tube in contact with the sediment, the upper stopper was loosened, and the glass wool plug and the overlying sediment was pushed up the tube to a height that would permit the insertion of a bottom stopper. A plug of glass wool was then inserted above the sediment in the tube. A rose bengal-ethyl alcohol solution (5 grams rose bengal in 1 liter 95% ethyl alcohol) was poured into the collecting tube and the bottom stopper was loosened. As the interstitial water drained from the tube and the level of the rose bengal-alcohol solution lowered, more was added. The bottom stopper was tightened when a color change indicated that the stain solution had replaced the interstitial water. The samples were then transported to the laboratory.

The rose bengal stain was used as a presumptive indicator of the living organisms in the collected sediments. It was mixed with alcohol instead of water to kill or immobilize the organisms quickly and thus prevent their migration within the sediment. The bottom glass wool plug was required to prevent the downward movement of sediment when the stopper was loosened to drain off liquid and the upper plug minimized the disturbance of the sediment surface as liquids were added to the tube. A glass collecting tube was used because propylene oxide, a chemical used in the processing of the core, reacts adversely with plastic.

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[1]Frankel, L., (1970), A technique for investigating microorganism associations. Jour. Paleontology, v. 44, 575-577.

In the laboratory the rose bengal-ethyl alcohol solution in the collecting tube was drained and 95% ethyl alcohol was added and drained until the liquid issuing from the bottom of the tube no longer had a rose color. This was done to prevent excessive staining and to continue the dehydration of the tissues of the organisms in the sediment in preparation for their infiltration by the epoxy resin mixture.

The samples remained in alcohol until sufficient time was found to complete the processing. This varied from a few days to a week. The alcohol was then drained from the collecting tube by loosening the bottom stopper. As this was happening, propylene oxide was added to the tube. The alcohol had to be removed from the intergrain spaces and the tissues because it would inhibit the polymerization of the epoxy resin; propylene oxide mixes with the epoxy resin. To do this, a volume of propylene oxide approximately 3 times that of the sediment in the collecting tube was run through the sediment. Then the tube was refilled with propylene oxide and the bottom stopper was reinserted. The sediment soaked in propylene oxide for at least 3 hours before the epoxy resin mixture was added.

First the upper glass wool plug was removed from the collecting tube. Then, the solid bottom stopper was removed and replaced by a one-hole stopper. When the level of propylene oxide fell to the surface of the sediment, the epoxy resin mixture was added to the collecting tube. The epoxy resin mixture used was composed of 159 milliliters dodecenyl succinic anhydride (DDSA), 88 milliliters nadic methyl anhyride (NMA), 200 milliliters Epon 812 and 7 milliliters dimethyl amino methyl phenol (DMP-30).

The sediment in the collecting tube was infiltrated by and soaked in the epoxy resin mixture for at least 12 hours. The collecting tube was then placed in an oven at 60 degrees Centigrade and cured for 72 hours.

The processing described above preserves the sedimentary features and stains and preserves the cytoplasm and nuclear materials of the interstitial organisms without the shrinkage common in epoxy embedments.

The upper 6 centimeters of the sediments in the polymerized cores were cut into vertically oriented slabs with a diamond saw and these were mounted on glass slides and ground into thin-sections approximately 120 microns thick.

In addition to the thin-sections, untreated sediment samples were also collected for study of the biota and, when possible, the following environmental data were obtained for each site when sediments were collected: air, water, and sediment temperatures; interstitial salinity, pH, dissolved oxygen content, hydrogen sulfide content; and carbon content of the sediment. These data are recorded in Tables 3-12.

The methods and/or instruments used to obtain these data are as follows:

Carbon Content: Weight loss on ignition at 650°C for two hours.

Salinity: American Optical Co. model 10419 Goldberg temperature compensated refractometer calibrated with Copenhagen water.

pH: Hach model 17-F Brom Thymol Blue test kit with dechlorinating solution.

Dissolved Oxygen: Hach model OX-2P dissolved oxygen test kit.

Hydrogen Sulfide: Hach model HS-6 hydrogen sulfide test kit; upper limit 3 mg/l.

## OBSERVATIONS

### Absorption and Retention of Crude Oil

The three investigated areas differed not only in the amount of crude oil that was absorbed in the study plots, but also in the manner in which it penetrated into the sediments. At Harkness Memorial State Park the oil was rapidly and seemingly evenly absorbed into the sediments over the 2 meter study plot. However, in the study plots in the Barn Island Wildlife Management Area and the Bluff Point Coastal

Reserve the oil rapidly sunk into the sediments in some places and in other places it ran over the surface or was only very slowly absorbed from puddles. (See figure 8.) As a result of this the crude oil was unevenly distributed in the sediments of the study plots in these areas.

This investigation also showed that the sediments differed greatly in their ability to retain the absorbed crude oil. This was seen following the oilings of the study plots and in subsequent spills of oil made to determine why this happens. Some of the oil absorbed by the sediments when they were exposed at low tide was expelled from them as the tide rose. The cause of this appears to be the buoying up of oil globules by the water filling the interstitial spaces. How much of the oil was expelled and how rapidly this happened apparently depended primarily on the amount and distribution in the sands of mucilaginous materials that are the secretive and autolytic products of the microorganisms. This mucilaginous material stabilizes the sand by binding grains together, and by filling interstitial spaces it apparently acts as a barrier to the upward migration and escape of oil globules. The filling of interstices apparently also prevents the rapid penetration of oil into the sediments and this probably was the cause of the uneven absorption previously noted for the Barn Island Wildlife Management Area and the Bluff Point Coastal Reserve. In addition, the mucilaginous material apparently entraps the oil in other ways. This was demonstrated by Stainken's[1] study of #2 fuel oil accumulation by Mya arenaria. He found that many oil droplets and globules became bound in mucus secreted by the gills and that the mucus bound particles were either ingested or they were ejected by the clam's ciliary system. Similarly, in experiments with the ciliate Euplotes diadaleos at the

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[1]Stainken, D. M., (1975), Preliminary observations on the mode of accumulation of #2 fuel oil by the soft shell clam, Mya arenaria, p. 463-468 in Proceedings of Conference on Prevention and Control of Oil Pollution, San Francisco, California, March 25-27, 1975.

U.S. Coast Guard Research and Development Center at Groton, Connecticut, J. J. Lanier and M. Light[1,2] found that in the presence of Empire Mix crude oil *Euplotes* secretes mucus and that oil is bound to the mucus.

At Harkness Memorial State Park, where the sediments contain only a small amount of mucilaginous material, crude oil was rapidly absorbed and then rapidly removed from the loose littoral sands. No oil was detected in the samples collected 15 days after the oiling. At the Barn Island Wildlife Management Area where the sediments contain a moderate amount of mucilaginous material oil was detected in the samples collected up to 45 days after the oiling but not thereafter. At the Bluff Point Coastal Reserve where the sediments contain large amounts of mucilaginous materials oil was detected in the samples collected up to 61 days after the oiling. At this locality a mousse was present at the edge of the low tide zone 12 days after the oiling but it was not seen at subsequent collecting times.

## Environmental Parameters

The measurements of several characteristics of the sediments and the surface and interstitial waters were obtained when possible at the oiled and unoiled plots in each study location at approximately monthly intervals. These data are recorded in Tables 3 to 12. The only measurements that may be directly related to the application of crude oil to the sediments are the high hydrogen sulfide and low dissolved oxygen content of the interstitial waters of the oiled plot at the Barn Island Wildlife Management Area during the early part of the study. However, even these relationships are tenuous because these conditions were sometimes present in the locality during the late spring and summer for several years prior to the oiling. The

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[1] Lanier, J.J., and Light, M., (1978), Ciliates as bioindicators of oil pollution; Proceedings, Conference on Assessment of Ecological Impacts of Oil Spills, June 14-17, 1978, Keystone, Colorado; Amer. Inst. Biol. Sci.

[2] Lanier, J.J. and Light, M. (1978), Inquiry into the suitability of protozoa as biological indicators of pollution. Report No. CG-D-83-78. U.S. Coast Guard Office of Research and Development, Washington, D.C.

Table 3. Surface water characteristics at Harkness Memorial State Park 5-25-77 to 10-19-77.

| Date | Air Temperature °C | Water Temperature °C | pH | Salinity 0/00 | Dissolved Oxygen mg/1 |
|---|---|---|---|---|---|
| 5-25-77 | 18.9 | 21.1 | 7.8 | 32 | 10.5 |
| 6-21-77 | 23.3 | 16.7 | 7.6 | 30 | 10.5 |
| 7-13-77 | 22.2 | 19.4 | 7.8 | 31 | 8.5 |
| 8-10-77 | 28.9 | 22.2 | 7.8 | 31 | 7.7 |
| 9-9-77 | 20.0 | 18.9 | 7.9 | 31 | 8.0 |
| 10-19-77 | 11.1 | 11.7 | 7.2 | 17 | 7.0 |

Table 4. Sediment and interstitial water characteristics at Harkness Memorial State Park 5-25-77 to 10-19-77.

| Date | Salinity 0/00 O-U[a] | Dissolved Oxygen mg/1 O-U | pH O-U | Hydrogen Sulfide mg/1 O-U | Sediment Temperature °C O-U | Ignition Weight Loss % O-U |
|---|---|---|---|---|---|---|
| 5-25-77 | b | b | b | b | 17.2-17.2 | .36-1.11 |
| 6-21-77 | 30-30 | 4.0-4.0 | 7.0-7.2 | 0-0 | 19.4-19.4 | .52- .83 |
| 7-13-77 | 30-30 | 4.0-2.6 | 7.1-7.1 | 0-0 | 19.4-19.4 | 1.24- .76 |
| 8-10-77 | 30-30 | 2.9-2.0 | 7.3-7.1 | 0-0 | 21.7-21.7 | 1.14- .59 |
| 9-9-77 | 30-30 | 3.8-4.0 | 7.3-7.3 | 0-0 | 20.6-20.6 | 2.89- .49 |
| 10-19-77 | 24-22 | 1.4-2.4 | 6.9-7.0 | 0-0 | 12.2-12.2 | 1.35-1.44 |

a) O = oiled study plot; U = unoiled study plot

b) interstitial water not obtained

Table 5. Surface water characteristics at Bluff Point Coastal Reserve 5-24-77 to 12-5-77.

| Date | Air Temperature °C | Water Temperature °C | pH | Salinity 0/00 | Dissolved Oxygen mg/1 |
|---|---|---|---|---|---|
| 5-24-77 | 19.4 | 23.9 | 7.2 | 21.5 | 9.0 |
| 6-24-77 | 24.4 | 21.7 | 7.5 | 25.0 | 9.3 |
| 7-11-77 | 25.0 | 26.7 | 7.5 | 28.0 | 13.0 |
| 8-9-77 | 31.7 | 27.8 | 7.6 | 29.0 | 9.5 |
| 9-7-77 | 26.1 | 24.1 | 7.6 | 29.5 | 10.0 |
| 10-21-77 | 19.4 | 14.4 | 7.4 | 22.0 | 10.0 |
| 11-9-77 | 15.6 | 12.2 | 7.8 | 19.0 | 8.0 |
| 12-5-77 | 1.1 | 4.4 | 7.9 | 23.0 | 7.0 |

Table 6. Surface water characteristics at Bluff Point Coastal Reserve 1-4-78 to 7-27-78

| Date | Air Temperature °C | Water Temperature °C | pH | Salinity 0/00 | Dissolved Oxygen mg/l |
|---|---|---|---|---|---|
| 1-4-78 | -6.7 | a | a | a | a |
| 2-3-78 | -7.8 | a | a | a | a |
| 3-2-78 | -5.0 | a | a | a | a |
| 4-14-78 | 8.3 | 7.2 | 7.4 | 18.0 | 10.5 |
| 5-4-78 | 7.2 | 13.3 | 7.7 | 24.0 | 9.5 |
| 5-31-78 | 20.0 | 21.7 | 7.9 | 10.0 | 12.5 |
| 6-27-78 | 22.8 | 22.2 | 7.7 | 25.0 | 8.5 |
| 7-27-78 | 26.7 | 25.0 | 7.8 | 26.0 | 9.5 |

a) no surface water; sediment covered by ice

Table 7. Sediment and interstitial water characteristics at Bluff Point Coastal Reserve 5-24-77 to 12-5-77.

| Date | Salinity 0/00 O-U[a] | Dissolved Oxygen mg/l O-U | pH O-U | Hydrogen Sulfide mg/l O-U | Sediment Temperature oC O-U | Ignition Weight Loss % O-U |
|---|---|---|---|---|---|---|
| 5-24-77 | 2-6 | 7.5-4.5 | 5.5-6.6 | 0-0 | 23.3-23.3 | .64-.54 |
| 6-24-77 | 3-8 | 8.0-6.0 | 5.7-5.5 | 0-0 | 20.5-20.5 | .41-.58 |
| 7-11-77 | 15-12 | 5.4-4.0 | 6.8-6.3 | T-T[b] | 24.4-24.4 | .30-.67 |
| 8-9-77 | 25-20 | 5.0-2.5 | 6.9-6.8 | 0-0 | 26.1-26.1 | 1.18-1.53 |
| 9-7-77 | 18-24 | 5.2-3.9 | 6.8-6.8 | 0-0 | 22.7-22.7 | .84-1.20 |
| 10-21-77 | 5-8 | 3.5-3.4 | 6.8-6.5 | T-0 | 13.9-13.9 | .51-.53 |
| 11-9-77 | 4-4 | 7.0-7.0 | 6.9-6.9 | 0-0 | 12.2-12.2 | 1.10-.78 |
| 12-5-77 | 4-2 | 6.0-6.0 | 6.2-6.3 | 0-0 | 3.9-3.9 | .92-.97 |

a) O = oiled study plot; U = unoiled study plot

b) T = trace, not measurable

Table 8. Sediment and interstitial water characteristics at Bluff Point Coastal Reserve 1-4-78 to 7-27-78.

| Date | Salinity 0/00 0-U[a] | Dissolved Oxygen mg/l 0-U | pH 0-U | Hydrogen Sulfide mg/l 0-U | Sediment Temperature oC 0-U | Ignition Weight Loss % 0-U |
|---|---|---|---|---|---|---|
| 1-4-78[c] | b | b | b | b | 1.1-1.1 | .33-.53 |
| 2-3-78[c] | b | b | b | b | 0.6-0.6 | .75-.49 |
| 3-2-78[c] | 0-0 | 7.0-7.0 | 6.1-5.7 | 0-0 | 1.1-2.8 | .42-.43 |
| 4-14-78 | 14-4 | 8.5-8.5 | 6.8-6.8 | 0-0 | 7.8-7.2 | 1.30-.82 |
| 5-4-78 | 10-10 | 8.5-8.5 | 6.0-5.9 | 0-0 | 12.2-12.2 | .64-.52 |
| 5-31-78 | 1-1 | 6.5-6.5 | 6.7-6.8 | 0-0 | 20.0-19.4 | .75-.84 |
| 6-27-78 | 0-19 | 6.0-3.5 | 5.6-5.5 | 0-0 | 20.6-20.6 | .63-.74 |
| 7-27-78 | 1-12 | 7.5-4.5 | 6.4-6.5 | 0-0 | 24.4-25.0 | .73-.75 |

a) 0 = oiled study plot; U = unoiled study plot

b) interstitial water not obtained

c) sediment covered by ice

Table 9. Surface water characteristics at Barn Island Wildlife Management Area 5-26-77 to 12-7-77.

| Date | Air Temperature °C | Water Temperature °C | pH | Salinity 0/00 | Dissolved Oxygen mg/1 |
|---|---|---|---|---|---|
| 5-26-77 | 22.8 | 18.9 | 7.8 | 30.0 | 10.0 |
| 6-25-77 | 23.9 | 20.6 | 7.8 | 25.0 | 7.0 |
| 7-12-77 | 21.7 | 22.2 | 7.7 | 30.0 | 7.0 |
| 8-8-77 | 27.8 | 27.2 | 7.5 | 30.0 | 6.0 |
| 9-5-77 | 19.4 | 21.1 | 7.5 | 29.0 | 7.0 |
| 10-10-77 | 15.6 | 13.3 | 7.1 | 20.0 | 3.8 |
| 11-7-77 | 11.1 | 11.1 | 7.5 | 29.0 | 4.5 |
| 12-7-77 | -1.7 | 2.8 | 7.1 | 14.0 | 8.0 |

Table 10. Surface water characteristics at Barn Island Wildlife Management Area 1-5-78 to 7-28-78

| Date | Air Temperature $^oC$ | Water Temperature $^oC$ | pH | Salinity 0/00 | Dissolved Oxygen mg/1 |
|---|---|---|---|---|---|
| 1-5-78 | -0.6 | a | a | a | a |
| 2-2-78 | -6.7 | a | a | a | a |
| 3-3-78 | -3.9 | a | a | a | a |
| 4-4-78 | 3.9 | 5.0 | 7.2 | 13.0 | 14.0 |
| 5-2-78 | 11.7 | 10.6 | 7.7 | 27.0 | 8.5 |
| 5-30-78 | 22.2 | 20.6 | 6.9 | 14.0 | 7.5 |
| 6-28-78 | 30.6 | 26.1 | 7.6 | 22.0 | 7.5 |
| 7-28-78 | 20.6 | 23.3 | 7.4 | 28.0 | 6.5 |

a) no surface water; sediment covered by ice

Table 11. Sediment and interstitial water characteristics at Barn Island Wildlife Management Area 5-26-77 to 12-7-77.

| Date | Salinity 0/00 0-U[a] | Dissolved Oxygen mg/1 0-U | pH 0-U | Hydrogen Sulfide mg/1 0-U | Sediment Temperature °C 0-U | Ignition Weight Loss % 0-U |
|---|---|---|---|---|---|---|
| 5-26-77 | 20.25 | 0-2.5 | 6.8-6.8 | 2.0-0.5 | 18.9-18.9 | .64-.73 |
| 6-25-77 | 25-24 | 1.0-2.0 | 6.8-7.1 | 3+ -T[b] | 20.0-20.0 | .52-.40 |
| 7-12-77 | 28-28 | 1.6-2.4 | 6.9-6.9 | .30-.20 | 21.1-21.1 | .88-.56 |
| 8-8-77 | 30-30 | T-T | 7.0-7.1 | 3+ -1.0 | 26.1-26.1 | .57-.57 |
| 9-5-77 | c | c | c | c | 20.6-20.6 | .79-.73 |
| 10-10-77 | 25-25 | 1.0-2.4 | 6.9-6.9 | 1.5-T | 13.3-13.3 | .96-1.07 |
| 11-7-77 | c | c | c | c | 12.2-12.2 | 1.50-.88 |
| 12-7-77 | 25-24 | T-2.2 | 6.8-6.8 | 3+ -.20 | 3.9- 3.9 | .88-.69 |

a) 0 = oiled study plot; U = unoiled study plot

b) 3+ = greater than 3 mg/1; T = trace, not measurable

c) interstitial water not obtained

Table 12. Sediment and interstitial water characteristics at Barn Island Wildlife Management Area 1-5-78 to 7-28-78

| Date | Salinity 0/00 O-U[a] | Dissolved Oxygen mg/l O-U | pH O-U | Hydrogen Sulfide mg/l O-U | Sediment Temperature $^{o}C$ O-U | Ignition Weight Loss % O-U |
|---|---|---|---|---|---|---|
| 1-5-78[d] | c | c | c | c | 1.1-1.1 | .50-.52 |
| 2-2-78[d] | 20.12 | 1.4-4.4 | 6.9-7.0 | 0-0 | 0-0 | .50-.49 |
| 3-3-78[d] | 26-26 | 2.6-4.8 | 6.9-6.9 | T[b]-0 | .6-1.1 | .47-.56 |
| 4-4-78 | 18-18 | 5.0-6.0 | 6.9-6.9 | 0-0 | 4.4-5.0 | .51-.53 |
| 5-2-78 | 26-26 | 3.4-3.2 | 6.7-6.7 | 0-0 | 11.1-10.6 | .62-.62 |
| 5-30-78 | 18-20 | 2.8-3.0 | 7.0-7.1 | 0-0 | 18.9-18.9 | .69-.70 |
| 6-28-78 | 23-24 | 3.0-2.8 | 6.7-7.1 | T-0 | 23.3-23.9 | .54-.67 |
| 7-28-78 | 28-28 | 2.0-2.5 | 7.1-7.1 | 0-0 | 23.2-23.3 | .58-.65 |

a) O = oiled study plot; U = unoiled study plot

b) T = trace, not measurable

c) interstitial water not obtained

d) sediment covered by ice

variations in the other measurement cannot be related to the application of crude oil to the sediments. They are probably caused by the interaction of many factors which include the time of year and the tidal condition when samples were collected, the flow of groundwater and fresh water from rivers and streams, wave activity, etc.

Biota

To determine what effect the crude oil had on the biota of the littoral sediments 1010 thin-sections cut from epoxy-impregnated cores and 75 unimpregnated sediment samples from the oiled and unoiled study plots were examined and compared in an attempt to answer the following questions: 1) Do the sediments from the unoiled study plots contain organisms that are not present in those from the oiled plots and vice versa? 2) Where the same organisms are present in the oiled and unoiled plots, are their relative abundances the same and, in relation to the date of oiling, does this occur synchronously?

Several hundred different kinds of organsisms were seen in the thin-sections and the unimpregnated sediments examined in this study. The most abundant of there were algae belonging to the Fragilarinaea, Achnanthineae, Naviculineae, Surirellineae, Chroococcaceae, and Chamaesiphonaceae; Ciliophora, particularly those belonging to the Frontoniidae, Holophryidae, Amphileptidae, Oxytrichidae, Euplotidae, Vorticellidae, and Vaginicolidae; and Nematoda. Less abundant but prominent were Rotifera; protists belonging to the Euglenoidida, Dinoflagellida, Phytomonadida, Testacida, and Foraminiferida; Ostracoda, and Polychaeta. Organisms belonging to other taxomomic groups were not common.

No significant differences were seen in either the types of organisms present or their frequencies of occurrence in the oiled and unoiled study plots at Harkness Memorial State Park. The reason for this apparently is that the crude oil was retained by the sediments for only a very short time.

Although they were somewhat different in composition, the interstitial biotas at the Barn Island Wildlife Management Area and the Bluff

Point Coastal Reserve responded in a similar manner to the crude oil applied to the sediments. The collections which were made 12 days after the oiling at the Bluff Point Coastal Reserve and 15 days after the oiling at the Barn Island Wildlife Management Area showed that species diversity in the oiled and unoiled study plots were essentially the same but that, with a few exceptions that will be discussed later, there was a slightly reduced number of individuals in many of these species in the oiled plots. Collections made at the Bluff Point Coastal Reserve 43 days after the oiling and at the Barn Island Wildlife Management Area 45 days after the oiling showed approximately the same species diversity but a greater discrepancy in the number of individuals in the oiled and unoiled plots. In addition, there was considerably more mucilaginous materials and skeletal debris (e.g., empty diatom frustules, tests of foraminifera, etc.) in the oiled plots. These differences were still apparent at the Bluff Point Coastal Reserve 61 days after the oiling and at the Barn Island Wildlife Management Area 62 days after the oiling; however, at these times the differences between the numbers of individuals in the oiled and unoiled study plots were significantly reduced. No significant differences in either species diversity or the number of individuals in these species were seen in the samples collected from both areas 89 days after the oilings, and this condition continued for the remainder of the study.

Significant differences in the number of individuals in the oiled and unoiled plots were apparent for only three organisms. These are Trochammina ochracea (Williamson), a Foraminiferida (Figures 9 and 10); Cyphodaria ampulla (Ehrenberg), a Testacida (Figure 11); and Trachylomonas sp., a Euglenoidida (Figure 12).

Trochammina ochracea is a nearly cosmopolitan species that lives in marine and brackish waters. The usual size of adults is in the 100-200 micron range. They are basically sessile organisms that attach over depressions in mineral grains (Frankel)[1]. They are usually not dislodged

[1] Frankel, L., (1974), Observations and speculations on the habitat and habits of Trochammina ochracea (Williamson) in subsurface sediments. Jour. Paleontology, v. 48, p. 143-148.

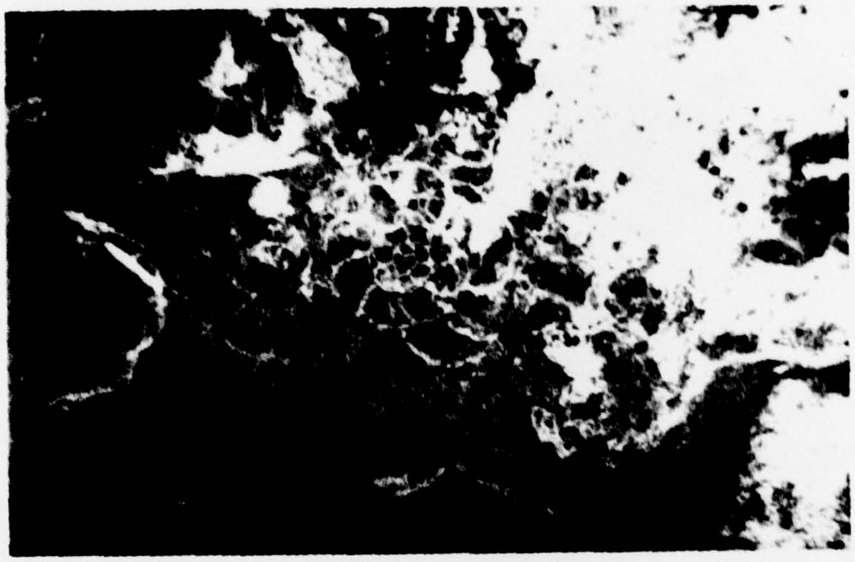

Figure 9. Thin-section photomicrograph of a large specimen of <u>Trochammina ochracea</u> (Williamson) attached to a pebble surface (130x).

Figure 10. Thin-section photomicrograph of Trochammina ochracea (cross-section) attached over a depression in a grain surface (225X).

Figure 11. Thin-section photomicrograph of Cyphodaria ampulla (Ehrenberg) attached to a grain (430X).

Figure 12. Thin-section photomicrograph of four specimens of Trachylomonas in an interstice (215X).

from the grains during the normal processing of samples and, hence, unless the grains are carefully examined their presence in a sediment sample is not usually noted. Even then, reliable estimates of their abundance in sediments are difficult to obtain if conventional sample preparation techniques are used because specimens commonly are hidden by algal overgrowths and the tests collapse on drying to amorphous masses.

Live Trochammina ochracea were seen in all the study areas. At Harkness Memorial State Park no differences in their abundance were noted in the oiled and unoiled study plots. However, at the Barn Island Wildlife Management Area and at the Bluff Point Coastal Reserve considerably fewer live specimens were seen in the samples collected from the oiled plots than those collected from the unoiled plots for the period up to 62 days after the oilings. Samples collected from the oiled and unoiled plots in both areas 89 days after the oilings has about equal numbers of live Trochammina ochracea and this condition continued for the remainder of the study.

Cyphodaria ampulla has a retort shaped test composed of pseudochitinous material that is covered by cemented siliceous scales arranged in diagonal rows. The length of the test ranges from about 60 to 200 microns. This species is almost universally described as an inhabitant of mosses and the oozes of freshwater ponds and lakes. However, large numbers of these organisms have been found at times in the intertidal and shallow water subtidal sands in some estuaries along the Connecticut shoreline. They are present in the intertidal sands of the study plots in the Barn Island Wildlife Management Area and the Bluff Point Coastal Reserve. Live individuals were considerably more abundant in the sediments of the unoiled plots than those of the oiled plots in both areas for up to 62 days after the oilings. In addition, during this time many of the live specimens in the oiled plots had encysted nuclei. No specimens with encysted nuclei were seen in the unoiled plots. In the collections made 89 days after the oilings about equal numbers of live Cyphodaria ampulla were seen in the oiled and unoiled study plots in both areas and this condition continued for the remainder of the study.

The specimens of Trachylomonas shown in Figure 12 have brownish, ovate, unormanted loricas about 28 microns long and 14 microns wide. Specimens of this organism were seen only in the Barn Island Wildlife Management Area. Live individuals were present in low frequencies in the unoiled study plot in the collections made up to 43 days after the oiling and none were seen in the sediments collected from this plot 61 days after the oiling and subsequently. Live specimens of this organism were about eight times more abundant in the sediments of the oiled plot than those from the unoiled plot for the period up to 45 days after the oiling. Live specimens were present in the oiled plot 62 days after the oiling but their numbers were reduced to about that seen in the unoiled plots earlier. No live specimens of this species were seen in the sediments collected from the oiled plot 89 days after the oiling and thereafter.

An examination of thin-sections made from sediment cores collected in this area for a few years prior to the start of this investigation did not reveal the presence of this organism at these times.

# CONCLUSIONS

The observations made in this field study and the conclusions drawn from them must be viewed in terms of many experimental limitations, among which are the following: 1) a specific type of oil, Arabian Light Stabilized crude, was used; 2) the oil was applied to the sediments in the late spring; 3) the oiled areas were of small size; and 4) the sediments were coarse grained. By modifying these conditions different results might have been obtained. For example, a different oil could have been more or less toxic to the organisms; the application of the oil at a different time could have more closely corresponded to crucial stages in life histories, such as reproduction, for some organisms, or other seasonal organisms, perhaps more susceptible to the oil, could have been present; a larger plot could have made it more difficult for some vagrant microorganisms to avoid the oil; a somewhat different biota would have been present if finer-grained sediments were utilized, and perhaps the oil would have been retained by these sediments for a longer time.

The results of this investigation suggest that it would not be practical to use microorganisms to assess the cleanup of coarse grained littoral sands after a "one time" spill of Arabian Light Stabilized crude oil. The conclusion is based on the following observations: 1) Species diversity was about the same in the oiled and unoiled study plots throughout the study. 2) No organism present in the unoiled plots was absent from the oiled plots and vice versa. 3) The number of individuals varied from place to place in the oiled and unoiled plots and, hence, unless a comprehensive study is made, the slight diminution in the number of individuals for most species that was seen in the oiled plots would be very difficult to detect. 4) The prospects of finding the organisms that were most affected by the crude oil, such as *Cyphodaria ampulla* and *Trochammina ochracea*, in random samples are very low either because they are not abundant or because they are difficult to detect in sediments.

The absorption and retention of crude oil by littoral zone sediments seems to be greatly influenced by the amount and distribution of contained mucilaginous materials that are mainly the secretive and autolytic products of the microorganisms living in the sediments. It is possible that a synthetic substance of similar composition applied to a beach just prior to its inundation by spilled oil could prevent the deep penetration of oil into the sediments and, hence, simplify cleanup operations.

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