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by

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ABSTRACT

Primary productivity, heterotrophic potential, chlorophyll *a* and phaeophytin, ATP, and direct bacterial counts were measured in the sea surface layer under controlled spills of La Rosa and Murban crude oils following immediate dispersal with the EXXON dispersant, Corexit 9527. Tests were conducted off New York on November 9, 1978.

The response of productivity to dispersed oil appeared as an initial increase in rates, a reduction to minimal rates 1-2 hours after dispersion, and a return to pre-spill rates after 3 hours. Comparison of productivity measurements from the two tests suggested a more efficient and rapid dispersion of the somewhat lighter La Rosa crude. Heterotrophic uptake at one meter below the La Rosa spill mirrored productivity results; missing samples at the Murban spill prohibited interpretation.

While oil and dispersant appeared to have little effect on chlorophyll-*a* and direct bacterial counts, ATP concentrations decreased to below detectable limits shortly after the application of the dispersant. Phaeophytin, expressed as a percentage of chlorophyll, varied inversely with ATP in both tests.

INTRODUCTION

Advantages and disadvantages of chemically dispersing spilled oil have resulted in considerable controversy among those governmental agencies and industries most directly concerned with protection of the marine environment. Demonstrated advantages listed by Canevar (1977) include (1) an increase in the rate of microbial degradation following dispersion of oil into the water column, (2) reduction in fire hazard and damage to waterfowl as oil is removed from the surface, (3) prevention of oil impingement on solid surfaces such as beach sand, shore structures and boats, and (4) prevention of the formation of tar residues. The primary dissension in the use of chemical dispersants has stemmed from the discovery that certain dispersant and oil mixtures are more toxic to marine life than is the oil itself (Hsiao et al., 1978).

Tests on the effects of oil and dispersant were conducted at sea, south of Long Island, on November 2, 3 and 9, 1978. These tests, sponsored by the American Petroleum Institute, utilized small, controlled spills of two crudes, Murban and La Rosa, and the Exxon dispersant, Corexit 9527. Experiments were designed to measure the effectiveness of immediate dispersal and dispersal delayed for two hours after a spill.

Biological measurements designed to test the effects of oils and dispersant on the lower trophic levels had the following objectives:

(1) To measure the effect of four separate oil spills on biomass, productivity and heterotrophic potential at specified depths below the spill.

(2) To measure acute toxicity of oil and dispersed oil to one or more species of zooplankton frequenting near-surface waters at the test site.

Success of the combined efforts of several ships and aircraft demanded close coordination and fair weather over a ten-day period.

This report presents results of biological observations at the test site on November 9, based on methodology rapidly amended after frequent weather delays and vessel breakdowns, and necessitated by use of a substitute vessel having a different mission within the sampling program. Tests with delayed addition of dispersant on November 2-3 were conducted in the absence of biologists, who were prevented from reaching the site by the first of two mechanical breakdowns on the biological vessel.

METHODS AND MATERIALS

The need to avoid a contaminated surface layer while making *in situ* measurements of toxicity and productivity and in sampling for biomass and heterotrophic potential was incorporated in our original sampling design through the use of a separate biological vessel. This vessel, after establishing control and test site buoys from which zooplankton cages and productivity vials were to be suspended at specific depths below the surface, was to tow cocked samplers below the surface to the test buoy after addition of oil and/or dispersant to the sea surface. Also, in a similar manner, the biological vessel was to retrieve and replace strings of suspended cages and vials during several hours of the test period. Water samples so obtained were also to be used for measurements of chlorophyll-*a*, phaeophytin, alkalinity, ATP, heterotrophic potential and direct microbial counts. Detailed methodology for these measurements was presented in the proposal (Grant, Kator and Zubkoff, August 1978) and will not be repeated here.

After the first mechanical failure of the biological vessel, D. T. Boyles of British Petroleum was transferred along with his sampling gear, to the chemistry vessel from which he successfully conducted experiments on all four test spills (Boyles, 1978). The initial two test spills (delayed addition of dispersant to Murban and La Rosa crudes) had both been completed before the second departure and breakdown of the biological vessel. The final failure of the biological vessel to reach the test site prompted a decision on which of the several biological measurements could still be performed on the final two tests, within the confinements of space left on the chemistry vessel and the portability (via commercial air travel) of

required equipment and supplies. Zooplankton studies were quickly ruled out because (1) equipment and supplies were too bulky for quick transport and (2) the number of scientists required would preclude making any of the other measurements as space for only three was available on the chemistry vessel and (3) the ability to tow cages into the test slick was lost.

In situ productivity measurements were also discounted and replaced by box incubation because the primary mission of the chemistry vessel precluded towing in situ sampling apparatus. Despite the obvious disadvantages of sampling through the JBF Corporation pumping system rather than with sterile bag samplers, three scientists were sent to join the chemistry vessel to measure box-incubated productivity, heterotrophic potential, chlorophyll-a, phaeophytin and ATP, and to make direct microbial counts. Methodology for these measurements was as follows:

Primary Productivity

Water samples of 20 ml were incubated in Pyrex^{R} test tubes with Teflon^{R}-lined screw caps after innoculation with 0.5 ml (2 µCi) of ^{14}C-$NaHCO_3$. Tubes for dark incubation were inserted into opaque polyethylene bags. Innoculated samples were incubated under constant light (four 40 watt fluorescent bulbs) and ambient water temperature for 4 hours, then fixed with approximately 0.1 ml of borate-buffered formalin (2%) and stored in the dark until laboratory processed (Biological Methods Panel, 1969). The ^{14}C-labelled particulate matter of each sample resulting from the incubation was trapped on a 25 mm 0.5 µm cellulose acetate membrane filter (Millipore^{R} EH) using a low vacuum of approximately

50 mm Hg. After filtration, the filters were placed in counting vials and 3.0 ml of Aquasol^{R} (New England Nuclear) added. The samples were then counted at 90% efficiency to 2% error or better using a liquid scintillation counter (Beckman LS-150). When necessary, an internal standard was added to determine efficiency and quenching. Computation of productivity (mg C $m^{-3}h^{-1}$) was then completed using the known radioactivity of the added ^{14}C-$NaHCO_3$ and the measured alkalinity.

Heterotrophic Potential (Vmax)

The heterotrophic potential (Vmax) was determined by incubating 20 ml aliquots (in triplicate) of the water sample with ^{3}H-glucose(U) with quantities of 0.41, 0.82, 2.058, and 4.12 µCi glucose l^{-1} in the dark at ambient temperature for 4 hours (Azum & Holm-Hansen, 1973). Approximately 0.1 ml of 2% neutralized formalin was added for the control sample (blank) or to terminate the reaction in test vials. The samples were stored in the dark until returned to the laboratory for further processing, as in the case of primary productivity samples, instead of being processed immediately aboard ship. The ^{3}H-labelled particulate matter was then collected on 0.5 µm cellulose acetate filters (Millipore^{R} EH) using a low vacuum of approximately 50 mm Hg. After filtration, filters were placed in counting vials and 3.0 ml of Aquasol^{R} (New England Nuclear) added. The samples were then counted at 34-35% efficiency to 2% error or better with a liquid scintillation counter (Beckman LS-150). When necessary, an internal standard was added to determine efficiency and quenching (Zubkoff and Warinner, 1977a, 1977b).

The rate of uptake of the substrate at any given concentration is calculated using the pseudo-first order equation of enzyme kinetics (Parsons and Strickland, 1962).

$$v = \frac{c(Sn + Sa)}{C\mu t} \qquad [1]$$

where

v = velocity of uptake of the substrate ($\mu g\ l^{-1}h^{-1}$)
c = counts taken up by heterotrophic population
Sn = natural substrate concentration
Sa = added substrate concentration
C = number of counts per μCi of substrate
μ = number of μCi added to incubation medium
t = time of incubation (hours)

When equation [1] is combined with a modified form of the Michaelis-Menten equation [2]:

$$\frac{(Sn + Sa)}{v} = \frac{K_t}{Vmax} + \frac{(Sn + Sa)}{Vmax}, \text{ where} \qquad [2]$$

K_t = transport constant
Vmax = maximum velocity of uptake at saturation of substrate

the resulting equation [3] is:

$$\frac{C\mu t}{c} \text{ or } \frac{(Sn + Sa)}{v} = \frac{(K_t + Sn)}{Vmax} + \frac{1}{Vmax}(Sa) \qquad [3]$$

When c is plotted against Sa, the result is a straight line with slope = $\frac{1}{Vmax}$. The value of Vmax, previously defined as the heterotrophic potential and the reciprocal of the slope, has units of μg glucose $l^{-1}h^{-1}$. Although several kinetic parameters may be calculated, Vmax is the most useful one for describing the relative functional distributions of the microbial populations in this study.

The kinetic data obtained are undoubtedly the result of heterogeneous assemblages of organisms which have active transport systems. Vmax is the rate of uptake observed at a substrate concentration high enough to completely saturate the transport mechanisms of the natural microbial populations under the experimental condition. In these studies, Vmax, glucose is an indicator which reflects the size

of the viable natural population of microbial organisms at the time of sampling; Vmax is an experimentally measured number which is the resultant of the endemic community's size, number, and state of viability as a function of temperature (Williams, 1973).

Chlorophyll-a and Phaeopigments

Chlorophyll a and phaeopigments were determined fluorometricly using a Turner Model 111 filter fluorometer (excitation filter = 5-60; emission filter = 2-64) according to Holm-Hansen et al. (1965). The fluorometer was previously calibrated using known solutions of chlorophyll-a and phaeophytin obtained from the Analtycial Quality Control Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268. One hundred ml of sea water sample was filtered using glass fibre filters (Whatman GF/A), immediately frozen on dry ice, and returned to the laboratory instead of being processed aboard ship. The filter was ground with 10.0 ml of 90% acetone, filter debris removed by centrifugation (approximately 2000 x G for 5 minutes), and the fluorescence measured with the fluorometer. A second reading was made after the extract was acidified with apprixmately 0.1 ml of 1 N HCl.

ATP Determination

At each surface station replicate 100 ml surface water samples were collected for determination of ATP concentrations. Water samples were immediately filtered through 0.45μ membrane filters using minimal suction. ATP was extracted in boiling Tris buffer after the method of Strickland and Parsons (1972). Extracts were frozen for storage prior to laboratory assay. Three replicate aliquots were assayed per sample with an ATP photometer (SAI Technology Corporation) adjusted for a one minute integration interval. Mean ATP concentrations were calculated using standard curves prepared at the time of assay.

Direct Bacterial Counts

Twenty ml volumes of seawater were collected in sterile test tubes from pump effluents at all depths and intervals sampled. Samples were fixed by addition of a 0.05% glutaraldehyde solution (1 ml/10 ml sample) and refrigerated prior to staining.

Direct counts of bacteria were made using the basic technique of Hobbie et al. (1977) with some variations. Nuclepore membranes (0.22μ, 25 mm dia.) were stained overnight in a 0.2% Irgalan black solution (in 2% acetic acid), rinsed, and stored in sterile distilled water. Stained Nuclepore membranes were placed over an ordinary 0.45μ membrane on a stainless steel frit in a microanalysis filtration unit. Ten ml of sample was placed in the microanalysis tower, 200 μl of a proflavin dye solution (0.033%) added, swirled, and stained for one minute before application of minimal suction. Residual dye was then washed through the membrane with 10 ml sterile seawater and the filter carefully removed under suction to facilitate water removal. The membrane was then placed over a drop of non-fluorescing immersion oil on a slide, a second drop placed on the filter followed by a coverslip applied with gentle pressure.

Bacterial cells were counted using a Zeiss Standard microscope equipped with an epifluorescence illuminator, a 100 W halogen lamp, a 450-490 nm excitation filter, an LP 520 barrier filter, and a FT 510 beam splitter. A reticle containing a grid subdivided into 49 squares, all of which were counted for a given sample, was employed. Seawater blanks were routinely processed as procedural controls.

Both of the remaining test spills, immediately dispersed with Corexit, were conducted on a single day (November 9) in advance of approaching adverse weather at the site. The number of measurements taken of the above parameters and at which depths is given in Table 1.

Table 1. Time, depth and number of biological observations at test spill site off New York, 9 November 1978 (N = number of replicates).

A. La Rose Crude, Immediate Dispersal (1015-1035 hrs)

Type of Measurement	Depth (meters)	Time (EST) 1005	1052	1125	1215	1320
		N				
Phytopigments	1	1	1	1	1	1
	3	1	1	1	1	1
	6	1	1	1	1	1
Productivity	1	3	3	3	3	3
	3	3	3	3	3	3
	6	3	3	3	3	3
Alkalinity	1	1	1	-	-	1
Heterotrophic Potential	1	4	4	4	-	4
ATP	1	2	2	2	2	2
Direct Counts	1	1	1	1	1	1
	3	1	1	1	1	1
	6	1	1	1	1	1

B. Murban Crude, Immediate Dispersal (1415-1430 hrs)

		Time (EST) 1400	1438	1507	1535	1610	1647
Phytopigments	1	1	1	1	1	1	1
	3	-	1	1	1	1	1
	6	1	1	1	1	1	1
Productivity	1	3	3	3	3	3	3
	3	-	3	3	3	3	3
	6	3	-	3	3	3	4
Alkalinity	1	1	1	1	-	1	1
Heterotrophic Potential	1	4	-	-	4	4	4
ATP	1	2	2	2	2	2	2
Direct Counts	1	1	1	1	1	1	1
	3	-	1	1	1	1	1
	6	1	1	1	1	1	1

RESULTS

The abbreviated and modified set of biological observations at the test site were chosen to provide a rapid first-order approximation of rate processes occurring in the microplankton (^{14}C-$NaHCO_3$ uptake by phytoplankton and ^{3}H-glucose uptake by the heterotrophic community), and comparative estimates of biomass (chlorophyll-a, ATP and microbial counts). However, the imposed necessity of sampling from the chemistry vessel (R/V Annandale) through the JBF Corporation pumping system removed the control and avoidance of surface contamination built into the original sampling design. Nevertheless, certain of our results were indicative of changes occurring during the early hours following a dispersed oil spill and they are presented below under (1) rate processes and (2) biomass estimates.

Rate Processes

Phytoplankton Productivity

Phytoplankton productivity was measured at three depths (1, 3 and 6 m) beneath each spill (Table 2). All measurements were low (mostly <1.0 µg C l^{-1} hr^{-1}), and increased to a maximum just after addition of the dispersant.

In the case of the La Rosa spill (Table 2, Fig. 1), the ^{14}C-$NaHCO_3$ productivity at all strata was highest immediately following the spill (1052 hrs) and reached minimal post-spill levels in the waters at 1125 or 1215 hrs; by 1320 hrs (3 hours post-spill), the levels of productivity had attained their prespill levels. Results indicate an immediate dispersal of La Rosa crude throughout the water column following addition of dispersant.

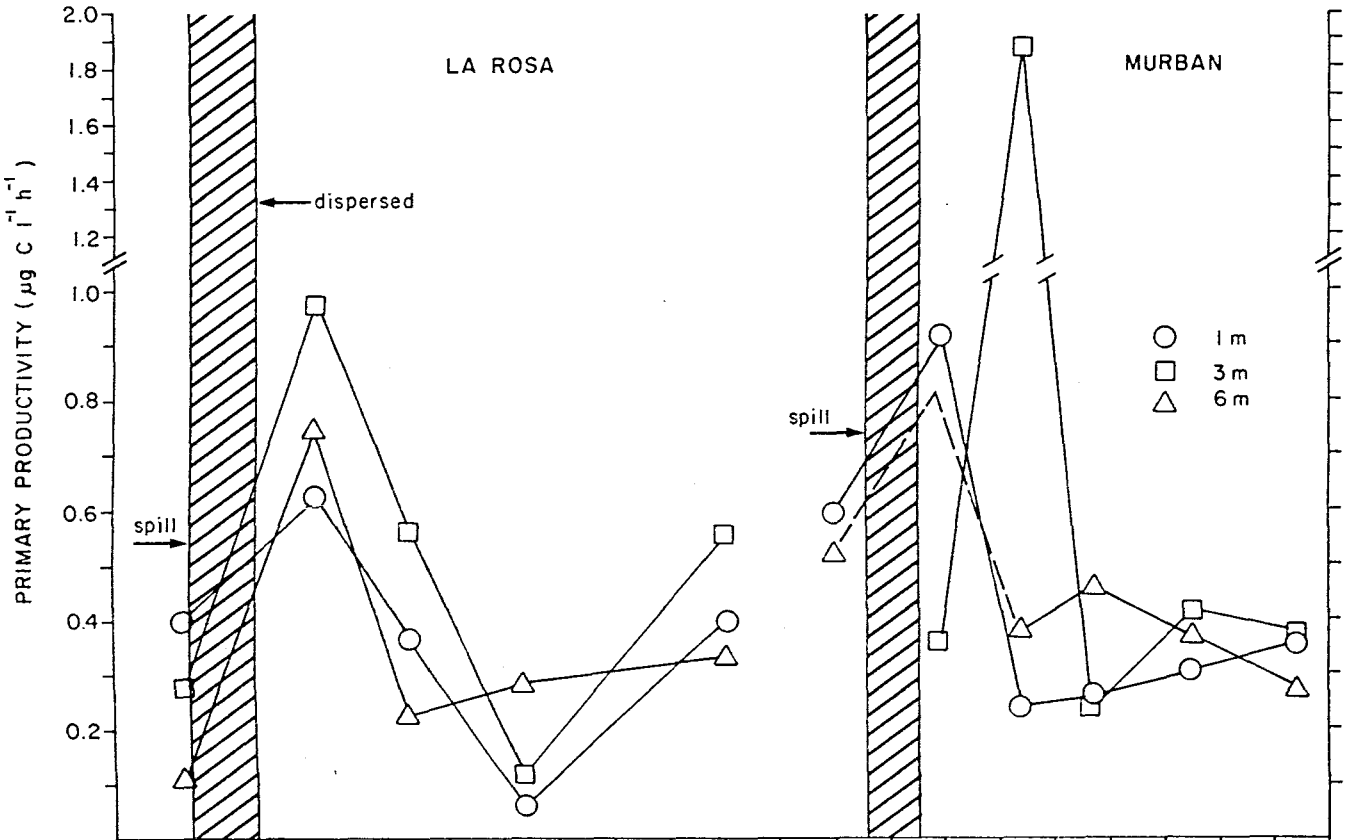

Fig. 1. Primary productivity (^{14}C-$NaHCO_3$ uptake) beneath experimental oil spills with immediate addition of the Exxon dispersant, Corexit 9527, 9 November 1978, off New York.

Table 2. Measurements of productivity ($\mu gC\ l^{-1}hr^{-1}$) and heterotrophic potential (pg glucose $l^{-1}hr^{-1}$) beneath dispersed spills of LaRosa and Murban crudes off New York, 9 Nov 1978.

LA ROSA

Depth (m)	Measurement	Pre-spill	Time (EST) Post-spill			
		1005	1052	1125	1215	1320
1	Productivity	0.40	0.63	0.37	0.06	0.40
	Heterotrophy	5.80	12.53	0.47	--	2.28
3	Productivity	0.28	0.98	0.57	0.12	0.56
6	Productivity	0.11	0.75	0.23	0.29	0.33

MURBAN

Depth (m)	Measurement	Pre-spill	Time (EST) Post-spill				
		1400	1438	1507	1535	1610	1647
1	Productivity	0.60	0.92	0.24	0.26	0.31	0.36
	Heterotrophy	0.89	---	--	0.12	0.63	0.27
3	Productivity	--	0.36	1.91	0.24	0.41	0.38
6	Productivity	0.52	--	0.38	0.46	0.37	0.27

In the Murban spill, ^{14}C-$NaHCO_3$ uptake was increased initially after the addition of dispersant at 1 m (1438 hrs) in comparison to the pre-spill control (1400 hrs); this initial increase was observed at 3 m at 1507 hrs. An initial increase after addition of dispersant was not observed in any of the 6 m samples. Unlike the lighter La Rosa crude, dispersion of Murban crude appeared to be slower and less effective, based on productivity data.

Heterotrophic Potential

The heterotrophic potential (Vmax) was measured at a single depth (1 m) beneath each spill (Table 2). All measurements were within two orders of magnitude (0.47-12.53 μg glucose l^{-1} h^{-1}).

The heterotrophic uptake reached its maximal value immediately following dispersion of the La Rosa spill, and its minimal value at 1115 hrs; as in the case of primary productivity, at 1320 (5 h post-spill), the level of heterotrophic activity at the 1 meter level had nearly attained the level of the pre-spill condition.

In the Murban spill, the heterotrophic potential was depressed after the addition of dispersant. Unfortunately, two samples were missed (1438 and 1507 hrs), thus obviating an adequate description of dispersant and oil effects.

Biomass Estimates

ATP

Whereas the dispersed spill experiments were completed in only a few hours, no decrease in actual biomass was expected. However, a sharp drop in ATP was evident in both spills (Table 3). Except for disagreement between replicates once in each series of experiments

Table 3. Concentration of ATP at one meter below the surface of experimental spills of LaRosa and Murban crude oils, with immediate addition of the dispersant Corexit 9527, 9 Nov. 1978.

Petroleum Type	Sample Time (EST)	Replicate No.	ATP (ng/liter)
LaRosa	1005 (pre-spill)	1	0.86
		2	0.83
	1015 (oil spill)		
	1035 (dispersant)		
	1052	1	0.73
		2	<0.20
	1125	1	<0.20
		2	<0.20
	1215	1	0.68
		2	0.64
	1320	1	<0.20
		2	<0.20
Murban	1400 (pre-spill)	1	0.62
		2	0.68
	1415 (oil spill)		
	1430 (dispersant)		
	1438	1	<0.20
		2	0.25
	1507	1	<0.20
		2	<0.20
	1535	1	<0.20
		2	0.66
	1610	1	<0.20
		2	<0.20
	1647	1	<0.20
		2	<0.20

(1052 hours, La Rosa and 1535 hours, Murban), where contamination in the pumping system is suspected, ATP levels dropped to below detectable limits immediately after addition of oil and dispersant.

In the La Rosa spill, the initial level of ATP (0.83-0.86 ng l^{-1}) was depressed after the addition of dispersant. At 1215 hrs, the level had returned to that of the control level, but was again depressed at the 1320 sampling.

In the Murban spill, initial levels of ATP (0.62-0.68 ng l^{-1}) were reduced to below detectable limits (<0.20 ng l^{-1}) immediately after dispersion and remained so throughout the test, except for disagreement between replicates at 1535 hrs (Table 3).

Other Biomass Measures

Oil and dispersant appeared to have little, if any, effect on chlorophyll-a and direct bacterial counts (Table 4), with measurements remaining low (chlorophyll-a less than 2.0 µg/liter) or relatively constant (with bacterial counts from 0.6 to 9.2 x 10^8 cells/liter) throughout the experiments. The amount of phaeophytin measured through the course of the experiments, when expressed as percentage of total chlorophyll, varied inversely with ATP (Fig. 2). ATP concentrations decreased shortly after the application of the dispersant Corexit 9527 to below the limit of detection (0.20 ng l^{-1}), while the percentage of phaeophytin in total chlorophyll increased to over 40% within one hour after the oil was dispersed. Return of both measurements to approximate control levels occurred once in each experiment (1215 hrs, La Rosa; 1535 hrs, Murban), suggesting that sampling was conducted apart from the actual location of dispersed oil. It would appear that toxic effects of oil and/or dispersant can be detected over such short periods in

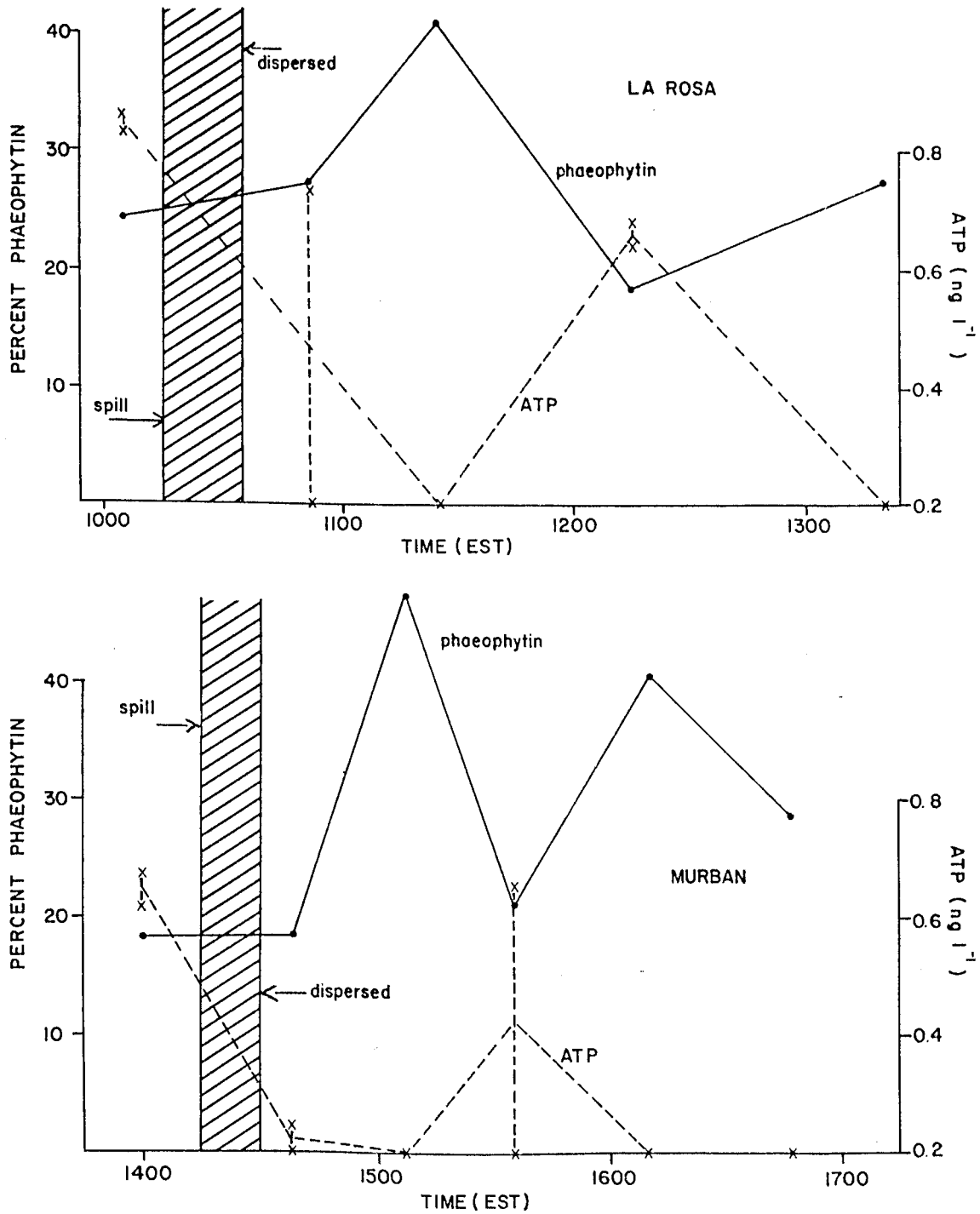

Fig. 2. Apparent inverse relationship of ATP measurements and those of phaeophytin (as percent of total chlorophyll) before and after immediately dispersed spills of LaRosa and Murban crudes. From samples taken one meter below the surface.

increases in pigment degradation products (or in disruption of energy storage mechanisms as measured by ATP analyses), but not in gross measures of biomass.

Table 4. Direct bacterial counts and concentrations of chlorophyll-a and phaeophytin at depths of 1, 3 and 6 meters off New York, 9 Nov 1978, during controlled, immediately-dispersed, spills of LaRosa and Murban crudes.

A. LaRosa (oil spill at 1015, dispersant at 1035 hrs EST)

Depth (m)	Biomass Indicator	Sample Time (EST) 1005*	1052	1125	1215	1320
1	Bacterial Counts (X $10^8 l^{-1}$)	3.4	4.7	5.4	6.8	5.5
	Chlorophyll-a (µg l^{-1})	1.52	1.64	1.52	1.52	1.52
	Phaeophytin (µg l^{-1})	0.37	0.45	0.62	0.28	0.42
3	Bacterial Counts	4.8	5.5	5.4	--	9.2
	Chlorophyll-a	1.47	1.64	1.52	1.64	1.58
	Phaeophytin	0.38	0.40	0.52	0.45	0.46
6	Bacterial Counts	4.6	5.0	6.4	4.1	6.5
	Chlorophyll-a	1.41	1.58	1.52	1.64	1.64
	Phaeophytin	0.34	0.61	0.42	0.30	0.40

B. Murban (oil spill at 1415, dispersant at 1430 hrs EST)

Depth (m)	Biomass Indicator	Sample Time (EST) 1400*	1438	1507	1535	1610	1647
1	Bacterial Counts (X $10^8 l^{-1}$)	5.5	3.9	--	6.1	--	4.9
	Chlorophyll-a (µg l^{-1})	1.64	1.76	1.82	1.52	1.52	1.70
	Phaeophytin (µg l^{-1})	0.30	0.33	0.86	0.32	0.62	0.49
3	Bacterial Counts	--	2.2	5.7	--	0.6	4.7
	Chlorophyll-a	--	1.76	1.94	1.68	1.58	1.88
	Phaeophytin	--	0.29	0.60	0.41	0.51	0.41
6	Bacterial Counts	4.6	2.6	4.9	--	5.1	--
	Chlorophyll-a	1.47	1.76	1.64	1.70	1.76	1.76
	Phaeophytin	0.38	0.48	0.65	0.33	0.24	0.43

* pre-spill control.

CONCLUSIONS

1. Productivity measurements at 1, 3 and 6 meters depth indicated a difference in the efficiency of Corexit 9527 in dispersing La Rosa and the somewhat heavier Murban crude oils. In the test using La Rosa crude, productivity was elevated at all depths immediately following addition of the dispersant, decreased to lowest levels after 1-2 hours, and returned to pre-spill rates 3 hours after the spill. Immediate changes in productivity following the Murban spill, on the other hand, were seen only at 1 meter, were somewhat delayed at 3 meters, and absent from the 6 meter depth.

2. Heterotrophic potential in the La Rosa test reached a maximal value immediately after the spill, with subsequent changes mirroring effects on productivity. Missing samples in the Murban test prevented conclusions as to trend.

3. Chlorophyll-a measurements remained low and quite constant throughout the experiments; direct bacterial counts also varied little during the test spills. Viable bacteria counts were not available due to the imposed changes in sampling techniques.

4. ATP decreased below detectable limits immediately after dispersion of both crudes. Conversion of average bacterial count data to ATP concentrations (one bacterial cell contains 2.5×10^{-10} µg ATP) yielded concentrations close to the minimum ATP value detectable for the volume filtered. This could be interpreted to mean that planktonic components in the water other than bacteria contributed the major portion of the ATP measured in pre-spill samples, and these components no longer contributed to particulate ATP following application of oil and dispersant.

5. The percent of phaeophytin in plant pigments increased sharply after addition of oils and dispersent, reflecting degradation of chlorophyll-a in phytoplankton cells. Over the time periods of the two tests, changes in phaeophytin percentage were inverse to those in ATP levels.

6. Reversal of trends in both ATP and phaeophytin data at one sampling period in each test suggests a separation of the sampling vessel from the actual slick area. This might be confirmed through results of hydrocarbon sampling (JBF Corp.).

7. Acute effects of oil and dispersant on zooplankton species residing in the sea surface layer could not be determined within restrictions imposed by sampling design changes, but should be included in any future studies of this type.

8. Certain reservations are mandatory with the above biological measurements. Departure from the original sampling design dictated the elimination of a control station that would have provided data on ambient conditions close to, but removed from, the actual test site. Control station data would have added information on population patchiness and onour implicit assumption that we were sampling a single endemic population at the test site, as well as have added validity to our conclusions that changes in biological measurements at the test site were, in fact, due to addition of oil and/or dispersent.

A most significant limitation in the design change was the substitution of a pumping system for sterile bag samplers. With any pumping system, there remains the possibility that measurements are being affected by contamination (sloughing of adsorbed cells or release of adsorbed chemicals).

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