

**ECOLOGICAL INVESTIGATIONS OF
PETROLEUM PRODUCTION PLATFORMS
IN THE CENTRAL GULF OF MEXICO**

VOLUME I—POLLUTANT FATE AND EFFECTS STUDIES

Part 4—Trace Metals Studies in Sediment and Fauna

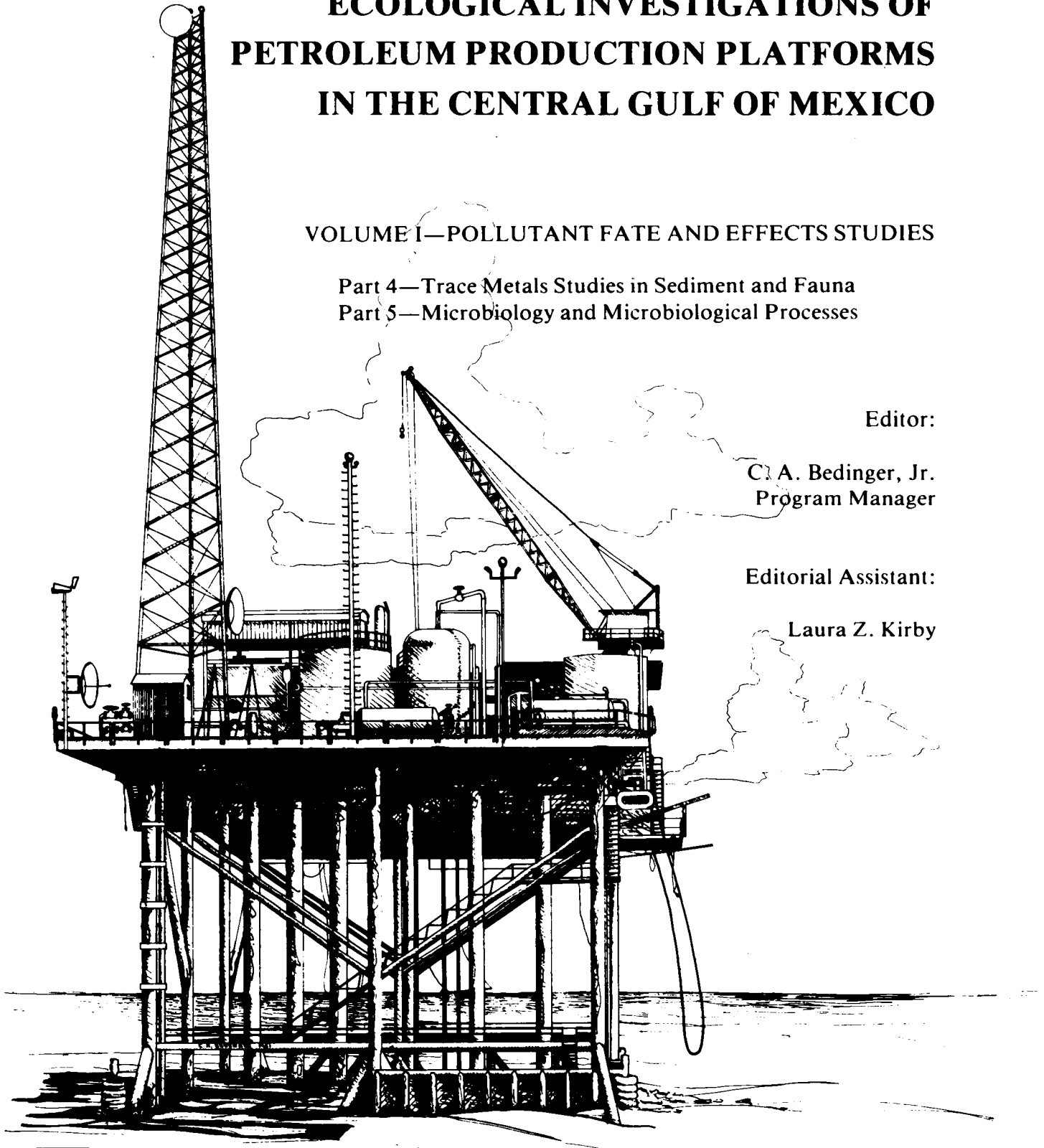
Part 5—Microbiology and Microbiological Processes

Editor:

C. A. Bedinger, Jr.
Program Manager

Editorial Assistant:

Laura Z. Kirby



SOUTHWEST RESEARCH INSTITUTE

SAN ANTONIO

HOUSTON

DISCLAIMER

“This report has been reviewed by the Bureau of Land Management and approved for publication. Approval does not signify that the contents necessarily reflect the view and policies of the Bureau, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.”

This report is not available from the sponsor. A limited number of printed copies are available from Southwest Research Institute, 2200 W. Loop South, Suite 690, Houston, Texas 77027, Attention: Dr. C.A. Bedinger, Jr. Photostatic copies will be available from the National Technical Information Service.

**ECOLOGICAL INVESTIGATIONS OF PETROLEUM
PRODUCTION PLATFORMS IN THE
CENTRAL GULF OF MEXICO**

Submitted to:

Bureau of Land Management, New Orleans OCS
Attention: Frances Sullivan
Hale Boggs Federal Building
500 Camp Street, Suite 841
New Orleans, Louisiana 70130

**COAR
COPY**

SwRI Project 01-5245

In fulfillment of Contract
AA551-CT8-17

by:

Southwest Research Institute
6220 Culebra Road
P.O. Drawer 28510
San Antonio, Texas 78284

1981

GUIDE TO USERS

This report is in six separate bindings:

- 1 VOLUME I —POLLUTANT FATE AND EFFECTS STUDIES
Part 1 —Background, Program Organization and Study Plan
Part 2 —Sediment Physical Characterization
Part 3 —Organic Chemical Analyses
- 2 VOLUME I —POLLUTANT FATE AND EFFECTS STUDIES
Part 4 —Trace Metals Studies in Sediment and Fauna
Part 5 —Microbiology and Microbiological Processes
- 3 VOLUME I —POLLUTANT FATE AND EFFECTS STUDIES
Part 6 —Benthic Biology
Part 7 —Normal Histology and Histopathology of Benthic Invertebrates and Demersal and Platform-Associated Pelagic Fishes
- 4 VOLUME I —POLLUTANT FATE AND EFFECTS STUDIES
Part 8 —Summary Data Set
- 5 VOLUME II —THE ARTIFICIAL REEF STUDIES
- 6 VOLUME III—EXECUTIVE SUMMARY

CONTENTS OF THIS BINDING

VOLUME I —POLLUTANT FATE AND EFFECT STUDIES	
Part 4 —Trace Metals Studies in Sediment and Fauna	1
Part 5 —Microbiology and Microbiological Processes	123

VOLUME I—POLLUTANT FATE AND EFFECTS STUDIES
Part 4—Trace Metals Studies in Sediment and Fauna

by

John B. Tillery
Principal Investigator
Trace Metals

Herbert L. Windon
Consultant
Trace Metals

Richard E. Thomas
Data Synthesis
Trace Metals

Southwest Research Institute
Department of Environmental Sciences
Division of Chemistry and
Chemical Engineering
P. O. Drawer 28510
San Antonio, Texas 78284

Skidaway Institute of Oceanography
Savannah, Georgia

Editor: C. A. Bedinger, Jr.
Editorial Assistant: Laura Z. Kirby

TABLE OF CONTENTS

	Page
I. INTRODUCTION	1
A. Objectives of the Study	1
B. Literature Review	1
C. Study Area and Sampling Sites	2
II. MATERIALS AND METHODS	5
A. Sample Collection and Preparation	5
1. Sediments	5
a. Surficial Sediments	5
(1) Partial Digestion	5
(2) Total Digestion	5
b. Downcore Sediments	5
(1) Trace Metal Analysis	5
(2) Downcore Dating	5
2. Biota	6
a. Collection	6
b. Initial Preparation and Digestion	6
(1) Pelagic Fish	6
(2) Macroepifauna and Demersal Fish	6
c. Preparation for Neutron Activation Analysis	7
(1) Barium	7
(2) Vanadium	7
(3) Hydrated Antimony Pentoxide (HAP) Column Preparation	7
B. Instrumentation	7
1. Atomic Absorption Spectrophotometry	7
2. Neutron Activation Analysis	7
3. Lead-210	7
C. Sample Analyses and Quality Control	7
1. Sediments	7
2. Biota	9
III. RESULTS	11
A. Sediments	11
B. Biota	11
IV. DISCUSSION	21
A. Sediments	21
1. Surficial Sediments	21
a. Physical Influence of Platforms on Sediment Metal Concentrations	21
b. Percent Clay Fraction Transformation	21
c. Leachable Iron Transformation	21
(1) Barium/Iron Ratios	22
(2) Cadmium/Iron Ratios	22
(3) Chromium/Iron Ratios	22
(4) Copper/Iron Ratios	22
(5) Lead/Iron Ratios	22
(6) Nickel/Iron Ratios	22
(7) Vanadium/Iron Ratios	22
(8) Zinc/Iron Ratios	22
2. Downcore Sediments	23
a. Geochronological Dating by Lead-210 Method	23
b. Trace Metals Analyses	23
B. Biota	23
1. <i>Archosargus probatocephalus</i>	24
2. <i>Chaetodipterus faber</i>	24
3. <i>Micropogon undulatus</i>	24
4. <i>Lutjanus campechanus</i>	24
5. <i>Penaeus aztecus</i> and <i>Penaeus setiferus</i>	25
C. Evaluation of Species for Monitoring of Trace Metal Pollution	25
D. Data Synthesis	25
1. Task 1: Correlation of Trace Metal Concentrations in Sediments and Biological Samples with Proximity to and Age of Platforms	25
2. Task 2: Correlation of Trace Metal Concentrations in Downcore Sediments with Proximity to and Age of Platforms	28

TABLE OF CONTENTS (cont'd)

	Page
3. <i>Task 2a: Correlation of Trace Metals in Downcore Sediments with Historical Development of the Study Area</i>	28
4. <i>Task 3: Determination of Impacts of Known Spills, Discharges and Other Sources of Petroleum Activity-Related Contaminants</i>	28
a. <i>Known Spills</i>	28
b. <i>Discharges</i>	29
c. <i>Other Sources of Trace Metal Contaminants</i>	29
5. <i>Task 4: Impact of Seafood Containing Elevated Trace Metal Concentration</i>	29
E. <i>Recommendations for Further Studies</i>	30
V. CONCLUSIONS	31
VI. ACKNOWLEDGEMENTS	33
VII. LITERATURE CITED	35
VIII. PERSONAL COMMUNICATIONS	37
APPENDICES	
APPENDIX A—Abbreviations used in this work	41
APPENDIX B—Metal Concentrations in Surficial Sediments by Platform and Station in $\mu\text{g/g}$ Dry Weight	45
APPENDIX C1—Graph of Metal Concentration vs. Fe Concentration for 5N HNO_3 Leach of Surficial Sediments	59
APPENDIX C2—Metal Concentrations vs Fe Concentrations from 5N HNO_3 Leach of Surficial Sediments by Platform and Station	69
APPENDIX D—Metal Concentrations vs Percent Clay Content of Surficial Sediments	79
APPENDIX E—Metal Concentration vs Hydrocarbon Concentration in Surficial Sediments by Platform and Station	91
APPENDIX F1—Concentration of Trace Metals in Downcore Sediments.....	103
APPENDIX F2—Concentration of Trace Metals in Downcore Sediments.....	107
APPENDIX G—Downcore Sediment Pb-210 Data from Control Site 22	119

LIST OF FIGURES

Figure	Page
1. Maps of the study area—(Top)Location of study area (Bottom) Study area showing sampling sites	3

LIST OF TABLES

Table	Page
1. Instrument parameters—AAS	8
2. Trace concentrations and percent recovery in NBS SRM 1645 River Sediment ($\mu\text{g/g}$ dry wt).....	9
3. Trace concentrations and percent recovery in NBS SRM 1577 Bovine Liver (mg/g dry wt).....	9
4. Comparison of the mean trace metal concentrations ($\mu\text{g/g}$ dry wt) in surficial sediments with other Gulf of Mexico studies.....	11
5. Biota samples collected for trace metal analysis	12
6. Biota samples common to other Gulf of Mexico marine environmental studies	13
7. Comparison of trace metal concentrations ($\mu\text{g/g}$ dry wt) in <i>Archosargus probatocephalus</i> (sheepshead) organs with other Gulf of Mexico studies	14
8. Comparison of average trace metal concentrations ($\mu\text{g/g}$ dry wt) in <i>Chaetodipterus faber</i> (spadefish) organs with other Gulf of Mexico studies	15
9. Comparison of average trace metal concentrations ($\mu\text{g/g}$ dry wt) in <i>Micropogon undulatus</i> (Croaker) organs with other Gulf of Mexico studies	16
10. Comparison of average trace metal concentrations ($\mu\text{g/g}$ dry wt) in <i>Lutjanus campechanus</i> (Red Snapper) organs with other Gulf of Mexico studies	17
11. Comparison of average trace metal concentrations ($\mu\text{g/g}$ dry wt) in <i>Penaeus aztecus</i> (brown shrimp) organs with other Gulf of Mexico studies	18
12. Comparison of average trace metal concentrations ($\mu\text{g/g}$ dry wt) in <i>Penaeus setiferus</i> (white shrimp) organs with other Gulf of Mexico studies	19
13. Number of individuals which must be analyzed to detect a given percentage difference in <i>Penaeus aztecus</i> muscle tissue.....	26
14. Number of individuals which must be analyzed to detect a given percentage difference in <i>Micropogon undulatus</i> muscle tissue	26
15. Chart	27
16. Chart	28
17. Chart	29

ABSTRACT

In 1978 and 1979, a multidisciplinary study of the effects of offshore petroleum production platforms on the marine environment was funded through the Bureau of Land Management and done by Southwest Research Institute. Twenty platforms in the Gulf of Mexico offshore Louisiana and west of the Mississippi River delta were studied. Four Primary Platforms and four Control Sites were visited in each of three seasons; May 1978 (Cruise I), August-September 1978 (Cruise II), and January 1979 (Cruise III), and 16 Secondary Platforms were sampled during Cruise II. Trace metal research examined surficial sediments, downcore sediments and selected biological samples collected up to 2000 m from these platforms. Concentrations of Cd, Cr, Cu, Fe, Ni, Pb, and Zn were determined in these samples by atomic absorption spectrophotometry; Ba and V were determined by neutron activation analysis.

Surficial sediment trace metal concentrations did not show strong evidence of contamination from the platforms. However, at 100 m of some platforms there were elevated concentrations of metals (Ba, Cr, Cu, Pb, and Zn) that were not related to natural geochemical processes. These elevated concentrations of metals were not correlated with the age of the platforms, the quantity of petroleum production, or the number of wells on the platforms. Sediment flow from the Mississippi River is thought to "mask" any trace metal sediment concentrations around the platforms.

Unsuccessful attempts to determine the chronological age of downcore sediments by Pb-210 dating techniques are thought to be due to one or more of the following:

- (1) excessive levels of Ra-226 supported Pb-210
- (2) high sedimentation rates
- (3) sediment reworking
- (4) the possibility of sediment mixing during sample collection (piston coring).

Downcore sediment trace metal concentrations were relatively constant with depth. However, concentrations of Ba, Cd, and Zn showed an increase with depth.

Concentrations of Cr, Cu, Fe, and Ni in sheepshead (*Archosargus probatocephalus*), spadefish (*Chaetodipterus faber*), and red snapper (*Lutjanus campechanus*) associated with the platform structures suggest a relationship with surficial sediment concentrations. No evidence of bioaccumulation was observed.

I. INTRODUCTION

A. Objectives of the Study

The Outer Continental Shelf (OCS) Environmental Studies Program of the Bureau of Land Management (BLM) was initiated in 1973. The program was designed to provide information with which BLM and other governmental agencies could better assess the environmental impact of petroleum production on the OCS.

The present study was a part of this program, and had the following overall objectives:

- (1) to establish the long-term fate and effects of pollutants associated with or derived from offshore exploration and production platforms
- (2) to identify indicators of pollution which can be used in future monitoring or assessment studies
- (3) to provide information on the "artificial reef" effect of platform structures
- (4) to make specific recommendations to BLM for the design of future research efforts.

The objectives of the trace metal portion of this study were:

- (1) to determine the concentrations of nine selected trace metals (Ba, Cd, Cr, Cu, Fe, Ni, Pb, V, Zn) in surficial and downcore sediments and biota samples collected in the vicinity of petroleum production structures and control sites
- (2) to determine if variations in concentrations can be related to petroleum production structures or activities
- (3) to determine if trace element concentrations in biota inhabiting the area around platform structures reflect bioaccumulation
- (4) to identify organisms that may be useful as possible indicators of metal pollution in the marine environment
- (5) to provide recommendations for future trace metal studies.

B. Literature Review

A number of studies have examined trace metal concentrations in sediments and biota of the Gulf of Mexico, but until recently none have addressed the specific objectives listed above. Of the recent studies with similar objectives, several have been part of the BLM OCS program but none have focused on the present study area.

Trace metals studies on sediments of the Gulf of Mexico OCS done prior to the more recent BLM OCS studies are described in Young (1954); Potter et al. (1963); Tiech et al. (1973); Holmes (1973); and Trefry and Presley (1976a, 1976b). With the exception of the work of Trefry and Presley these studies have primarily described the distribution of trace metals in surficial sediments and have not attempted to correlate distributions with anthropogenic inputs. Trefry and Presley (1976a) more specifically addressed anthropogenic inputs and effects of dredging on trace metal redistribution.

Trefry and Presley (1976b) did the only investigation of sediment trace metals near the present study site.

These authors found that the trace metal concentrations varied considerably from site to site due to variation in grain size and organic matter. However, when metal concentrations were normalized to iron the trace element composition was shown to represent a regional level of concentration based on sediment holding capacity. Deviations from these regionally constant ratios were interpreted as being the result of recent anthropogenic inputs. From analyses of nearshore and shelf sediments near the Mississippi River delta, Trefry and Presley (1976b) concluded that over the past 25 to 30 years there has been a 60% increase in the Pb flux and a 100% increase in the Cd flux to the OCS sediments from the Mississippi River. Other metals (Co, Cr, Cu, Mn, Ni, and Zn) studied did not show a significantly increased influx to this region.

Accumulation rates of sediments on the Louisiana shelf near the Mississippi River delta were determined by Shokes (1976) using Pb-210 dating techniques. Shokes concluded that sediments on the delta's contiguous continental slope accumulate at a rate less than 0.1 g/cm² per year, whereas at the river mouth the rate is about 1.5 g/cm² per year. The sedimentation rates in the nearshore areas of the delta were too high (>2 g/cm² per year) to be measured by the Pb-210 technique. The nearshore sedimentation rates are controlled by the terrigenous sediment fluxes while those farther offshore (deep water) are apparently controlled by pelagic contributions (Shokes, 1976).

Several more recent studies, although not done on the Louisiana OCS, expanded understanding of trace metal concentrations and distributions in other Gulf of Mexico OCS areas. As stated above, some of these were initiated as a part of the BLM OCS program and addressed some of the objectives of the present study.

Investigations on the Mississippi, Alabama and Florida (MAFLA) outer continental shelf sponsored by BLM from 1977 to 1978 (Dames and Moore, 1979) provided information on the baseline concentrations of Al, Ba, Ca, Cd, Cr, Cu, Fe, Ni, Pb, V and Zn in sediments, suspended particulate matter, epibenthos and demersal fish. At the time of these studies there were no significant anthropogenic inputs to this study area and no petroleum related activities. This study can, therefore, be used as a benchmark for comparison of trace metal concentrations from this area with those of other regions such as the Central Gulf OCS where petroleum production development is extensive.

The BLM sponsored South Texas OCS study (STOCS) (Berryhill, 1979; Presley and Booth, 1979), conducted from 1974 to 1978, covered the continental shelf between San Antonio Bay to the north and the Rio Grande River on the south. Sediments and biota were analyzed for ten trace metals (Al, Ca, Cd, Cr, Cu, Fe, Ni, Pb, V, and Zn). Zooplankton, fish muscle, gill and liver tissue, and shrimp muscle and hepatopancreas tissue were analyzed. No significant differences in the annual mean trace metal concentrations of these samples were observed. There were significant differences in certain trace metal concentrations at different sampling stations but no consistent trends were noted. Sediments had elevated concentrations of Ba where exploratory

wells had been drilled and also showed elevated concentrations of Cd, Cr, Mn, Ni, V and Zn in areas of known gas seeps. Lead concentrations were lower in offshore zooplankton samples but Cd concentrations were higher.

The "Rig Monitoring Study" (White, Turgon, and Blizzard, 1977), a part of the MAFLA program, examined an offshore drilling site prior to, during, and after drilling operations. Sediments and biota were analyzed for Ba, Cd, Cr, Cu, Fe, Ni, Pb, and V. Increased Ba concentrations were observed in bottom sediments both during and after drilling operations. Increases in Fe concentrations in epifauna were also observed but did not appear to be related to drilling activities; resuspension of sediments due to the passage of a storm was a more probable causative factor.

A study on the impact of oil production on marine ecology in Timbalier Bay, Louisiana and adjacent offshore areas, entitled the Offshore Ecology Investigation (OEI), was done by the Gulf Universities Research Consortium (GURC) (Montalvo and Brady, 1974a, b; Williams and Jones, 1974; and Ward, Bender and Reish, 1979). Sediment and water samples were analyzed over a two-year period (1972-74) for 17 trace metals. Only Ba concentration in sediments were found to be unusually high, apparently related to the dumping of drilling muds containing barite. Arsenic, Cd, Hg, Pb and Zn concentrations in water samples from Timbalier Bay were higher than those of water sampled in the offshore oil field. Concentrations of Cd, Pb and Zn in offshore waters decreased with distance from the production platforms. Near bottom and surface water samples had higher Pb and Zn concentrations than mid-depth samples. A major conclusion of the study was that any effect the oil drilling operations had on the marine environment of Timbalier Bay and adjacent OCS was overshadowed by the sediment input from the Mississippi River.

A four-year environmental study of the Buccaneer Gas/Oil Field (BGOF), funded through the National Oceanic and Atmospheric Administration by the Environmental Protection Agency, is presently being completed. This study is investigating the effect an active production platform has on the marine environment. The study site is located approximately 50 km SSE of Galveston, Texas. Results from the second year (1977-78) of the study (Anderson and Schwarzer, 1979) indicate there are decreasing trace metal gradients in surficial sediments away from the platform structures. Elevated concentrations of Ba, Pb, Sr, and Zn were observed in surficial sediments within 180 m of the structures. Sediment cores had significantly higher concentrations of Ba, Hg, Pb, Sr, and Zn in the surficial

layers as compared to the subsurface layers, indicating a recent increase in trace metal input to the sediments. Suspected sources of the trace metal concentrations are platform structures, corrosion, metal debris on the bottom, used drilling muds, and production water.

Cluster analysis of sediment trace metal data from the first and second years of the BGOF study (Wheeler et al., 1980) was performed using three different populations of control samples. There are limitations to this method of evaluating the data, but the authors' conclusions are that Ba, Cd, Co, Pb, and Sr are possible contaminants. The suggested sources included drilling muds (Ba), production water (Sr), corrosion of platform sacrificial electrodes or metallic debris on the sea floor (Cd, Co, Pb) and gasoline engines of recreational boats (Pb).

Sediment data from the third year of this study confirms earlier observations of metal concentration gradients decreasing away from the platforms (Tillery, 1980a). These gradients do not appear to be related to the hydrous iron fraction, grain size, or percent CaCO₃ in the sediments. These results suggest that the metals (Ba, Cd, Cr, Cu, Mn, Pb, Sr, and Zn) are coming from the structures, activities on the platforms, or post-drilling operations.

C. Study Area and Sampling Sites

Louisiana offshore oil fields occur in the region to the east and west of the Mississippi River delta. The present study area and locations of the petroleum production platforms and control sites visited are shown in Fig. 1. Primary Platforms (P1-P4) were sampled for surficial sediments along a north-south and east-west transaxis at 100, 500, 1000, and 2000 m in three seasons: May, August/September, and January. Secondary Production Platforms (S5-S20) were sampled along a north axis at the same distance intervals as the Primary Platforms in August/September only. Control Sites (C21-C24) were located in lease-blocks where no prior petroleum exploration or production activities had taken place but which had physical characteristics and influences similar to the areas where Primary and Secondary Platforms were located. They were sampled each season.

Fish and epifaunal samples were also collected at Primary, Secondary, and Control Sites. Diving, angling and trawling were all used in the collection of these samples. Demersal fish and macroepifauna were taken by trawls in the N500 to N2000 area except when lack of sufficient organisms necessitated collection of platform species. Pelagic fish were taken at the platforms.

All samples were analyzed for Ba, Cd, Cr, Cu, Fe, Ni, Pb, V, and Zn. Selected downcore sediments were analyzed for Pb-210 to obtain the chronological age and history of the sediments in the study area.

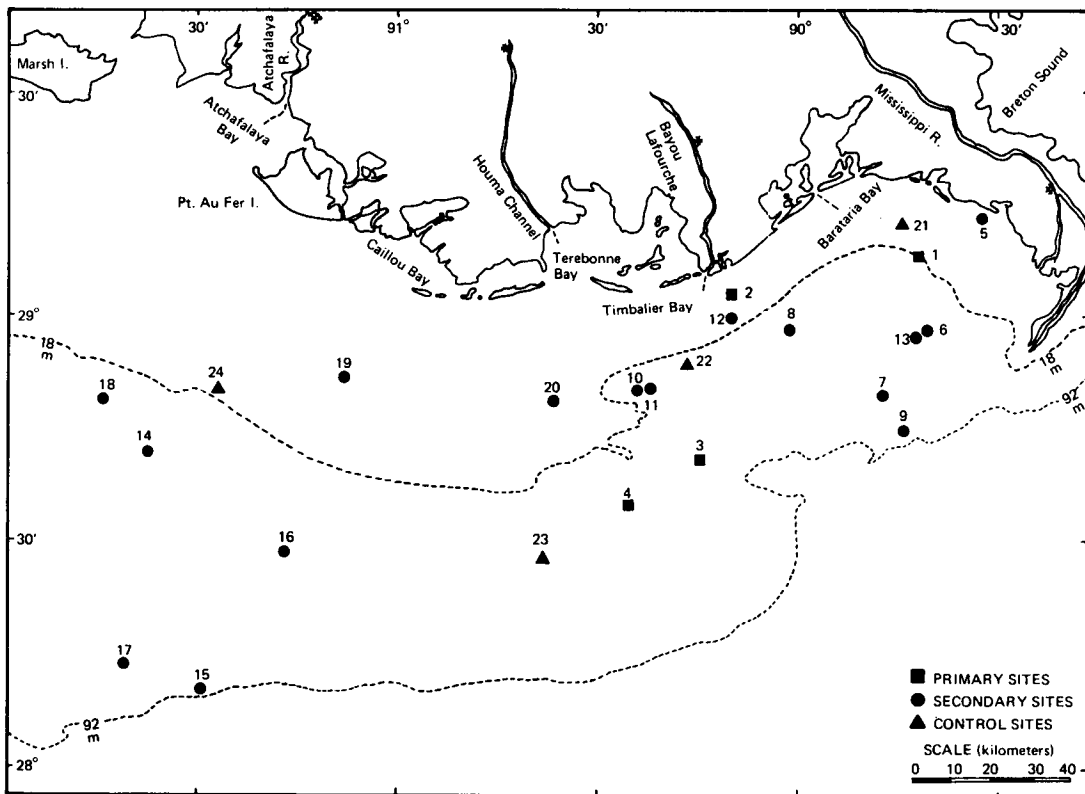
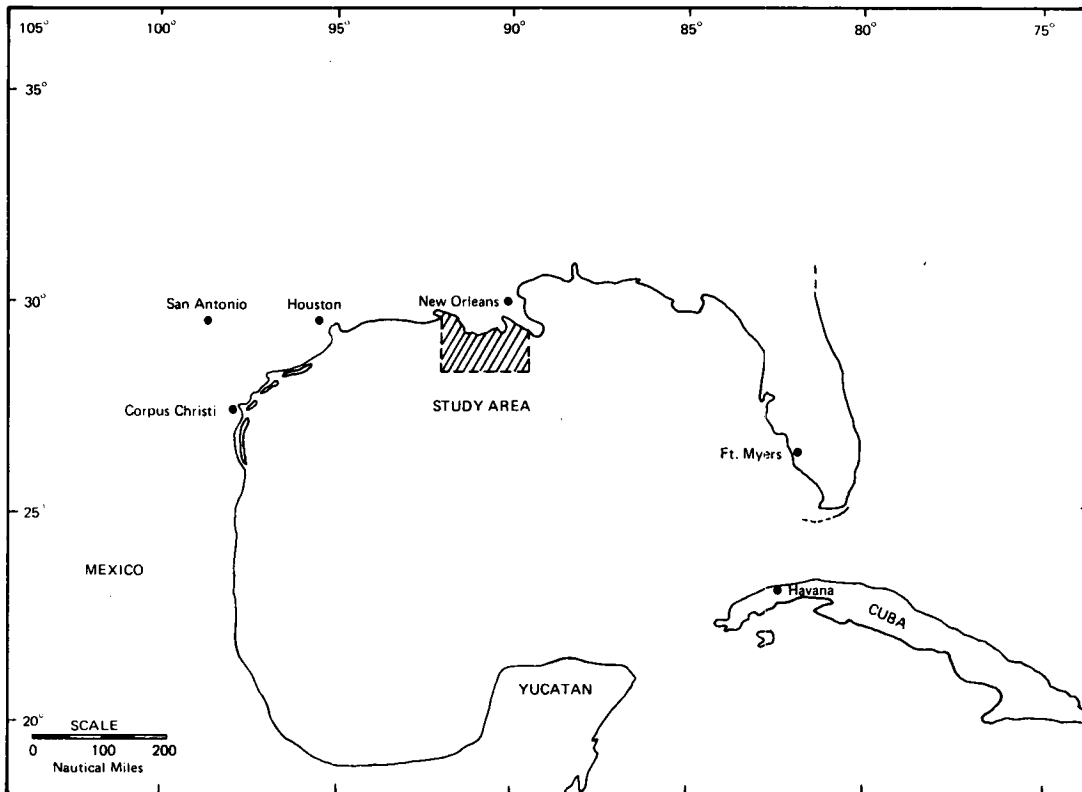


FIG. 1. Maps of the study area—(Top) Location of study area (Bottom) Study area showing sampling sites.

II. MATERIALS AND METHODS

A. Sample Collection and Preparation

1. Sediments

a. Surficial Sediments

Surficial sediment samples for trace metals analysis were collected with a stainless steel Smith-McIntyre grab (Kahlsico Model 214WA250). Subsamples were taken from the top 5 cm in an area away from the sides of the metallic sampler using a Lexan® coring device (5 × 5 cm). The subsample cores were placed in acid-washed polyethylene jars, labeled and kept frozen until preparation for analysis.

Partial digestion was done on all sediment samples. On 25% of the sediment samples, a total digestion was also done.

(1) Partial Digestion

Sediment subsamples were removed from the freezer and allowed to thaw completely and equilibrate with room temperature. An acid-cleaned glass rod was used to thoroughly mix the wet sediment. Approximately 60-80 g of the wet sediment was weighed into a tared polyethylene beaker and particles 3 mm or larger in any dimension were removed using Teflon-coated forceps. The beaker was then covered with a thin sheet of tissue paper and placed in a drying oven (60 C) until the sediment reached a constant dry weight. The sample was reweighed to determine water loss then ground in a mortar and pestle and stored in an acid-cleaned polyethylene bottle.

A 5-g aliquot of the dried sediment was weighed into a 250-ml polyethylene, screw-cap, Erlenmeyer flask, and 25 ml of 5N HNO₃ (redistilled) was added to the sample. The flask was sealed and placed on a mechanical shaker at low speed for 2 hrs. The sample was quantitatively transferred to a 50-ml polyethylene centrifuge tube using three distilled water rinsings of the Erlenmeyer flask. The sample was then centrifuged at 2500-3000 RPM for 20 min in order to separate suspended silica material from the leachate, thereby preventing an interference in the flame and flameless atomic absorption spectrophotometric (AAS) determination of the analyte metals. The leachate was quantitatively transferred to a 50-ml polyethylene volumetric flask and brought to volume with distilled water. This leachate was used for metals analysis.

(2) Total Digestion

A 10-g subsample of the dried sediment (as prepared above for partial digestion) was sieved through a 150 μm (100 mesh) stainless steel screen (ATM Corporation, Milwaukee, Wisconsin) using an ATM Sonic Sifter. Contamination of Cr, Fe, and Ni from the stainless steel screens was minimized by using new screens, washing and drying them thoroughly after each use, and by daily inspection for corrosion. A 5-g subsample of the preground, presieved sample was weighed into a 250-ml Teflon beaker. Twenty-five ml of concentrated HCl was added to the sample and the beaker was

covered with a Teflon watchglass. The sample was placed on a hot plate (90-100 C) for one hour to digest. Following the HCl digestion, the sample was allowed to cool on a clean bench and 15 ml of concentrated HNO₃ was added. The beaker was then returned to the hot plate for another 45 min of heating (90-100 C). The beaker was removed and allowed to cool before 25 ml of 48% HF was added to break down the crystalline lattice of the sediment. The sample was returned to the hot plate for a third time and heated for 2 hrs. After this final digestion, the sample was cooled and quantitatively transferred to a 50-ml polyethylene volumetric flask. The beaker was rinsed three times with distilled water and the flask brought to volume with distilled water. Aliquots of this digest were repeatedly evaporated with HNO₃ to remove chlorine which would interfere with the neutron activation analysis (NAA) determination of V.

b. Downcore Sediments

Downcore sediments were collected using a 1-m x 0.05-m piston coring device (Kahlsico Model 217WA260). Cores for trace metal analyses and Pb-210 analyses were collected in polyethylene core liners. Cores were capped on both ends with PVC caps and frozen in a vertical position until prepared for analysis.

(1) Trace Metal Analysis

For trace metal analysis, the polyethylene core liner was split using a surgical saw with a stainless steel blade. The frozen core was split in half along its long axis with a stainless steel knife. Each half of the core was subdivided at 1.0-cm intervals. Composite samples were made by combining ten adjacent 1.0-cm cuts throughout the total length of the core. The composite samples were thoroughly homogenized with a Teflon spatula, placed into acid-washed polyethylene jars, labeled and later processed by the methodology described above for surficial sediment.

(2) Downcore Dating

Downcore sediment samples for Pb-210 analysis were prepared the same way as for trace metal analysis, but 0.5-cm cuts were used and no composites were made. Several grams of sediment from each 0.5-cm layer were dried for 3 hrs at 400 C to destroy the organic matter which could interfere with the extraction of lead for determination of Pb-210. The organic content of the sediment was determined by the weight loss of the sediment. The residue was leached with 6N HCl by heating to near boiling for one hour then filtered. The leachate was evaporated to dryness and the residue was dissolved in 1.5 N HCl. The hydrated silica was removed by centrifugation of the solution.

Ten to 30 mg of stable lead in the form of Pb-210-free nitrate solution was added to each dissolved sediment sample as a carrier. The solution was then passed through an anion exchange column (AG1-X1 100-200 mesh resin). The Pb-210 was precipitated as the sulfate by the addition of a saturated solution of sodium sulfate. The precipitate was dried at 100 C to a constant weight and mounted on a planchet for counting.

Lead-210 was determined by measuring the growth of the bismuth-210 (Bi-210) daughter (Beta of 1.2 Mev). The Bi-210 activity was determined utilizing a gas proportional anti-coincidence counting system. The system has a background of approximately 0.5 cpm and a counting efficiency of 30% for Bi-210 beta particles. Final assays were made 20 to 40 days after the isolation of Pb-210 (i.e. to allow sufficient Bi-210 growth) from the sediment samples.

2. Biota

a. Collection

Fish and epifaunal samples were collected by diving, angling, and trawling. The proposed method of obtaining pelagic fish attracted to the platforms was by angling, with emphasis on snappers and grouper. However, after little angling success on Cruise I, this was modified to include diving on a special "fishing" expedition, Cruise II-B. When "pelagic" fish were not angled, divers speared fish at platforms to meet the contract requirements for "pelagic" fish. Divers also collected attached bivalved mollusks to meet "epifauna" requirements when these were not met by trawling for epifauna and demersal fish.

Equipment (fishhooks, spears, tongs, etc.) was of stainless steel whenever possible and rigorously cleaned plastic; i.e., acid-washed ice chest for temporary storage and uncoated nylon trawls and dive bags. Samples taken during diving went directly from the water into ice chests and those from trawls into a stainless steel sorting tray where they were immediately hand-sorted using acid-washed rubber gloves. Individual samples were then placed in prelabeled, acid-washed polyethylene bags and frozen. For both trace metals and hydrocarbons samples, care was taken to avoid contamination from on-deck equipment and activities by immediate processing in an established routine. Experience indicates two keys to avoiding on-deck contamination: maintaining the sample processing area upwind of engine, galley and other exhausts, and frequent washdown of everything with plenty of seawater.

b. Initial Preparation and Digestion

(1) Pelagic Fish

On arrival at the onshore laboratory, transfer of custody documents was completed and all samples were inventoried according to the preprinted sample inventory form and stored in a walk-in freezer. For analysis, five individual sample specimens were thawed and dissected on a clean bench using Teflon-coated forceps and stainless steel surgical instruments. Tissues (flesh, gills, liver, and gonads) were removed and pooled in acid-washed, preweighed 250-ml polyethylene beakers. Pooled samples were composed of individual organisms of approximately the same size and developmental stage which were collected from approximately the same area. During dissection procedures, separate instruments were used for separate species and tissue groups to avoid cross-contamination. Between use, all dissecting instruments were cleaned according to normal laboratory procedures then washed with 0.1N nitric acid and rinsed with distilled water. Before dissection procedures began, all samples were rinsed with distilled water.

The first dissection procedure was to open the visceral cavity from the gular region to the vent with stainless steel scissors. The alimentary system was snipped above the stomach and pulled from the abdominal cavity, mesenteries when necessary, exposing the liver and gonads (where developed). This allowed for excision of the liver and gonads. The second dissection procedure involved removing filets of skeletal muscle from the lateral musculature. The epidermis and scales were removed and the muscles from both sides of the specimen were fileted with a stainless steel knife. The third dissection procedure was excision of the gills. The operculum was raised and the gill arches removed at the dorsal and ventral connections of the gill cavity with stainless steel scissors. Gills were collected from all specimens, whether the gonads were developed or not, as a backup for insufficient gonadal tissue. The entire gill structure—arches, rakers, and gill filaments—was analyzed.

After the five specimens within a group were dissected, each tissue—liver, flesh, gonads, and gills—was placed in a pre-weighed 250-ml beaker. The beakers were weighed to obtain wet weights of the tissues. Beakers were sealed with polyethylene sheets, frozen (0°C) and placed in a Labconco Model 75010 freeze dryer for 48 hrs. The freeze-dried samples were reweighed to determine water loss, then ground to ensure complete mixing of the sample in a Virtis "45" homogenizer (The Virtis Company, Inc., Gardiner, New York) using stainless steel blades.

The possibility of Cr, Fe, and Ni contamination of the samples through microcorrosion of the stainless steel blades was investigated by Tillery (1980a). Shrimp tissues were prepared with the Virtis "45" homogenizer and were also ground with an agate mortar and pestle. No evidence of contamination was found.

The finely ground samples (0.5 g) were weighed into tared Pyrex® ashing boats and placed into a low temperature asher (LTA-505, LFE Corporation, Waltham, Massachusetts). They were ashed for 16 hrs at 450 watts of forward power using an oxygen plasma. The ashing boats were removed from the asher and 1 ml of 70% HNO₃ (Suprapur) was added to solubilize the ash and retain it in the ashing boat during the transfer to a clean bench. The ash was quantitatively transferred into a Teflon bomb with distilled water and 3 ml of 70% HNO₃ (Suprapur) was added. The Teflon bomb was sealed and placed in a steam bath (90-110°C) for 2 hrs. The bomb was allowed to cool and the digestant was quantitatively rinsed into a 15-ml polyethylene centrifuge tube using three rinsings (both cap and cylinder) of distilled water. The sample was centrifuged for 10 min at 3000 RPM and the supernatant decanted into a 25-ml volumetric flask without disturbing the precipitate. The precipitate was rinsed with 2 ml of distilled water and re-centrifuged for 10 min. This rinse was then decanted into the volumetric flask and brought to volume with distilled water. This solution was used to determine the different analyte metals using flame or flameless AAS and NAA. Concentrations of the different elements determined the method of AAS analysis. Aliquots were taken for Ba and V analyses by NAA.

(2) Macroepifauna and Demersal Fish

Pooled samples of macroepifauna and demersal fish were composed of individual organisms of

approximately the same size and developmental stage which were collected from approximately the same area. Organisms which had limited fleshy portions (<100 g) were used *in toto* after removal of the outermost layer. For organisms which were larger than 100 g, only the muscle tissue was used for analysis if practical. For example, muscle tissue from shrimp was used and the soft tissues were removed from the shells of bivalves.

After dissection, the sample tissue was treated similarly to the pelagic fish. A sample was placed in a preweighed 250-ml polyethylene beaker and wet-weight taken. The sample was freeze-dried, then reweighed to determine water loss. The freeze-dried sample was ground in a Virtis homogenizer and a 0.5 g portion was weighed into a Pyrex® ashing boat. At this point, the sample was ashed, digested, and analyzed according to the procedure given for pelagic fish analyses.

c. Preparation for Neutron Activation Analysis

(1) Barium

A 5-ml aliquot of each digested biological sample was placed in a small acid-washed polyethylene vial using an Oxford Macro-Set Transfer Pipet System. The vial was heat sealed, leak tested, and rinsed with acetone to remove oily films from the outside of the polyethylene vial that might contain contaminants that would interfere with NAA. The vial was placed in 5 × 15-cm polyethylene Zip-Loc® bag which was heat sealed and shipped to the NAA laboratory.

(2) Vanadium

A 10-ml aliquot of the digested sample solution (see above) was pipetted into a 100-ml Teflon beaker, placed on a hot plate (200 C), evaporated to near dryness and removed. After cooling, 2 ml of 8N HNO₃ (Suprapur) was added to the sample. The 2-ml sample was poured onto a prepared hydrated antimony pentoxide (HAP) column (see below). The column effluent was collected in a second 100-ml Teflon beaker and evaporated to near dryness as before. The sample was dissolved and transferred to the counting vial with four 250-μl rinses: (1) deionized water, (2) deionized water, (3) 8N HNO₃, and (4) deionized water. The total sample volume was approximately 1 ml. The vial was heat sealed, leak tested, and rinsed with acetone. Vials were shipped to the NAA contractor for analysis.

(3) Hydrated Antimony Pentoxide (HAP) Column Preparation

The HAP was preconditioned in 8N HNO₃ (Suprapur) for two weeks prior to use (Reed, 1977). One column was set up per sample. The column was an acid-washed disposable polypropylene Oxford pipet tip. A quartz wool plug was placed in the end of each column and 1.5 g of preconditioned HAP was added to the column with a Teflon-coated spatula. The column was rinsed with 20 ml of 8N HNO₃ to determine the flow rate. The flow rate was adjusted to less than 2 ml per min. Once the column was rinsed, the end of the column was stoppered and the HAP material kept under an 8N HNO₃ layer until ready for sample application.

B. Instrumentation

1. Atomic Absorption Spectrophotometry

A Perkin-Elmer Model 306 Atomic Absorption Spectrophotometer with an HGA-2000 Graphite Furnace and a Perkin-Elmer Model 506 AAS with an HGA-2100 Graphite Furnace were used for the determination of Cd, Cr, Cu, Fe, Ni, Pb, and Zn in all digested sample matrices.

2. Neutron Activation Analysis

A TRIGA MARK III Nuclear Reactor was used to irradiate all samples for Ba and V determinations. Detection and counting of samples was by Ge (Li) detector. An on-line NOVA 800 process computer was used for data storage, analysis and control of the ND-2200 multichannel analyzer.

3. Lead-210

Beta counting of the Bi-210 decay was by a low background proportional system (Beckman WIDE-II).

C. Sample Analyses and Quality Control

1. Sediments

Atomic absorption analyses were performed on the partial and total digests of sediment samples using flame or flameless analyses depending upon the analyte metal and its concentration. Instrument parameters were those recommended by the manufacturer. Table 1 summarizes the AAS analytical parameters and technique used for each metal.

Samples for Ba analysis were irradiated in the rotary sample rack of the TRIGA MARK III Nuclear Reactor at 3×10^{12} n/cm²/sec in batches of 40, including standards, blanks, and flux monitors. The irradiation time was 14 hrs, cooling time was from 14 to 24 days, and counting time was 2 hrs. The ¹³¹Ba activity was determined by measuring the intensity of the 496 KeV peak with a Ge(Li) detector (37 cc). The ¹³⁹Ba isotope could not be used because its half-life is short as compared to the cooling time required for these samples. Corrections for decay and neutron flux changes between batches were introduced as necessary. Spectra were recorded on magnetic tape and processed.

Samples for V analyses were placed in polyethylene "rabbits" with an aluminum flux monitor attached to each sample. The rabbit was transferred by the pneumatic transfer system into the TRIGA-Reactor core and irradiated for 5 min at 5×10^{12} n/cm²/sec. After irradiation, the rabbit was returned to the terminal, opened, and the sample was transferred to a Ge(Li) detector for gamma spectrometric measurement. The flux monitor was transferred to a scintillation counter. After a fixed cooling time (3 to 5 min) the ⁵²V activity was measured by counting the 1434 KeV peak. An on-line computer was used to store the data on magnetic tape and to subsequently process the data. All ⁵²V measurements were normalized with the flux monitors.

Verification of the methodologies and quality assurance for the sediment analyses involved spiked, homogeneous samples and standard reference sample

TABLE 1. Instrument parameters - AAS

<i>Sample Type</i>	<i>Instrument Parameters</i>	<i>Cd</i>	<i>Cr</i>	<i>Cu</i>	<i>Fe</i>	<i>Ni</i>	<i>Pb</i>	<i>Zn</i>
Surficial Sediment (partial & total) Downcores	wavelength (nm)	228.8	357.9	324.7	248.3	232.0	283.3	213.9
	slit (nm)	0.7	0.7	0.7	0.2	0.2	0.7	0.7
	atomization ¹	HGA	air/H ₂ C ₂	air/H ₂ C ₂	air/H ₂ C ₂	air/H ₂ C ₂	air/H ₂ C ₂	air/H ₂ C ₂
Paint Chips	wavelength (nm)	228.8	357.9	324.7	248.3	232.0	283.3	213.9
	slit (nm)	0.7	0.7	0.7	0.2	0.2	0.7	0.7
	atomization ¹	air/H ₂ C ₂	air/H ₂ C ₂	air/H ₂ C ₂	air/H ₂ C ₂	air/H ₂ C ₂	air/H ₂ C ₂	air/H ₂ C ₂
Pelagic & Demersal Fish and Macro- crustacea Flesh	wavelength (nm)	228.8	357.9	324.7	248.3	232.0	283.3	213.9
	slit (nm)	0.7	0.7	0.7	0.2	0.2	0.7	0.7
	atomization ¹	HGA	HGA	HGA	air/H ₂ C ₂	HGA	HGA	air/H ₂ C ₂
Pelagic Fish: gills, gonads and liver	wavelength (nm)	228.8	357.9	324.7	248.3	232.0	283.3	213.9
	slit (nm)	0.7	0.7	0.7	0.2	0.2	0.7	0.7
	atomization ¹	HGA	HGA	HGA	air/H ₂ C ₂	HGA	HGA	air/H ₂ C ₂

1. Type of atomication: HGA = graphite furnace (flameless)
 air/H₂C₂ = air/acetylene (flame)

analyses. The standard reference sample used in this program was the National Bureau of Standards (NBS), Standard Reference Material 1645, River Sediments. Table 2 gives the results of our analyses of this reference material using the partial digestion procedure previously described.

Replicate analyses (7) of an actual surficial sediment sample gave the following variabilities (coefficient of variation in percent): Ba, 22%; Cd, 17%; Cr, 13%; Cu, 18%; Fe, 9%; Ni, 7%; Pb, 18%; Zn, 5%; and V, 6%. For many of the metals the variability is greater than expected due to analytical precision and probably results from sample inhomogeneity.

2. Biota

Digested biota samples were analyzed by both AAS and NAA as described above for sediments. More flameless AAS analyses were required for the biota samples because of the low concentrations of analyte metals in the samples.

Verification of the methodologies and quality assurance for the biota samples involved the analysis of spiked, homogeneous tissues (shrimp muscle) and standard reference samples. National Bureau of Standards, Standard Reference Material 1577, Bovine Liver samples were routinely analyzed. Table 3 gives the results of analysis of this NBS reference material.

TABLE 2. Trace concentrations and percent recovery in NBS SRM 1645 River Sediment¹($\mu\text{g/g}$ dry wt)

	<i>Ba</i>	<i>Cd</i>	<i>Cr</i> ²	<i>Cu</i>	<i>Fe</i> ³	<i>Pb</i>	<i>Ni</i>	<i>V</i>	<i>Zn</i>
Reported Concentration	--	10.2 \pm 1.5	2.96 \pm 0.28	109 \pm 19	11.3 \pm 1.2	714 \pm 28	45.8 \pm 2.9	--	1720 \pm 169
Determined Concentration	20.2	7.23	2.26	84.2	3.94	710	23.6	ND ⁴	1519
% Recovery	--	71	76	77	35	99	52	--	88

1. by partial digestion procedure
2. weight %
3. value not certified
4. not determined

TABLE 3. Trace concentrations and percent recovery in NBS SRM 1577 Bovine Liver (mg/g dry wt)

	<i>Ba</i>	<i>Cd</i>	<i>Cr</i> ¹	<i>Cu</i>	<i>Fe</i>	<i>Pb</i>	<i>Ni</i> ¹	<i>V</i>	<i>Zn</i>
Reported Concentration	--	0.27 \pm 0.04	--	193 \pm 10	270 \pm 20	0.34 \pm 0.08	--	--	130 \pm 10
Determined Concentration	4.78	0.43	1.19	188	252	0.31	0.19	0.90	138
% Recovery	--	119	--	97	93	91	--	--	106

1. by partial digestion procedure

III. RESULTS

A. Sediments

Mean trace metal concentrations in surficial sediments from all sampling stations are compared with similar data from other investigations in the Gulf of Mexico in Table 4. Distribution of the trace metal concentrations by platform and station is given in Appendix B.

Computer generated scatter plots of surficial sediment trace metal concentrations versus Fe concentrations are given in Appendix C1. The surficial sediment trace metal concentrations normalized to the Fe concentration are listed by platform and station in Appendix C2. All trace metal concentrations normalized to percent clay are listed in tabular form by platform and station in Appendix D1. Normalization of surficial sediment trace metal concentrations with the total hydrocarbon content (%) (Nulton et al., 1980) are presented in Appendix E by platform and station.

Tabular and graphical presentations of the downcore sediment trace metal data are presented in Appendices F1 and F2, respectively. Downcore sediment Pb-210 data are tabulated in Appendix G.

B. Biota

Table 5 lists the types of biota samples collected and also the number of pooled and individual samples available for analysis. Table 6 lists species of biota common to other environmental investigations in the Gulf of Mexico.

Table 7 is a comparison of the mean trace metal concentrations in organs of *Archosargus probatocephalus* (sheepshead) with similar data (muscle tissue) from the BGOF (Tillery, 1980a). A comparison of the mean trace metal concentrations in organs of *Chaetodipterus faber* (spadefish) with similar data from the BGOF (Tillery, 1980a) is presented in Table 8.

Table 9 is a comparison of the mean trace metal burdens in organs of *Micropogon undulatus* (Atlantic croaker) with similar data from the Strategic Petroleum Reserve (SPR) program baseline studies (Shokes, 1978) and the STOCs environmental survey (Horowitz and Presley, 1977).

Mean trace metal concentrations in tissues of *Lutjanus campechanus* (red snapper) are compared to similar data from the STOCs study (Presley and Booth, 1979) in Table 10.

Table 11 is a comparison of the mean trace metal concentrations in muscle tissue of *Penaeus aztecus* (brown shrimp) with data from the SPR baseline survey (Tillery, 1980b), the STOCs study (Horowitz and Presley, 1977), and the MAFLA baseline survey (Johnson, 1979).

Table 12 is a comparison of the mean trace metal concentrations in muscle tissue of *Penaeus setiferus* (white shrimp) with similar data from the SPR studies (Shokes, 1978; Tillery, 1980b), the STOCs study (Presley and Booth, 1979), and the MAFLA study (Gould and Morbert, 1979).

TABLE 4. Comparison of the mean trace metal concentrations ($\mu\text{g/g}$ dry wt) in surficial sediments with other Gulf of Mexico studies

		Ba	Cd	Cr	Cu	Fe ¹	Ni	Pb	Zn	V
Central Gulf ²	mean \bar{x}	77	0.30	8.9	11-	0.69	10.2	18.8	44	9.8
	CV (%)	175	64	39	49	34	27	96	60	54
Platform Study	Range n=147	0-1515	0.01-0.92	2.3-19	1.0-45	0.15-2.0	3.9-17.2	0-136	14-193	0-44
Northwest Gulf ³ of Mexico (Trefry & Presley, 1976a)	mean \bar{x}	NA ⁴	0.3	NA	11.4	2.18	22.6	16.5	73.8	NA
	Range		0.02-0.7		2.0-24.8	0.53-3.34	5.1-38.8	4.9-34.4	17.6-132.3	
SPR-Weeks ² Island, Summer 1978 (Tillery, 1980b)	mean \bar{x}	37	0.05	5.09	2.0	0.45	6.2	5.7	22	NA
	CV (%) n=9	45	37	17	40	10	8	22	8	

1. concentration in wt %
2. 5N HNO₃ leach at room temperature
3. 16N HNO₃ + 5N HCl leach with heat
4. NA - metal not determined

TABLE 5. Biota samples collected for trace metal analysis

<i>Generic Name</i>	<i>Common Name</i>	<i>Number of Pooled Samples</i>	<i>Number of Individuals</i>
<i>Agriopoma texasiana</i>	clam	6	44
<i>Amusium papyraceum</i>	scallop	2	15
<i>Anadara ovalis</i>	oyster	1	6
<i>Arca baughmani</i>	clam	1	5
<i>Archosargus probatocephalus</i>	sheepshead	11	54
<i>Arius felis</i>	catfish	3	15
<i>Callinectes sapidus</i>	blue crab	1	5
<i>Callinectes similis</i>	blue crab	1	16
<i>Centropristis philadelphica</i>	sea bass	3	17
<i>Chaetodipterus faber</i>	spadefish	18	97
<i>Citharichthys spilopterus</i>	bay whiff	1	18
<i>Coryphaenus hipperus</i>	dolphin	1	5
<i>Crassostrea virginica</i>	oyster	3	22
<i>Cynoscion arenarius</i>	sea trout	2	10
<i>Diplectrum bivittatum</i>	perch	1	10
<i>Eutropus crossotus</i>	fringed flounder	1	10
<i>Lutjanus campechanus</i>	red snapper	5	23
<i>Lutjanus griseus</i>	grey snapper	1	5
<i>Lutjanus synagris</i>	lane snapper	1	4
<i>Macoma pulleyi</i>	clam	1	9
<i>Micropogon undulatus</i>	Atlantic croaker	11	69
<i>Ostrea equestris</i>	oyster	3	36
<i>Paranthias furcifer</i>	creole	2	11
<i>Penaeus aztecus</i>	brown shrimp	12	76
<i>Penaeus setiferus</i>	white shrimp	2	10
<i>Squilla empusa</i>	squilla	2	23
<i>Syacium papillosum</i>	dusky flounder	1	8

TABLE 6. Biota samples common to other Gulf of Mexico marine environmental studies

<i>Study/Species</i>	<i>Central Gulf Study (BLM)</i>	<i>MAFLA</i>	<i>MAFLA Rig Monitoring</i>	<i>Strategic Petroleum Reserve (SwRI)</i>	<i>Strategic Petroleum Reserve (SAI)</i>	<i>Buccaneer Oilfield Third Year</i>
Sheepshead <i>Archosargus probatocephalus</i>	(11)					X
Spadefish <i>Chaetodipterus faber</i>	(18)					X
Dusty flounder <i>Syacium papillosum</i>	(1)	X				
Croaker <i>Micropogon undulatus</i>	(11)				X	
Broken neck shrimp <i>Trachypenaeus constrictus</i>	--		X	X		X
Brown shrimp <i>Penaeus azteus</i>	(12)		X	X		
White shrimp <i>Penaeus setiferus</i>	(2)		X	X	X	
Various species of shrimp	--	X		X		
Sugar shrimp <i>Trachypenaeus similis</i>	--					X
Squilla <i>Squilla empusa</i>	(2)		X			

() = number of pooled samples analyzed

TABLE 7. Comparison of trace metal concentrations ($\mu\text{g/g}$ dry wt) in *Archosargus probatocephalus* (sheepshead) organs with other Gulf of Mexico studies

Study	Organ	Parameter ¹	Ba	Cd	Cr	Cu	Fe	Ni	Pb	V	Zn
	Muscle n=11 (54) ²	\bar{x}	<21.0	0.022	0.73	1.13	19.6	1.11	0.14	<3.5	14.1
		σ	--	0.019	0.70	0.49	9.1	0.63	0.05	--	2.5
		CV (%)	--	87	96	44	46	57	35	--	17
	Gills n=10 (49) ²	\bar{x}	<21.0	0.251	2.40	17.2	376	4.38	0.60	<3.5	144
		σ	--	0.145	3.76	18.6	160	5.83	0.40	--	26
		CV (%)	--	58	156	108	43	133	66	--	18
Central Gulf Platform Study	Gonads n=7 (34) ²	\bar{x}	<21.0	0.059	0.57	1.45	24.2	<0.30	0.38	<3.5	8.1
		σ	--	0.035	0.66	0.82	29.4	--	0.62	--	2.4
		CV (%)	--	59	115	56	122	--	160	--	30
	Liver n=10 (49) ²	\bar{x}	<21.0	1.64	0.49	168	1470	0.50	1.50	<3.5	384
		σ	--	0.80	0.21	78	536	0.36	2.16	--	152
		CV (%)	--	49	44	47	36	72	144	--	40
Buccaneer Oilfield Third year (Summer 1978)	Muscle n = 8	\bar{x}	<0.88	0.031	0.25	0.59	8.5	0.56	0.13	ND ⁴	13.5
		σ	--	0.032	0.06	0.17	2.8	0.26	0.03	--	1.1
		CV (%)	--	103	22	29	33	46	26	--	8

1. detection limit values used in obtaining \bar{x}
2. n = number of pooled samples; () = number of individuals
3. BGOF - Tillery, 1980a
4. ND = not determined

TABLE 8. Comparison of average trace metal concentrations ($\mu\text{g/g}$ dry wt) in *Chaetodipterus faber* (spadefish) organs with other Gulf of Mexico studies

Study	Organ	Parameter ¹	Ba	Cd	Cr	Cu	Fe	Ni	Pb	V	Zn
	Muscle n = 18 (97) ²	\bar{x}	<21.0	0.068	0.61	1.14	18.2	0.86	0.23	<3.5	17.3
		σ	--	0.070	0.85	0.34	8.3	0.64	0.31	--	2.7
		CV (%)	--	102	140	30	45	74	135	--	16
	Gills n = 12 (67) ²	\bar{x}	<21.0	0.450	2.41	7.94	490	1.85	<0.27	<3.5	87
		σ	--	0.416	3.91	3.22	385	1.04	--	--	19
		CV (%)	--	92	162	41	78	56	--	--	22
Central Gulf Platform Study	Gonads n = 7 (35) ²	\bar{x}	<21.0	0.517	0.33	4.52	88.1	1.61	0.65	<3.5	77
		σ	--	0.453	0.14	1.13	58.9	0.77	0.75	--	34
		CV (%)	--	88	43	25	67	48	115	--	44
	Liver n = 18 (97) ²	\bar{x}	<21.0	6.31	0.36	16.7	1308	0.43	0.38	<3.5	80
		σ	--	8.47	0.22	33.2	1422	0.30	0.21	--	80
		CV (%)	--	134	61	199	109	73	54	--	100
Buccaneer ³ Oilfield Tillery (Summery 1978)	Muscle n = 8	\bar{x}	<0.88	0.032	0.22	0.53	8.8	1.04	0.13	ND ⁴	18
		σ	--	0.022	0.06	0.08	3.3	0.75	0.04		2
		CV (%)	--	69	27	15	38	72	31		11

1. detection limit values used in obtaining \bar{x}
2. n = number of pooled samples; () = number of individuals
3. BGOF - Tillery, 1980a
4. ND = not determined

TABLE 9. Comparison of average trace metal concentrations ($\mu\text{g/g}$ dry wt) in *Micropogon undulatus* (Croaker) organs with other Gulf of Mexico studies

Study	Tissue	Parameter ¹	Ba	Cd	Cr	Cu	Fe	Ni	Pb	V	Zn
Central Gulf Platform Study	Muscle	x	<21.0	0.042	0.32	1.59	19.5	0.83	0.23	<3.5	19.0
		σ	--	0.031	0.22	1.24	4.5	0.73	0.18	--	4.4
		CV (%) n = 11 (70) ²	--	72	67	78	23	87	76	--	23
	Gills	x	<21.0	0.768	1.07	5.37	644	1.72	1.21	<3.5	91
		σ	--	--	--	--	--	--	--	--	--
		CV (%) n = 1 (5) ²	--	--	--	--	--	--	--	--	--
	Liver	x	<21.0	1.46	0.31	11.9	953	0.64	1.29	<3.5	100
		σ	--	1.19	0.19	2.6	570	0.54	1.45	--	4
		CV (%) n = 2 (10) ²	--	82	60	22	60	86	113	--	4
	Gonads n = 1 (5) ²	x	<21.0	0.306	0.21	5.69	104	0.74	0.60	<3.5	136
		σ	--	--	--	--	--	--	--	--	--
		CV (%)	--	--	--	--	--	--	--	--	--
SPR- Texoma Sites ³	Muscle	x		0.014	0.007	1.69	20.1	0.045	0.124		20.5
		σ	ND ⁴	0.005	0.002	0.30	1.9	0.011	0.129	ND	2.8
		CV (%)		36	29	18	9	24	104		14
		n = (?)									
STOCS ⁵	Muscle	x	ND	0.1	7.3	1.7	24.0	2.7	0.8	ND	17.5

1. x (mean) includes detection limit values for samples below detection limit
2. n = number of pooled samples analyzed; () = number of total individuals
3. Shokes in SAI, 1978
4. ND = element not determined
5. Horowitz and Presley, 1977

TABLE 10. Comparison of average trace metal concentrations ($\mu\text{g/g}$ dry wt) in *Lutjanus campechanus* (Red Snapper) organs with other Gulf of Mexico studies

Study	Tissue	Parameter ¹	Ba	Cd	Cr	Cu	Fe	Ni	Pb	V	Zn
Central Gulf Platform Study	Muscle	x	<21.0	0.085	0.72	0.79	21.1	0.82	0.19	<3.5	13.2
		σ	--	0.066	0.59	0.45	9.1	0.48	0.10	--	3.1
		CV (%) n = 4 (20) ²	--	78	83	57	43	58	55	--	23
	Gills	x	<21.0	0.753	2.20	5.01	463	1.86	0.33	<3.5	94.6
		σ	--	0.533	1.21	1.24	20	1.11	0.11	--	10.7
		CV (%) n = 3 (15) ²	--	71	55	25	4	60	31	--	11
	Liver	x	<21.0	0.762	0.358	8.05	251	0.94	0.30	<3.5	71.0
		σ	--	0.137	0.248	2.01	61	1.16	0.05	--	8.4
		CV (%) n = 3 (15) ²	--	18	68	25	24	123	15	--	12
	Gonads	x	<21.0	0.079	0.259	1.30	10.9	0.30	0.49	<3.5	23.2
		σ n = 3 (15) ²	--	0.051	0.066	0.49	3.5	--	0.53	--	24.1
	STOCS-- 1976/1977	Muscle	x	ND	0.03	0.03	0.8	5.4	0.06	0.03	0.20
σ			ND	0.00	0.01	0.3	2.8	0.02	0.01	0.10	10
CV (%) n = 17			--	--	33	38	52	33	33	50	83
Gills ³		x	ND	0.5	0.1	1.5	110	0.4	0.5	0.60	70
		σ	ND	0.4	0.0	0.6	30	0.2	0.3	0.20	30
		CV (%) n = 6	ND	80	70	40	27	50	60	33	43
Liver ³		x	ND	1.2	0.04	14	540	0.1	0.2	0.25	110
		σ	ND	0.4	0.05	4	180	0.0	0.07	0.14	18
		CV (%)	ND	33	125	3	33	-- ⁵	35	56	16

1. x (mean) includes detection limit values for samples below detection limit
2. n = number of pooled samples analyzed; () = number of total individuals
3. Presley and Boothe in STOCS 1979
4. ND = element not determined
5. insufficient sample

TABLE 11. Comparison of average trace metal concentrations ($\mu\text{g/g}$ dry wt) in *Penaeus aztecus* (brown shrimp) organs with other Gulf of Mexico studies

STUDY	PARAMETER ¹	Ba	Cd	Cr	Cu	Fe	Ni	Pb	V	Zn
Central Gulf Platform Study	x	<21.0	0.118	0.32	24.0	46.7	0.91	0.17	<3.5	54
	σ	--	0.091	0.18	5.8	33.4	0.64	0.12	--	10
	CV (%) n = 12 (76) ²	--	77	56	24	72	70	74	--	19
SPR-Summer ³ 1978 (West Hack-berry Site)	x	4.9	0.17	0.17	26.0	48.0	0.70	<0.09	ND ⁴	62
	σ	3.7	0.06	0.10	4.7	27.4	0.27	--	--	12
	CV (%) n = 5 (32) ²	75	33	56	18	57	38	--	--	20
(Weeks Island Site)	x	13.2	0.17	0.11	35.0	0.94	0.94	<0.09	ND	62
	σ	29.2	0.06	0.05	4.2	23.8	0.50	--	--	2
	CV (%) n = 6 (12) ²	221	37	46	12	68	53	--	--	3
STOCS ⁵	x	ND	0.16	2.1	24.2	14.2	1.4	1.1	ND	47.7
	Maximum	ND	0.33	3.8	28.5	28.8	1.9	1.6	ND	57.5
	Minimum n = 9	ND	0.05	0.4	18.5	4.5	0.3	0.5	ND	20.5
MAFLA ⁶	x	ND	0.42	0.21	32.5	17.0	0.81	0.067	ND	48.6
	σ	ND	0.36	0.19	13.8	17.4	1.18	0.001	ND	13.1
	CV (%) n = 45	ND	86	90	42	102	147	1	ND	27

1. x (mean) includes detection limit values for samples below detection limit
2. n = number of pooled samples analyzed; () = number of total individuals
3. SPR - Tillery, 1980 b
4. ND element not determined
5. Horowitz and Presley, 1977
6. Johnson, 1979

TABLE 12. Comparison of average trace metal concentrations ($\mu\text{g/g}$ dry wt) in *Penaeus setiferus* (white shrimp) organs with other Gulf of Mexico studies

STUDY	LOCATION ^M	SEASON	PARAMETER	Ba	Cd	Cr	Cu	Fe	Ni	Pb	V	Zn
Louisiana Platform Study	Louisiana OCS		\bar{x}^2 σ CV (%) n = 2 (10) ³	<21.0 -- --	0.103 0.014 14	0.21 0.02 8	26 3 9	25 24 98	0.43 0.06 15	0.14 0.04 26	<3.5 -- --	62 1 1
SPR-SwRI 1978-79 Tillery ⁵	W. Hackberry	Summer 1978 n = 6	\bar{x}^2 σ CV (%)	1.6 1.3 81	0.11 0.08 76	0.17 0.08 49	34 3 10	41 19 46	1.30 1.01 78	0.10 0.01 13	ND ⁴	70 3 4
	Weeks Island	Winter 1979	\bar{x}^2 σ CV (%) n = 7 (61)	2.0 1.7 87	0.37 0.32 87	0.69 0.41 60	35 5 15	28 10 35	1.17 0.99 85	0.29 0.28 97	ND	43 1 3
SPR-SAI 1977-78 ⁶	W. Hackberry	Fall 1977- Winter 1979	Range	ND	0.010- 0.079	0.026- 0.043	ND	2.1- 9.7	0.034- 0.500	0.052- 0.101	ND	35.2- 60.9
Shokes	Weeks Island	Fall 1977 Winter 1978	Range	ND	0.032- 0.040	0.038	23.6- 27.3	6.4- 6.77	0.090- 0.240	0.001- 0.082	ND	49.4- 56.3
STOCS 1976-77 ⁷	Texas OCS	Winter, Spring & Fall 1977	\bar{x}^2 σ CV (%) n = 19	ND	0.03 0.03 100	0.04 0.02 50	23 4 2	5 7 140	0.08 0.02 25	<0.05 0.02 40	0.11 0.10 91	53 9 17
MAFLA 1977-78 ⁸	Miss, Ala, & FLA. OCS	Summer 1977	\bar{x}^2 σ CV (%) n = (7)	2.08 ⁹ 1.25 60	0.42 0.36 86	0.21 0.19 90	32.5 13.8 42	17.0 17.4 102	0.81 1.18 146	0.067 0.001 1.5	0.39 ⁹ 0.21 54	48.6 15.1 2.3

1. see text reference for detail description of study area
2. \bar{x} (mean) includes detection limit values for samples below the detection limit
3. n = number of pooled samples analyzed; () = number of total individuals
4. ND element not determined
5. SPR - Tillery, 1980 b

6. Shokes in SAI, 1978
7. Presley and Boothe in STOCS 1979; Chapter 6, Table 6.5, p. 6-26
8. Gould and Moberg in Dames and Moore 1979; Vol. II, Chapter 5
9. Shokes in Dames and Moore, 1979, Vol. II, Chapter 7, Table 51, p. 47 (Phylogenetics means

IV. DISCUSSION

A. Sediments

1. Surficial Sediments

Marine sediments contain trace metals bound in different ways. Trace metals bound in such a way that they are chemically inert in the marine environment can be referred to as "non-labile" forms. These include trace metals bound in crystalline lattices of refractory silicate minerals. Trace metals that may chemically react in the marine environment, exchanging between phases, can be referred to as "labile" forms. These include trace metals bound in organic, carbonate and hydrated iron oxide phases or in exchangeable sites on the surfaces of solids. A number of investigators have attempted to identify various trace metal forms in sediments (Gibbs, 1973) but have found results difficult to interpret.

Trace metals entering marine waters ultimately accumulate in bottom sediments. Because the labile forms of trace metals are potentially available to marine biota it is important to estimate the amount of the total trace metal concentration of marine sediments that is in these forms. Although there is no simple procedure for determining the labile forms, the acid leach technique used in this study does provide an estimate of relative concentrations available to biota.

Table 4, discussed previously, compares the overall mean of leached trace metal concentrations in the surficial sediments with data from other investigations in the same general area of the Gulf of Mexico. Trefry and Presley (1976a) suggest that their leached trace metal concentrations are representative of nonpolluted sediments from the Texas-West Louisiana coast and Mississippi River submarine delta. Mean concentrations of Cd, Cu, Pb, and Zn are close to the overall means for these metals found in the present study. Reported concentrations of Fe and Ni, however, were higher than those in this study and may reflect the stronger leaching solution (16N HNO₃ + 5N HCl) and high leaching temperature used.

Concentrations of Cd, Cu, Pb, and Zn in sediments at Weeks Island, approximately 25 nautical miles off Marsh Island, Louisiana (Tillery, 1980b) were lower than the concentrations observed in the present study area. The coarser sediments at the Weeks Island site probably account for these differences since overall the Central Gulf Platform Study samples had a finer median grain size.

Although the mean trace metal concentrations of surficial sediments found in this study are similar to those reported for unpolluted sediments, this is not sufficient reason to conclude that these sediments receive no metal inputs from the drilling platforms. Distribution patterns and the geochemical characteristics of the sediments surrounding individual platforms in relation to regional sediments must also be considered.

a. Physical Influence of Platforms on Sediment Metal Concentrations

To evaluate the trace metal distribution pattern around production platforms, the concentrations of the leachable trace metals in sediments in the vicinity of the platform structures can be used. From these data

(Appendix B), definite concentration gradients increasing toward the platform structures are apparent. With the exceptions of Cu, Fe, and Zn, all the other metals demonstrated sediment concentration gradients for at least one of the platforms studied. This suggests sources from the structure or associated activities. It is possible, however, that these gradients are the result of geochemical or biogeochemical processes that are modified by the platform structure. For example, finer grained sediment may preferentially accumulate in the vicinity of the platforms due to modifications of water movement. This would lead to increased metal levels associated with the finer sediments near the platform. Also, increased biological productivity in the vicinity of the platforms may result in higher total organic carbon (TOC) levels in sediments. Higher trace metal concentrations would be expected in sediments containing the higher TOC.

b. Percent Clay Fraction Transformation

To evaluate the effect of grain size on trace element gradients around the platforms, leachable trace metal concentrations were normalized to percent clay fraction in the sediments (Appendix D). The clay fraction also includes most of the organic matter in the sediments. This data transformation is an attempt to "filter out" the grain size and organic matter control on trace metal concentrations. Therefore, gradients in the ratios of metal to percent clay may better indicate trace metal releases from a platform itself.

Appendix C1 reveals higher trace metal to percent clay ratios near the platforms (especially P3) for most metals for Cruise I. Similar observations were made from Cruise II data. Nonetheless, the increases in the ratio in sediments near the platform are generally small as compared with sediments collected farther away.

c. Leachable Iron Transformation

Because the controls on trace metal concentrations in sediments are complex, many geochemists use elemental ratios to evaluate the importance of different processes. For example, in studies of atmospheric particulates, many investigators (Duce et al., 1976) normalized trace element concentrations to aluminum to estimate the importance of the continental source since aluminum can be considered to be almost exclusively derived from the continents.

A similar approach was used by Trefry and Presley (1976a) who normalized HNO₃-HCl leachable trace metals to leachable iron concentrations in sediments of the Gulf of Mexico. They used this data transformation to compensate for variabilities in leachable trace metal concentrations that were due to variations in the grain size, organic carbon, calcium carbonate content and mineralogy. They found that metals such as Ni, Pb, and Zn strongly correlated with iron. Trefry and Presley concluded that since sediments of a large region of the Gulf of Mexico (including the Louisiana OCS) had remarkably constant metal to iron ratios of the leached fraction, these ratios were representative of natural conditions, and that only where significant anthropogenic inputs occurred would the ratio be markedly increased.

Following the approach of Trefry and Presley in the present study, leachable trace metal concentrations were compared to that of iron in the sediments surrounding the platforms. Appendix C1 contains computer-generated scatter plots of metal concentrations against Fe concentration using all samples collected during Cruises I and II. Regression lines, regression equations, and correlation coefficients (r) for each cruise have been included. An r value greater than 0.2144 is significant at $p = 0.05$. These data (metal concentrations normalized to Fe) are summarized by platform and station in Appendix C2.

The plots of trace metals against iron can be used to determine if the data from sediments surrounding a production platform follow a trend consistent with the data collected on a more regional basis (Trefry and Presley, 1976a; Shokes, 1978). Outliers of any trends that might be observed would clearly suggest pollutant concentration. One P4 sample from Cruise I had unusually high Fe concentration and is not included in these scatter plots. It probably represents contamination from iron inputs such as metal debris in the sediments or shipboard contaminants. Iron releases from the platforms obviously could invalidate the comparison of present data with others using the approach just described. It is unlikely, however, that the rates of releases of iron and other trace metals from the platforms are constant and in the same proportions as their consolidation in regional sediments. Therefore, the approach seems reasonable.

For those scatter plots for which the information is available, regression lines, equations and r or r^2 values from Shokes (1978), Tillery (1980b), and Trefry and Presley (1976a) have been included for comparative purposes. Although different digestion solutions were used in each of these studies, the results should be comparable. The stronger digestion solutions would only shift the data points further to the right along the regression line.

(1) Barium/Iron Ratios

Figure 1 in Appendix C1 is the scatter plot of Ba against Fe. Ba is an alkaline earth metal, and of the metals studied is geochemically the least similar to iron. It is not surprising, therefore, that the correlation between Ba and Fe is poor for both cruises. Natural barium in sediments (i.e., not from anthropogenic inputs) is probably better correlated with the CaCO_3 fraction of the sediments. Calcareous sediments usually contain higher concentrations of Ba because of its chemical similarity to Ca. However, the correlation of the Ba concentration to the CaCO_3 content (%) of the sediments was rather weak ($r = 0.372$, significant at $p < 0.05$). Therefore, another explanation (e.g. possible contamination from the platform) must be considered for the distribution of this metal.

(2) Cadmium/Iron Ratios

The correlation between Cd and Fe (Appendix C1, Fig. 2) is also weak ($r = 0.257$ for Cruise I; $r = 0.390$ for Cruise II; both significant at $p < 0.05$) for both cruises. Most of the data points fall considerably off the Cd vs. Fe correlation of Shokes (1978). This suggests that the Cd concentrations in the sediments surrounding the platforms may come from the platforms.

Three of the four samples with the highest Cd levels were collected at the 100-m stations at S10, S14, and S17.

(3) Chromium/Iron Ratios

The relationships of Cr vs. Fe (Appendix C1, Fig. 3) are significant ($p < 0.05$) for both Cruise I ($r = 0.821$) and Cruise II ($r = 0.755$). No data points on this plot appear to be outliers; however, this plot indicates that the sediments from this study are naturally enriched in Cr with respect to Fe.

(4) Copper/Iron Ratios

The Cu vs. Fe scatter plot (Appendix C1, Fig. 4) has correlation coefficients of 0.671 and 0.385 for Cruises I and II, respectively. These values are significant at $p < 0.05$. Only one sample (collected at the N100-m station at S11) had a Cu concentration falling significantly away from the regression curve. Data from all other locations indicate that Cu concentrations in sediments result from natural processes.

(5) Lead/Iron Ratios

Scatter plots of Pb vs. Fe from Cruises I and II (Appendix C1, Fig. 5) show conspicuously different results. The Pb vs. Fe plot for Cruise I indicates a relationship consistent with the regional pattern ($r = 0.544$, significant at $p < 0.05$). Data from Cruise II, however, do not show this consistent relationship ($r = 0.010$, not significant at $p < 0.05$) because many of the samples had Pb concentrations considerably higher than might be expected from Cruise I data. Data falling significantly away from the regression curve were from the N100-m station at S7, S11, and S17; also the N500-m station at S17, suggesting Pb release from these structures.

(6) Nickel/Iron Ratios

The relationship for Ni vs. Fe (Appendix C1, Fig. 6) is significant ($p < 0.05$) for Cruise I ($r = 0.682$) and Cruise II ($r = 0.833$). This pattern is similar to that for regional sediment. No data points appear to be outliers.

(7) Vanadium/Iron Ratios

With the exception of a few data points, the V vs. Fe plots (Appendix C1, Fig. 7) are significantly ($p < 0.05$) correlated. The points excluded for the correlation (circled in Fig. 7) may reflect V releases from the platforms although two of the high V concentrations were found at the N1000-m station at S5 and S2000-m station at P2. No other sources of V at these more distant sites are known.

(8) Zinc/Iron Ratios

The regression line for the scatter plot of Zn vs. Fe (Appendix C1, Fig. 8) for Cruise I ($r = 0.454$, significant at $p < 0.05$) is similar to that of regional sediments. However, the plot (Fig. 8) from the Cruise II data ($r = 0.167$, not significant at $p < 0.05$) has a number of points that show Zn levels considerably higher than expected based on the regional pattern. The highest of these values are from the 100-m station at P4, S11, S14, and S17.

All of the approaches to data interpretation used above must be considered together to evaluate

whether or not sediment contamination occurs due to metals release from the platforms. This further evaluation is presented in the Data Synthesis section (Task 1).

The program design of this study required that "control stations" be sampled. Control stations were located in lease blocks where no previous petroleum development had taken place but which had characteristics (e.g., water depth, sediment characteristics, biota types, etc.) and influences (e.g., wind, wave action, currents, etc.) similar to nearby platform stations. The four control sites were designated C21, C22, C23, and C24 (Fig. 1).

Evaluation of the sediment trace metal data (Appendix B) from the control sites indicated that the data are representative of the background concentrations found beyond the 100-m stations at the platforms. Therefore, comparison of "Control" sediment trace metal concentrations with those from the platforms would not prove fruitful. However, the "Control Site" data has been retained in the data evaluations and correlations (Appendix C1, B2, D, and E).

2. Downcore Sediments

Downcore sediments were collected at all four Primary Platforms and the four Control Sites during Cruise 1. Separate core samples were taken at each site for trace metals determination and geochronological dating (Pb-210 method).

a. Geochronological Dating by Lead-210 Method

Downcore sediments from C22 were analyzed in detail for geochronology (see Appendix G for tabulation of Pb-210 data). The results of the Pb-210 analyses showed large variations, no trend toward decreasing activity with depth and no indication of increasing age with depth. These results indicated that either the sediments in this area were well mixed to the depth of the core or that the Pb-210 supported by Ra-226 in the sediment was much greater than the unsupported Pb-210. Because of these results, further attempts to establish the chronological age of the sediment cores were abandoned.

b. Trace Metals Analyses

Although attempts to date sediments with depth were unsuccessful, piston core samples up to 50 cm long were analyzed in sections for evidence of metals layering. With the exception of Ba, Cd, and Zn, trace metal concentrations in the downcore sediments were relatively uniform with depth (Appendix F2). Concentrations of Ba, Cd, and Zn increased with depth down to 20 cm then became relatively constant through the remaining core depth. The concentrations of these metals from the surface to 20 cm may reflect anthropogenic inputs followed by a discontinuation of the input activity. Though it is logical to presume this concentration to be associated with drilling activities and the "below 20-cm" discontinuity to be a predrilling condition, the data to support the presumption are lacking. This is a definite area for further research.

B. Biota

The objectives of the biota analyses were to (1) determine if any bioaccumulation of trace metals could be determined in marine organisms inhabiting the area

around petroleum production platforms and (2) determine which of these organisms might be useful as monitors of trace metal pollution. Both objectives were only partly accomplished due to the limited availability of organisms at the designated sampling sites.

The first objective requires the collection and analyses of a sufficiently large number of pooled samples of a species so that interspecies variability may be determined. Ideally, there should be a large enough data base (i.e., data from other investigators) available for each species so that statistically valid comparisons can be made.

One problem which limits the comparison to data from other Gulf studies is that previous studies were in open shelf waters, and the species analyzed are representative. Fish data from the present study focuses on those species which are residents of the platform structures (i.e., reef or other solid substrate) or pelagic forms attracted to the structure as temporary residents.

Individual species may be classified as to time of residency as follows:

Species	Association with Platform
Sheepshead	Long-term resident as adult, solitary
Spadefish	Long-term resident as adult, schooling
Croaker	Apparent infrequent migratory visitor (length of time not definitely known), demersal
Red Snapper	Seasonal resident (highest concentration in winter), usually demersal

Shrimp species analyzed are highly migratory and are not known to associate with platforms and associated structures, except for perhaps being temporarily impeded by pipelines.

This difference in the species of biological samples available at platforms versus open ocean sites was illustrated by the lack of similar species being available at the control sites and the platforms. This prevented a comparison of trace metal burdens between exposed and nonexposed sampling populations as originally planned.

Biological samples collected and analyzed for trace metals are listed in Table 5. The total number of individuals and the total number of pooled samples analyzed are also given.

Organisms analyzed that are common to other Gulf investigations are listed in Table 6. Three of these organisms, *Penaeus setiferus*, *Syacium papillosum*, and *Squilla empusa*, were not collected in sufficient numbers to make more than one or two pooled samples; therefore, they have limited value in statistical evaluation of the data. However, the number of individuals pooled to make these samples are sufficient to estimate the trace metal burdens of these species in the study area. For the remaining four organisms (Table 6) sufficient numbers of samples (and individuals) were collected and analyzed to describe their tissue trace metal burdens.

Accomplishing the second objective listed above requires sampling populations of sufficient size for statistical evaluation and also a diversity in the types of marine organisms sampled.

Demersal fish and epibenthic organisms include a number of species that are important to the commercial and sports fishing industries. Since these species are

consumed by man, any accumulation of toxic metal pollutants by these species may be detrimental to human health.

Other species may be food sources for animals higher in trophic order. As these species are consumed, their trace metal burdens could be magnified as they are passed up the food chain, eventually affecting man (Windon et al., 1976).

It has been shown (Pringle et al., 1968) that some marine organisms which accumulate high concentrations of toxic trace metals (e.g., Cd) will purge themselves and return to ambient levels once the source of exposure is removed. This capability can complicate any consideration of a species as an indicator for trace metal pollution.

Another important factor in considering a potential monitoring species is the partitioning of trace metals among different tissues of the animal. Horowitz (1977) showed that there can be several orders of magnitude difference in the concentration of metals in different tissues of the same animal. Pertinent points about each tissue analyzed are: (1) the liver provides a "filtering" mechanism for the circulatory system and should show the accumulative effect of exposure to high metal concentrations; (2) the gills are in intimate contact with the external environment of the fish and should indicate exposure to soluble and suspended particulate trace metals; (3) the gonads exposed to high concentrations of certain trace metals may prevent or inhibit the normal reproductive cycle of the individuals and thus reduce the species population with time; and (4) muscle tissue is the principle tissue consumed by humans - exposure to elevated concentrations of toxic trace metals in muscle would have an immediate impact on human health.

1. *Archosargus probatocephalus*

Table 7 summarizes the mean trace metal concentrations in *Archosargus probatocephalus* (sheepshead) tissues. Data from the BGOF (Tillery, 1980a) are included for comparison.

Trace metal concentrations in sheepshead muscle tissue from this study are very similar to those from the BGOF study. This is to be expected since the sheepshead habitats (i.e., petroleum production platforms) in both studies are similar. Concentrations of Cu, Fe, and Ni, however, appear to be higher in sheepshead from the Louisiana oil fields ($p=0.01$).

Different tissues showed significant differences in trace metal concentrations. Liver tissue concentrates higher levels of Cd, Cu, Fe, Pb, and Zn than the other tissues. Higher concentrations of Cu, Fe, and Zn may be explained on the basis of their biochemical functions. However, there are no known biochemical requirements for Cd and Pb, and the higher levels in the liver probably reflect the age of the fish and the detoxification function of the liver. Cadmium, Cr, Cu, Fe, Ni, Pb, and Zn concentrate in the gills. The gonads appear to concentrate Cd, Cr, and Pb even though the last three elements have no known biochemical function.

2. *Chaetodipterus faber*

Mean concentrations of trace metals in *Chaetodipterus faber* (spadefish) tissues are given in Table 8. Comparisons are also made with spadefish data from the BGOF study. There appear to be significantly ($p=0.01$) higher Cu and Fe concentrations in spadefish

muscle from the present study. Since these were collected during summer (1978), differences may reflect either spacial or exposure variations.

Liver tissues contained higher concentrations of Cd, Cu, and Fe than other tissues. Concentrations of Cu and Fe may reflect biochemical requirements for these metals. The high Cd concentrations, however, may reflect the detoxification function of the liver and age of the fish.

Higher concentrations of Cd, Cr, Cu, Fe, Ni, and Zn in the gill tissues were also observed in this species and the gonads had higher levels of Cd, Cu, Fe, Ni, Pb, and Zn as compared to muscle tissue. No other literature values were found for these particular tissues.

The muscle tissues of sheepshead (Table 7) and spadefish have similar concentrations of all metals except Pb. Except for Ni, Pb, and Zn, gill tissue metal concentrations are also similar, probably reflecting the similarity in the water columns from which both species were taken. The differences in Ni, Pb, and Zn concentrations may reflect differences in the water column concentrations of these metals.

The gonadal tissue of spadefish had higher concentrations of Cd, Cu, Fe, Ni, Pb, and Zn than that of the sheepshead. Liver tissues in sheepshead were higher in Cu, Pb, and Zn, whereas livers of spadefish were higher in Cd. These variations may reflect the different abilities of the species to accumulate certain metals, exposure levels or possible age differences.

3. *Micropogon undulatus*

Comparisons of trace metal concentrations in *Micropogon undulatus* (Atlantic croaker) muscle tissue with similar data from other studies in the Gulf are given in Table 9. Concentrations of Cd, Cr, Ni, and Pb from this study are lower than those reported by Horowitz and Presley (1977) but higher than those reported by Shokes (1978). The concentrations of the remaining metals (Cu, Fe, and Zn) are similar in all three studies. The differences in Cd, Cr, Ni, and Pb may be due to spacial and temporal variations or possibly due to analytical variability. Horowitz and Presley (1977) thought their Cr and Ni values may be influenced by contamination from the stainless steel instruments used in sample dissection.

Trace metal concentrations in muscle tissues of croaker, sheepshead (Table 7) and spadefish (Table 8) are very similar. Metal concentrations in gills and gonads of croaker are more closely matched to those in spadefish than to those in sheepshead. Liver concentrations of Cr, Cu, Fe, Pb, and Zn are similar to those of spadefish while Cd and Ni concentrations are closer to those of sheepshead. The number of croaker gills, gonads and liver samples available were not sufficient to make more than estimates of their trace metal concentrations.

4. *Lutjanus campechanus*

Table 10 is a comparison of the mean trace metal concentrations in *Lutjanus campechanus* (red snapper) tissues with data from the STOCs study (Presley and Boothe, 1979). This is the only fish species for which comparisons with another location can be made for tissues other than muscle.

Concentrations of Cr, Fe, Ni, and Pb are higher in snapper muscle samples from this study while Cd,

Cu, and Zn concentrations are similar to those reported by Presley and Boothe (1979). Gill samples from this study have higher concentrations of Cr and Pb. The remaining metal concentrations were similar in both studies.

The higher concentrations of Cr, Fe, Ni, Pb, and possibly Cu in muscle and gills may be the result of exposure to higher concentrations of these metals in the water column and sediments in the present study area.

5. *Penaeus aztecus* and *Penaeus setiferus*

Tables 11 and 12 summarize the mean trace metal concentrations in muscle tissue for *Penaeus aztecus* (brown shrimp) and *Penaeus setiferus* (white shrimp), respectively. Similar data from other Gulf studies are included for comparison.

Comparison of brown shrimp data (Table 11) with data from the Strategic Petroleum Reserve Program proposed brine disposal sites at West Hackberry and Weeks Island (Tillery, 1980b) indicates similar concentrations of all metals except Pb, which is higher in samples from this study. Compared with the STOCS data (Horowitz and Presley, 1977), present study concentrations of Cd, Cr, Ni, and Pb are lower. When compared to the MAFLA data (Gould and Moberg, 1978), shrimp from the present study have lower concentrations of Cd but higher concentrations of Fe and Pb. The concentrations from the MAFLA study are not restricted to brown shrimp but include all shrimp species collected.

Table 12 data (white shrimp) are based on only two samples (10 pooled individuals) and gives only estimates of the metal concentrations present. The concentrations, however, are similar to those in SPR samples from West Hackberry and Weeks Island (Tillery, 1980b) except for Ni. Compared to Shokes (1978) data from the West Hackberry site, the concentrations of Cd, Cr, Fe, Ni, and Pb in shrimp from the present study appear to be significantly higher. However, compared to the MAFLA data (Gould and Moberg, 1979) for the combined shrimp analyses, only Pb concentrations appear to be higher.

C. Evaluation of Species for Monitoring of Trace Metal Pollution

Several factors must be considered when selecting a species as a monitoring organism for trace metal pollution. These include: (1) availability of the species during all seasons, (2) the species' natural range of habitat, (3) its ability to accumulate specific trace metals of interest in response to environmental levels, (4) natural variability of trace metals in nonexposed individuals, (5) number of analyses necessary to detect a specific difference between mean concentrations, and (6) ability of the organism to purge itself of certain trace metals when removed from exposure.

The ideal species for monitoring trace metal accumulations should be available in the study area during all seasons in sufficient numbers so that collection will not be a problem. The species should irreversibly accumulate the trace metals of interest in a tissue that is of sufficient size for analysis. The natural variability of the trace metals in the species should be low and the ambient concentration should be low enough that accumulations can be readily detected.

The final factor to consider is the number of samples that must be analyzed so the lowest variance for a particular metal-matrix can be obtained relative to the analytical effort expended. Pooling of individuals in a species to make one analytical sample is a convenient means of reducing costly analytical efforts while obtaining reliable population means. Before pooling of samples can be done, it is necessary to sample a large population individually to determine the standard deviation between the individuals and the mean. This allows the number of individuals needed for pooling to be selected for a specific amount of variance to detect a real difference between population means (i.e., resolution). Resolution is dependent upon both metal and species; therefore, different sample sizes will be needed for different metal-matrix combinations.

Tables 13 and 14 give the number of individuals needed to detect a given difference (%) between population means for *P. aztecus* muscle and *M. undulatus* muscle, respectively.

In *P. aztecus*, a relatively low resolution (30%) requires that a large number of individuals (27 to 55) be pooled for Cd, Cr, Fe, Ni, and Pb. However, for Cu and Zn, only four individuals need to be pooled to see a 30% difference in means.

In *M. undulatus* a low resolution of 30% requires that between 38 and 67 individuals be pooled for Cd, Cr, Cu, Ni, and Pb. Only five individuals are needed for the same resolution in Fe and Zn.

D. Data Synthesis

The purposes of the data synthesis of the trace metal data were as follows:

1. To correlate concentration of trace metals in sediments and benthic and pelagic macrofauna with proximity to and age of the platforms studied, with emphasis on potentially toxic compounds.
2. To correlate concentrations of trace metals in downcore sediments to various ages with proximity to and age of the platforms studied with estimated age of the sediments analyzed, and with initiation of petroleum exploration, development, and production in the overall study area.
3. To determine the probable or possible impact of known spills, discharges, or other sources of petroleum-activity-related contaminants on the study area in general and on the samples collected and analyzed, to review other known sources of pollutants in the study area, and to discuss the Mississippi-Atchafalaya Rivers' discharges in terms of magnitude and contaminant loading, and potential impact on the study area.
4. To discuss the effects of human consumption of seafood products containing various levels of trace metals including contaminant levels and seafood consumption necessary to produce a probable effect.

The results of these synthesis tasks are discussed below.

1. *Task 1: Correlation of Trace Metal Concentrations in Sediments and Biological Samples with Proximity to and Age of Platforms*

As discussed earlier in this report, the difficulty in collecting prescribed species resulted in samples of a

TABLE 13. Number of individuals which must be analyzed to detect a given percentage difference in *Penaeus aztecus* muscle tissue

%	Number of samples						
	<i>Cd</i>	<i>Cr</i>	<i>Cu</i>	<i>Fe</i>	<i>Ni</i>	<i>Pb</i>	<i>Zn</i>
10	488	235	43	378	360	368	28
20	122	59	11	95	90	92	7
30	55	27	5	42	40	41	4
40	31	15	3	24	23	23	2
50	20	10	2	16	15	15	2
60	14	7	2	11	10	11	1
70	10	5	1	8	8	8	1
80	8	4	1	6	6	6	1
90	7	3	1	5	5	5	1
100	5	3	1	4	4	4	1

TABLE 14. Number of individuals which must be analyzed to detect a given percentage difference in *Micropogon undulatus* muscle tissue

%	Number of Samples						
	<i>Cd</i>	<i>Cr</i>	<i>Cu</i>	<i>Fe</i>	<i>Ni</i>	<i>Pb</i>	<i>Zn</i>
10	417	340	463	42	602	446	41
20	105	85	116	11	151	112	11
30	47	38	52	5	67	50	5
40	27	22	29	3	38	28	3
50	17	14	19	2	25	18	2
60	12	10	13	2	17	13	2
70	9	7	10	1	13	10	1
80	7	6	8	1	10	7	1
90	6	5	6	1	8	6	1
100	5	4	5	1	6	5	1

number of species being collected for analysis. This resulted in a minimum of information on intraspecies variability and an inability to distinguish what might in fact be a concentration indicative of trace metal pollution. Therefore, correlation of environmental metal levels to the presence of production platforms had to be based on sediment data.

The approach was to determine ratios of the concentration of the trace metals to:

- a) percent iron,
- b) percent clay,
- c) total hydrocarbons.

These relative concentrations were examined to see if there was a trend toward decreasing levels of the metals with distance from a platform. If a tendency was observed, other factors were examined that could explain the gradient on a basis other than introduction by petroleum related activities (i.e., geochemical processes altered by the existence of the platform). If an increase in concentration relative to Fe was observed along with no increase relative to clay content, then the difference in sediment mineralogy was considered a possible explanation for the differences among relative concentrations at the various distances. If a decrease with distance was observed relative to both Fe and clay, but Fe decreased relative to percent clay with distance from the platform, then the decrease could have been due to natural causes. The platforms were subsequently classified according to the extent to which they could be considered affected by environmental pollution arising from the petroleum activities. This is as follows:

- Probably affected: P1, S6, S7, S11, S17, S18, S19
- Possibly affected: P2, P3, P4, S13, S14, S15, S16, S20
- Probably not affected: S5, S8, S9, S10, S12

Probably affected implies that for one or more metals a tendency toward decreasing relative concentration was observed which could not be explained by relationships between that metal and percent clay or percent iron. Possibly affected implies that gradients were observed in relative concentrations but a possible explanation unrelated to the platform existed. Probably not affected means no meaningful tendencies were observed.

The strongest indications of trace metal contamination due to production were at Platforms S7, S11, and S17. At S7, five metals, Ba, Cr, Cu, Pb, and Zn demonstrated a gradient with distance from the platform, and Pb to Fe and Ba to Fe ratios (Appendix C2) of more than 100 were observed at the N100-m station. At S11, Ba, Cd, Cu, Pb, and Zn were cited as probable pollutants, and Ba, Cu, Pb, and Zn concentrations relative to Fe exceeded 100 at N100 m from the platform. The same five metals were indicated as pollutants at S17, with Ba, Pb, and Zn ratios relative to Fe in excess of 100 at the closest sampling site. Other "probably affected" sites and the metals indicated to have accumulated in the sediment from platform-related activities are listed below:

Platform	Indicated Pollutants
P1	Ba, Ni, Pb
S6	Cr, Ni
S18	Cu, Zn
S19	Zn

Data were obtained on the age of the platform, level of production, and type of production to determine if these correlated with the contingency table between pollution indication and other particular study factors.

Platforms were arbitrarily divided into three age groups: 5 to 8 years, 11 to 17 years, and 22 to 25 years. A contingency table is demonstrated by placing the actual number of observations in each category (termed D_{ij}) in the square and calculating an expected number of observations (termed E_{ij} in the inset box for that square. The results are indicated in Table 15.

If there was no relationship between the likelihood of trace metal pollution and the age of the platform, then the distribution of platforms by age would be mirrored by the distribution by age at each level of potential pollution. To evaluate this, expected frequencies were calculated by the formula:

$$E_{ij} = \frac{(R_i)(C_j)}{N}$$

where E_{ij} = expected frequency in row i, column j
 R_i = number of items in row i
 C_j = number of items in column j,
and N = total number of items investigated.

TABLE 15

Age	Probable Pollution		Possible Pollution		Probable No. Pollution		Total
5 to 8	2	1.75	3	2	0	1.25	5
9 to 17	4	3.5	3	4	3	2.5	10
22 to 25	1	1.75	2	2	2	1.25	5
Total	7		8		5		20

The test for independence is calculated as

$$\chi^2 = \sum_{ij} (O_{ij} - E_{ij})^2 / E_{ij}$$

where O_{ij} is the observed number of items in row i , column j . The test statistic follows an approximate chi-square distribution with degrees of freedom equal to the number of rows less one times the number of columns less one.

For the factor of age, a χ^2 of 2.98 with 4 degrees of freedom was calculated. The tabled value at the 5-percent level is 9.49 for 4 degrees of freedom, and the results are clearly not significant. The conclusion is, then, that no relationship can be assigned between platform age and pollution level.

A relationship between the productivity of the platform and the likelihood of trace metal contamination of the surrounding area was examined in a similar manner, using number of wells drilled as an indicator of activity level. The numbers of wells were divided into three classes: 1 to 7, 12 to 18, and 21 to 24. The resulting contingency table is given below (Table 16).

The chi-squared value for this contingency table was 8.50 with 4 degrees of freedom, again falling below the critical value of 9.59. The conclusion is that no relationship may be assessed between the activity level and the likelihood of pollution around the platform.

The final investigation examined the relationship between level of production of both oil and gas and the likelihood of trace metal contamination. Cumulative production figures were obtained for 17 of the 20 study platforms. Oil production in millions of barrels and gas production in MCF were used to construct contingency tables (Table 17).

The chi-squared values were 3.50 with 4 degrees of freedom and 10.30 with 6 degrees of freedom for oil and gas, respectively. The critical values from the chi-squared distribution at the five-percent level are 9.49 and 12.59 with 4 and 6 degrees of freedom, respectively. Therefore, no relationship is assessed.

The overall conclusion is that if some portion of the trace metal concentrations in sediments analyzed in this study are due to releases from platforms, this contamination cannot be related to the age of the platform, the level of activity, or the level or type of production.

2. Task 2: Correlation of Trace Metal Concentrations in Downcore Sediments with Proximity to and Age of Platforms

The investigation of trace metals in downcore sediments with respect to age of the platform and distance from it could not be successfully completed. The dating of the cores revealed that either extensive mixing had occurred naturally or during the sampling process or the activity of supported Pb-210 was too large and no accurate time frames could be established. In addition, a particle size analysis of the top ten centimeters did not correspond to the analysis of the surficial sediments from the same sites. This raises the possibility that the surficial layer was lost on sampling, which would also adversely affect the results of this investigation. The overall conclusion, then, is that no meaningful information can be obtained from these data, and that no response to this task is possible.

3. Task 2a: Correlation of Trace Metals in Downcore Sediments with Historical Development of the Study Area

The original plan to determine the petroleum "predevelopment" strata in the downcore sediments (by Pb-210 geochronological dating) was not accomplished because of the apparent well-mixed character of the upper (0 to 20+ cm) sediment layers. This well-mixed upper layer was either (1) an artifact of the sampling effort (see Volume I, Part 3, II.C.1.b.), (2) actually representative of rapid sedimentation in this area of the OCS, or (3) the result of the passage of a major storm front (Tropical Storm *Debra*, 1978).

Without the geochronological time frame of reference the trace metal data from the downcore sediments could not be analyzed to draw any further conclusion than those stated in Section IV A.2 of this report.

4. Task 3: Determination of Impacts of Known Spills, Discharges and Other Sources of Petroleum Activity-Related Contaminants

a. Known Spills

Oil spills are known to have occurred near S12 and S13 (Vol. I, Part 1, V.C.11). Crude oils usually have high concentrations of Ni and V, and concentrations of these two metals would have been higher at these two sites. However, no concentrations of trace metals in surficial sediments were observed that could be associated with those spills.

TABLE 16

<u>No. of Wells</u>	<u>Probable Pollution</u>	<u>Possible Pollution</u>	<u>Probable No. Pollution</u>	<u>Total</u>
1 - 7	0 0.7	0 0.8	2 0.5	2
12 - 18	6 4.55	5 5.2	3 5.25	14
21 - 24	1 1.75	3 2	0 1.25	4
Total	7	8	5	20

TABLE 17

OIL

<u>bb1 × 10⁻⁶</u>	<u>Probable Pollution</u>	<u>Possible Pollution</u>	<u>Probable No. Pollution</u>	<u>Total</u>
18.4 - 27.9	2 1.4	1 1.4	1 1.2	4
7.0 - 16.1	3 1.8	1 1.8	1 1.4	5
0.2 - 2.6	1 2.8	4 2.8	3 2.4	8
Total	6	6	5	17

GAS

<u>MCF</u>	<u>Probable Pollution</u>	<u>Possible Pollution</u>	<u>Probable No. Pollution</u>	<u>Total</u>
138.1 - 170.5	0 0.7	2 0.7	0 0.6	2
21.4 - 57.3	4 2.1	1 2.1	0 1.8	5
1.1 - 5.4	1 1.4	1 1.4	2 1.2	4
Total	6	6	5	17

b. Discharges

Produced waters from petroleum producing wells may contain concentrations of trace metals in excess of those found in the marine waters. These produced waters are treated in separators to reduce the volume of hydrocarbons associated with them. Trace metal concentrations, however, are not controlled in the produced waters and relatively large quantities may be introduced to the marine environment.

Platform 1, which contains some of the older producers in this study area, contributes up to 20,000 bpd of produced waters (John Burgbacher, personal communication). These produced waters may contribute to the concentrations of Ba and Pb found in surficial sediments at P1. Other platforms, such as S6 and S19, are also heavy contributors of produced waters. Surficial sediments surrounding both of these platforms have concentrations of Cr, Ni, and Zn that can be related to the platform structure and may reflect the volume of produced water from them. However, S11, which has little discharge (Dick Hickman, personal communication), also has surficial sediment burdens of Ba, Cd, Cr, Cu, Pb, and Zn that are related to the platform. Lack of information on the trace metal content of the produced waters limits the ability to correlate them with trace metal concentrations in the marine environment.

c. Other Sources of Trace Metal Contaminants

The nine trace metals determined in this program are known to be potentially released by various

petroleum production and drilling activities. Other important sources of these trace metals are the exhausts of internal combustion engines located on the platform structures on boats (supply, service or pleasure boats), flaring of natural gas, petroleum seepages, airborne terrestrial sources, riverine inputs, and sacrificial anodes on the platform and satellite structures.

Insufficient data prohibits a quantitative evaluation of the relationship between these sources and sediment trace metal concentrations. Trefry and Presley (1976a) suggest that Cd and Pb burdens in shelf sediments have increased within the last 25-30 years due to input from the Mississippi River. This could be an explanation of the sediment burdens of Cd and Pb at P1, S7, S11, and S17. These same investigators, however, state that there have been no increases in shelf sediment burdens of Co, Cr, Cu, Mn, Ni, or Zn from the Mississippi River over the same time period. This leaves the platform-related sediment concentrations of Ba, Cr, Cu, Ni, and Zn unexplained. The concentrations of Cd and Pb in sediments may suggest the Mississippi River as a source; however, the presence of the sediment concentration gradients with distance from a platform at P1, S7, S11, and S17 remains unexplained and may suggest a platform-related source.

5. Task 4: Impact of Seafood Containing Elevated Trace Metal Concentration

Ninety seven samples from 27 different organisms were analyzed for nine metals. This approach

provided for a rather broad examination of tissue levels of trace metals of biota from the entire study area but it provided very limited data for any single organism. More samples of fewer species would improve the reliability of the data and information regarding possible differences within the study area. Comparison of the values with other areas of the Gulf Coast (STOCS and MAFLA) for those organisms common to all three studies shows no consistent elevations in the Louisiana study area. The levels of the trace metals measured in biota are well below those necessary to cause public health concern.

E. Recommendations for Further Studies

1. Further development is needed of the data evaluation technique of linear regression of sediment trace metal concentrations with other chemical and physical parameters. This technique is a valuable tool in determining abnormal levels of trace metals in the sediments and could be expanded to investigate other chemical and physical parameters that correlate with trace metal

concentrations. More intensive investigations into the technique and its limitations are needed. Sufficient data to further evaluate this technique already exist from previous studies done in the Gulf of Mexico and elsewhere.

2. An in situ means of determining trace metal pollution from a point source (e.g., petroleum production platforms) is needed to eliminate extraneous factors that could prevent an accurate determination of a pollution event or of long-term chronic pollution.

Placing filter-feeding organisms (clams, oysters, etc.) at a petroleum production platform (in non-contaminating cages) and harvesting them 6 months to 1 year later would be one way of determining trace metal pollution. A similar control group of filter-feeding organisms would also be placed at a site such as an inactive production platform, buoy, sunken ship, etc. The trace metal concentrations in the soft tissues of the organisms from the active platform site would be compared to those from the control site; this comparison would provide data on the amount of trace metal pollution coming from an active petroleum production platform.

V. CONCLUSIONS

No strong evidence exists of high levels of trace metal contamination in sediments surrounding petroleum platforms. Generally, significant increases of trace metal concentrations above regional levels are observed at the 100 m stations from the platforms. Sediments collected farther away from the platforms had trace metal levels that usually could be explained by natural geochemical processes. Sediment trace metal concentrations that were not explained by natural processes could not be related to platform age, level of production, or number of wells.

Trace metal inputs from the Mississippi River probably exert the dominant influence on trace metal concentration in sediments in this study area. This influence masks the effects of trace metal release from petroleum platforms.

Attempts at establishing ages of sediment strata using the Pb-210 method failed. This failure is probably due to either the existence of high levels of Ra-226 supported Pb-210, high rates of sedimentation, sediment reworking, or a combination of these. Therefore, trace metal concentrations in downcore sediments could not be interpreted in a historical sense. Concentrations of most trace metals were relatively constant with depth, but for Ba, Cd, and Zn, concentrations in the sediments decreased toward the surface.

Concentrations of Cr, Cu, Fe, and Ni in sheepshead, spadefish and red snapper collected at the platforms may be related to sediment concentrations. Bioaccumulation, however, cannot be verified based on comparison of the overall mean tissue trace metal concentrations and ranges with similar data from other Gulf studies.

VI. ACKNOWLEDGEMENTS

Lead-210 analyses were performed by Controls for Environmental Pollution, Inc., Santa Fe, New Mexico.

Neutron activation analysis was performed by Mr. Andrzej H. Pradzynski, University of Texas, Austin, Texas.

Dr. Herbert L. Windom, Skidaway Institute of Oceanography, Savannah, Georgia, acted as consultant to the Trace Metal Work Group and provided valuable assistance in data interpretation and evaluation.

Laboratory work on this study was performed by Bonnie Fergus, Jack Trevino, Bernie Villasenor and Cara Froboese.

VII. LITERATURE CITED

- Anderson, J.B., and R.R. Schwarzer. 1979. Sedimentology and trace metal concentrations in sediments and organisms. *In* W.B. Jackson [ed.] Environmental assessment of an active oilfield in the northwestern Gulf of Mexico, 1977-1979. Vol. III, Physical and chemical investigations. Nat. Oceanogr. Atmos. Admin., Nat. Mar. Fish. Serv., Environmental Protection Agency.
- Berryhill, H.L., Jr., G.L. Shideler, C.W. Holmes, G.W. Hill, S.S. Barnes, and R. G. Martin, Jr. 1979. Geology. *In* Environmental studies, South Texas Outer Continental Shelf, 1975. Rep. to U.S. Bureau of Land Management. Prep. by U.S. Geological Survey.
- Dames and Moore. 1979. The Mississippi, Alabama, Florida, Outer Continental Shelf baseline environmental survey, 1977-1978. Vol. I-A. Program synthesis report. Final rep. to Bureau of Land Management.
- Duce, R.A., G.L. Hoffman, B.J. Ray, I.S. Fletcher, G.T. Wallace, J.L. Fasching, S.R. Piotrowicz, P.R. Walsh, E.J. Hoffman, J.M. Miller, and J.L. Heffler. 1976. Trace metals in the marine atmosphere: Sources and fluxes, pp. 77-120. *In* H.L. Windsor and R.A. Duce [ed.] Marine pollutant transfer. Lexington Books, Lexington, Massachusetts.
- Gibbs, R.J. 1973. Mechanisms of trace metal transport in rivers. *Science* 180:71-73.
- Gould, G., and B. Moberg. 1979. Macroepifauna and demersal fish trace metal analyses. *In* The Mississippi, Alabama, Florida, Outer Continental Shelf baseline environmental survey, 1977-1978. Vol. II, Chapter 5. Final rep. to Bureau of Land Management. Dames and Moore.
- Holmes, C.W. 1973. Distribution of selected elements in surficial marine sediments of the northern Gulf of Mexico continental shelf and slope. U.S. Geol. Surv. Prof. Paper 814. 7 p.
- Horowitz, A., and B.J. Presley. 1977. Trace metal concentration and partitioning in zooplankton, neuston and benthos from South Texas Outer Continental Shelf. *Arch. Environ. Contam. Toxicology*. 5(2):241-255.
- Johnson, D. 1979. Interpretation of tissue trace metal data. *In* The Mississippi, Alabama, Florida, Outer Continental Shelf baseline environmental survey, 1977-1978. Vol. II. Final rep. to Bureau of Land Management. Dames and Moore.
- Montalvo, J.G., and D.V. Brady. 1974a. Toxic metal determinations in Timbalier Bay water samples. Part 1. Final rep., Offshore Ecol. Invest., Gulf Univ. Res. Consortium, Houston, Texas.
- Montalvo, J.G., and D.V. Brady. 1974b. Toxic metal determinations in offshore water samples. Part 2. Final rep., Offshore Ecol. Invest., Gulf Univ. Res. Consortium, Houston, Texas.
- Nulton, C.P., C.F. Bohnstedt, D.E. Johnson, S.F. Martin, and R.E. Thomas. 1980. Ecological investigations of petroleum production platforms in the Central Gulf of Mexico. Vol. 1: Pollutant fate and effects studies. Part 3: Organic chemical analyses. Draft final rep., Bureau of Land Management. Southwest Research Institute, Houston, Texas. 143 p.
- Potter, P.E., N.F. Shimp and J. Witters. 1963. Trace elements in marine and fresh water argillaceous sediments. *Geochem. Cosmochem. Acta*. 27:669-694
- Presley, B.J., and P.N. Boothe. 1979. Trace metals in epifauna, zooplankton and macronekton, pp. 6-1 to 6-47. *In* Environmental studies, South Texas Outer Continental Shelf, Biology and Chemistry, 1977. Univ. Texas Mar. Sci. Inst., Port Aransas, Texas.
- Pringle, B.H., D.E. Hissong, E.L. Katz, and S.T. Mulawka. 1968. Trace metal accumulations by estuarine mollusks. *J. Sanit. Eng. Div. Amer. Soc. Civ. Engrs.*, 94(3):455.
- Shokes, R.F. 1976. Rate-dependent distributions of lead-210 and interstitial sulfate in sediments of the Mississippi River Delta. Ph.D. Dissertation. Texas A&M Univ., College Station, Texas.
- Shokes, R.F. 1978. Characterization of baseline conditions at the West Hackberry offshore brine disposal site-Texoma group strategic petroleum reserve program. Final rep. to U.S. Department of Energy. Prep. by Science Applications, Inc.
- Shokes, R.F. 1979. Macroepifauna and demersal fish barium and vanadium. *In* The Mississippi, Alabama, Florida, Outer Continental Shelf baseline environmental survey, 1977-1978. Vol. II, Chapter 7. Final rep. to Bureau of Land Management. Dames and Moore.
- Tiech, T.T., T.E. Pyle, D.H. Egger, and R.A. Nelson. 1973. Chemical variations in sedimentary facies of an inner continental shelf environment, northern Gulf of Mexico. *Sediment Geol.* 9:101-115.
- Tillery, J.B. 1980a. Trace metals. *In* W.B. Jackson, [ed.] Environmental assessment of an active oilfield in the northwestern Gulf of Mexico. Nat. Oceanogr. Atmos. Admin., Nat. Mar. Fish. Serv., Environmental Protection Agency.
- Tillery, J.B. 1980b. Trace metals. *In* W.B. Jackson and G.M. Fau [ed.] Biological/Chemical survey of Texoma and Capline sector salt dome brine disposal sites off Louisiana, 1978-1979. Vol. VII. NOAA Technical Memorandum NMFS-SEFC-31, 76 p.
- Trefry, J.H., and B.J. Presley. 1976a. Heavy metal transport from the Mississippi River to the Gulf of Mexico, pp. 39-75. *In* H.L. Windom and R.A. Duce [ed.] Marine pollutant transfer. Lexington Books, Lexington, Massachusetts.
- Trefry, J.H., and B.J. Presley. 1976b. Heavy metals in sediments from San Antonio Bay and the northwest Gulf of Mexico. *Environ. Geol.* 1:283-294.
- Ward, C.H., M.E. Bender, and D.J. Reish [ed.] 1979. The offshore ecology investigation. Effects of oil drilling and production in a coastal environment. *Rice Univ. Stud.* 65(4&5): 1-589.
- Wheeler, R.B., J.B. Anderson, R.R. Schwarzer, and C.L. Hokanson. 1980. Sedimentary processes and trace metal contaminants in the Buccaneer

- Oil/Gas Field, Northwestern Gulf of Mexico. *Environ. Geol.* 3:163-175.
- White, T.T., K.E. Turgon, and A.W. Blizzard. 1977. Rig monitoring. *In* Baseline monitoring studies, Mississippi, Alabama, Florida Outer Continental Shelf, 1975-1976. Vol. VI. Final rep. to U.S. Bureau of Land Management. State Univ. System Fla. Inst. Oceanogr.
- Williams, S.E., and J.J. Jones. 1974. The distribution and origin of bottom sediments in Timbalier Bay, Louisiana. Final rep., Offshore Ecol. Invest., Gulf Univ. Res. Consortium.
- Windom, H.L., W.S. Gardner, W.M. Dunstan, and G.A. Paffenhofer. 1976. Cadmium and mercury in a coastal marine ecosystem, pp. 135-158. *In* H.L. Windom and R.A. Duce [ed.] Marine pollutant transfer. Lexington Books, Lexington, Massachusetts.
- Young, E.J. 1954. Studies of trace metals in sediments. Ph.D. Thesis, M.I.T., Cambridge, Massachusetts.

VIII. PERSONAL COMMUNICATIONS

1. **John Burgbacher**
Shell Oil Company
One Shell Square Rm 3650
P.O. Box 60124
New Orleans, LA 70160

2. **Richard R. Hickman**
Exxon
P.O. Box 60626
New Orleans, LA 70160

APPENDICES

APPENDIX A
Abbreviations used in this work

Abbreviations used in this work

AAS	Atomic Absorption Spectrophotometry	NBS	National Bureau of Standards
Bi-210	Bismuth-210	NAA	Nuclear Activation Analysis
BGOF	Buccaneer Gas/Oil Field	OEI	Offshore Ecology Investigation
BLM	The Bureau of Land Management	OCS	Outer Continental Shelf
HAP	Hydrated Antimony Pentoxide	STOCS	South Texas Outer Continental Shelf
Pb-210	Lead-210	SPR	Strategic Petroleum Reserve
MAFLA	Mississippi, Alabama and Florida Study	TOC	Total Organic Carbon

APPENDIX B

Metal Concentrations in Surficial Sediments by Platform and Station in $\mu\text{g/g}$ Dry Weight

Transect	Distance				Mean
	0100	0500	1000	2000	
Station 01					
E	152.800	17.200	51.000	26.400	61.850
N	94.600	61.600	58.000	60.800	68.750
S	191.800	45.200		44.400	93.800
W			10.800	42.200	28.500
Mean	146.400	41.333	39.933	43.450	67.779
Station 02					
E	56.800	43.400	118.400	92.800	75.511
N	64.800	81.800	67.200	117.600	82.850
S	107.800		68.000	87.200	87.666
W	66.200	68.600	78.000		70.933
Mean	73.900	64.600	82.900	99.200	80.150
Station 03					
E	71.800			44.200	60.760
N	81.400	34.600	41.600		52.533
S		61.400		52.200	56.800
W	119.800	64.600	84.400	35.000	75.950
Mean	91.000	53.533	63.000	43.800	62.833
Station 04					
E	79.900	77.800		97.400	85.033
N		76.800	75.800	48.600	67.066
S	39.000	132.400		84.600	85.333
W	163.200	75.600	31.000		89.933
Mean	94.033	90.650	53.400	76.866	78.737
Station 21					68.700
Station 23					47.500
Station 24					26.600
Grand Mean	101.333	62.529	59.808	65.829	61.757

Transect	Distance				Mean
	0100	0500	1000	2000	
Station 01					
E	.350	.185	.406	.187	.289
N	.226	.412	.427	.281	.339
S	.637	.414	.182	.278	.377
W	.200	.184	.177	.363	.231
Mean	.353	.298	.298	.279	.307
Station 02					
E	.151	.223	.112	.272	.185
N	.173	.340	.190	.395	.274
S	.261	.252	.190	.274	.244
W	.319	.203	.316	.274	.278
Mean	.226	.254	.202	.303	.246
Station 03					
E	.050	.042	.034	.048	.044
N	.065	.075	.062	.208	.102
S	.045	.044	.047	.115	.062
W	.036	.075	.079	.249	.109
Mean	.049	.059	.055	.155	.079
Station 04					
E	.281	.098	.114	.140	.171
N	.211	.121	.155	.119	.151
S	.425	.140	.119	.341	.256
W	.430	.105	.083	.101	.179
Mean	.336	.116	.117	.175	.186
Station 21					
.					.359
Station 22					
.					.209
Station 23					
.					.124
Station 24					
.					.136
Grand Mean	.241	.181	.168	.228	.205

Transect	Distance				Mean
	0100	0500	1000	2000	
Station 01					
N	12.230	9.580	6.120	7.670	9.270
S	14.300	6.940	7.410	10.200	9.712
W	11.060	7.770	9.070	10.710	9.652
Mean	11.860	10.960	9.450	7.890	10.040
Mean	12.362	8.812	8.012	9.117	9.575
Station 02					
N	4.570	6.300	4.190	4.830	4.927
S	4.510	6.120	5.540	4.830	5.250
W	5.410	5.800	8.440	5.530	6.295
Mean	4.880	6.600	7.190	5.440	6.350
Mean	4.842	5.977	6.340	5.907	5.766
Station 03					
N	5.290	5.300	5.630	4.190	5.143
S	4.400	4.200	3.410	6.800	4.725
W	5.170	5.290	5.540	7.190	5.797
Mean	3.950	5.370	4.120	5.590	4.757
Mean	4.702	5.085	4.675	5.942	5.101
Station 04					
N	8.825	10.060	10.210	7.120	9.028
S	10.210	9.510	9.980	9.200	9.725
W	8.000	9.550	10.580	9.710	9.460
Mean	7.770	8.990	9.350	10.960	9.267
Mean	8.701	9.527	10.030	9.247	9.376
Station 21					
.					7.030
Station 22					
.					6.980
Station 23					
.					7.410
Station 24					
.					9.450
Grand Mean	7.651	7.350	7.264	7.553	7.586

Transect	Distance				Mean
	0100	0500	1000	2000	
Station 01					
E	14.528	14.464	11.774	11.027	12.234
N	14.542	9.307	11.716	13.481	12.261
S	15.366	13.176	9.837	13.686	13.016
W	13.891	12.688	11.690	12.154	12.605
Mean	14.581	12.408	10.254	12.587	12.457
Station 02					
E	5.849	10.122	3.830	9.745	7.215
N	5.821	13.906	8.982	10.329	9.759
S	11.570	9.345	11.548	11.716	11.044
W	12.300	6.358	12.715	16.110	11.870
Mean	8.885	9.932	9.268	11.975	10.015
Station 03					
E	2.459	4.081	3.723	2.375	3.081
N	2.451	3.217	2.518	10.122	4.577
S	2.566	2.566	2.851	7.966	3.987
W	3.294	3.600	4.051	7.847	4.698
Mean	2.692	3.366	3.285	7.077	4.105
Station 04					
E	16.250	10.741	9.695	10.037	12.188
N	16.538	10.193	11.336	9.552	11.904
S	20.184	10.877	9.766	10.570	12.849
W	10.986	11.027	10.025	10.479	10.629
Mean	15.989	10.709	10.205	10.159	11.765
Station 21					
.					13.248
Station 22					
.					10.877
Station 23					
.					7.654
Station 24					
.					10.724
Grand Mean	10.536	9.103	8.253	10.449	10.105

Transect	Distance				Mean
	0100	0500	1000	2000	
Station 01					
F	.974	.831	.556	.674	.782
N	1.060	.577	.696	.943	.819
S	.743	.729	.761	.991	.806
W	.916	.921	.846	.606	.822
Mean	.923	.764	.714	.803	.801
Station 02					
F	.426	.654	.383	.455	.473
N	.432	.711	.568	.480	.547
S	.592	.601	.788	.520	.625
W	.530	.580	.807	.972	.722
Mean	.495	.636	.630	.606	.593
Station 03					
F	.352	.373	.366	.380	.366
N	.343	.441	.263	.621	.417
S	.327	.364	.396	.624	.427
W	.256	.486	.361	.538	.410
Mean	.319	.416	.346	.540	.405
Station 04					
F	.830	.746	.852	.577	.760
N	1.060	.825	.925	.782	.898
S	.733	.892	.846	.934	.851
W	.646	.734	.698	1.990	1.017
Mean	.817	.799	.830	1.070	.879
Station 21					
.					.791
Station 22					
.					.737
Station 23					
.					.720
Station 24					
.					.882
Grand Mean	.638	.653	.631	.754	.726

Transect	Distance				Mean
	0100	0500	1000	2000	
Station 01					
E	9.925	9.990	9.380	8.450	9.490
N	12.430	9.510	10.670	11.350	10.990
S	10.990	11.120	8.570	12.330	10.752
W	10.100	11.280	10.030	10.280	10.422
Mean	10.861	10.475	9.662	10.602	10.400
Station 02					
E	6.220	10.450	6.680	9.250	7.935
N	7.610	12.080	8.780	9.700	9.542
S	10.280	10.050	10.450	10.410	10.297
W	10.410	8.760	12.640	12.750	11.140
Mean	8.630	10.335	9.637	10.527	9.782
Station 03					
E	6.005	5.980	5.980	4.450	5.648
N	4.310	5.030	4.760	10.450	6.137
S	5.430	6.480	6.690	6.820	6.355
W	3.920	6.180	5.910	8.480	6.122
Mean	4.916	5.917	5.835	7.550	6.054
Station 04					
E	12.650	8.140	10.030	9.640	10.396
N	12.960	9.610	11.490	9.400	10.865
S	15.550	11.350	9.820	9.980	11.675
W	10.540	8.280	7.930	10.870	9.395
Mean	12.925	9.335	9.817	9.972	10.512
Station 21					
.					12.355
Station 22					
.					8.900
Station 23					
.					9.200
Station 24					
.					9.910
Grand Mean	9.333	9.015	8.737	9.662	9.639

Transect	Distance				Mean
	0100	0500	1000	2000	
Station 01					
N	23.185	13.950	12.760	11.070	16.123
N	23.500	13.380	11.940	18.890	16.927
S	27.170	14.000	11.000	15.500	16.917
W	18.600	14.000	13.500	14.410	15.127
Mean	23.113	13.832	12.300	14.967	16.053
Station 02					
N	8.225	8.000	7.090	8.230	7.923
N	10.040	8.230	8.000	8.640	8.727
S	9.880	10.630	11.500	11.110	10.780
W	8.640	12.990	14.470	12.000	12.025
Mean	9.196	9.962	10.265	9.995	9.854
Station 03					
N	7.000	7.750	6.640	7.090	7.106
N	7.680	6.500	4.730	7.500	6.602
S	7.000	6.000	6.500	19.490	9.747
W	7.530	7.680	5.350	7.820	7.095
Mean	7.302	6.982	5.805	10.475	7.641
Station 04					
N	30.125	18.160	18.500	19.760	22.579
N	17.500	17.000	22.440	15.000	17.983
S	13.170	20.670	18.000	21.550	18.347
W	44.450	17.720	17.720	17.250	24.285
Mean	26.311	18.387	19.165	18.390	20.563
Station 21					
.					12.335
Station 22					
.					14.170
Station 23					
.					17.935
Station 24					
.					21.260
Grand Mean	16.480	12.290	11.883	13.456	14.976

Transect	Distance				Mean
	0100	0500	1000	2000	
Station 01					
E	9.500	7.000	9.100	4.300	7.475
N	9.800	8.900		7.200	8.633
S	13.200	9.200	11.800		11.400
W	8.300	6.700	6.700	7.100	7.200
Mean	10.200	7.950	9.200	6.200	8.387
Station 02					
E	8.100	7.900	4.800	4.500	6.522
N	5.100	12.000	6.900	7.700	7.925
S	6.400	5.600	8.700	25.400	11.525
W	3.000	6.000	7.100	13.600	7.425
Mean	5.650	7.875	6.875	12.800	8.300
Station 03					
E		4.800	3.400		4.100
N	29.900	2.900	3.300	7.900	11.000
S			5.300	8.100	6.700
W			4.100	13.100	8.600
Mean	29.900	3.850	4.025	9.700	11.868
Station 04					
E		8.800	11.300	10.900	10.333
N	9.000	10.600	11.500	8.600	9.925
S	13.400	10.800	12.200	12.800	12.300
W	8.400	14.500	9.100	15.700	11.925
Mean	10.266	11.175	11.025	12.000	11.116
Station 21					8.300
Station 22					6.800
Station 23					11.550
Station 24					7.500
Grand Mean	14.004	7.712	7.781	10.175	9.227

Transect	Distance				Mean
	0100	0500	1000	2000	
Station 01					
N	68.550	35.200	47.200	32.700	48.427
S	48.700	51.600	41.600	48.300	47.550
W	80.600	42.100	31.000	42.700	49.100
Mean	42.300	39.400	37.900	43.100	40.675
Mean	60.037	42.075	39.425	41.700	45.809
Station 02					
N	19.700	25.000	19.200	25.700	22.100
S	21.500	30.800	21.400	27.500	25.100
W	30.300	26.800	33.100	30.800	30.250
Mean	28.200	29.100	34.900	33.600	31.450
Mean	24.925	27.925	27.150	29.400	27.350
Station 03					
N	18.600	20.000	20.000	19.200	19.355
S	19.300	18.600	16.400	24.000	19.625
W	19.700	18.200	19.400	32.000	22.325
Mean	18.200	25.000	21.600	25.700	22.625
Mean	19.000	20.450	19.350	25.225	21.006
Station 04					
N	93.400	31.800	35.500	35.900	54.066
S	41.300	33.600	45.400	33.600	38.475
W	48.200	43.000	34.600	41.600	41.850
Mean	99.000	33.900	30.200	37.000	50.025
Mean	70.475	35.575	36.425	37.025	44.875
Station 21					
.					40.250
Station 22					
.					33.700
Station 23					
.					34.300
Station 24					
.					37.200
Grand Mean	43.609	31.506	30.587	33.337	35.561

Trace Metals in Surficial Sediments - Second Cruise

	Cd	Cr	Cu	Fe	Ni	Pb	Zn	Ba	V
Station 05									
0100	.417	5.630	5.254	.437	7.840	.5.230	23.400	103.400	4.600
0500	.556	5.510	8.780	.494	8.970	12.290	36.300	86.000	7.500
1000	.430	9.010	13.423	.775	15.330	11.170	42.400	83.800	44.300
2000	.789	5.880	17.411	.583	12.530	13.600	42.400	123.800	9.600
Station 06									
0100	.500	15.750	15.213	.865	15.020	29.290	78.500		11.900
*.667	14.670	15.071	.889	11.870	28.030	62.200	34.000	11.300	
0500	.400	16.160	15.586	.851	15.230	29.540	65.000		14.400
*.509	17.150	16.033	.968	15.430	30.280	78.800	51.400		
1000	.524	13.760	16.526	.852	11.950	30.340	68.400		
2000	.532	15.390	14.656	.897	11.760	24.260	50.600		9.800
Station 07									
0100	.316	18.700	14.318	.808	13.790	97.800	61.800	98.800	12.900
0500	.253	13.060	13.572	.799	14.510	27.310	52.700	62.000	12.100
1000	.230	15.230	12.678	.818	15.430	27.310	54.700	49.200	11.200
2000	.373	13.040	12.098	.808	10.600	22.170	45.800	60.800	
Station 08									
0100	.412	9.170	11.614	.722	9.640	16.940	46.800	79.200	7.800
0500	.312	9.430	14.169	.677	13.480	15.140	49.900		10.200
1000	.374	6.910	13.132	.500	9.060	17.530	49.100	60.600	10.700
2000	.235	11.140	12.827	.832	14.200	16.880	49.200	37.200	17.900
Station 09									
0100	.388	9.430	11.338	.762	9.750	13.600	44.400		13.400
*.568	11.600	12.678	.851	13.580	37.230	63.200		13.000	
0500	.347	9.390	13.206	.573	10.410	24.070	48.500	89.400	20.600
1000	.378	12.510	11.891	.828	11.230	21.130	42.900	15.400	19.600
2000	.415	13.890	13.412	.955	13.460	23.640	47.500		14.500
Station 10									
0100	.746	11.270	19.357	1.100	15.050	11.290	43.400		
*.570	10.780	20.732	1.160	17.180	15.140	65.300		15.200	
0500	.604	8.050	13.688	.899	13.030	6.690	32.300		
*.436	8.190	11.932	.827	12.550	8.190	47.600		7.400	
1000	.366	5.830	11.214	.585	8.870	9.160	37.100	60.800	7.500
2000	.368	8.190	9.918	.747	12.350	9.180	40.100		12.600
Station 11									
0100	.575	6.480	44.856	.410	7.520	51.010	82.500	149.800	5.200
0500	.201	7.360	7.010	.602	11.110	6.950	32.500		6.500
1000	.418	5.610	10.550	.571	8.870	7.320	31.300	35.200	6.100
2000	.273	8.810	12.827	.745	14.710	10.430	42.000	13.000	9.200
Station 12									
0100	.528	7.770	19.330	.723	13.300	16.220	48.000	118.200	11.300
0500	.550	8.580	15.486	.808	11.760	9.200	35.200		
1000	.536	6.800	17.411	.605	10.800	14.130	35.700	75.000	9.300
2000	.384	5.670	12.247	.514	10.030	10.200	36.300	72.800	7.700

* Entry skewed four places to the left

Trace Metals in Surficial Sediments - Second Cruise

	Cd	Cr	Cu	Fe	Ni	Pb	Zn	Ba	V
Station 13									
0100	.599	12.910	13.827	.788	9.320	21.330	65.100		8.100
*.609	17.100	15.064	.902	13.990	34.750	87.300		8.600	
0500	.584	15.000	14.725	.889	12.080	24.260	37.100		2.300
1000	.599	16.180	15.555	.925	12.720	27.190	56.900	60.400	13.900
2000	.430	16.370	15.511	.949	15.330	30.040	66.000		11.600
Station 14									
0100	.920	12.520	13.427	.587	10.030	44.730	167.300	240.800	7.000
0500	.273	9.300	7.259	.696	8.690	13.390	36.200		8.400
1000	.117	10.470	7.308	.790	11.830	17.130	44.400		10.800
2000	.193	9.560	7.466	.722	9.850	10.670	34.700	79.400	11.600
Station 15									
0100	.323	13.680	9.695	.724	14.820	46.170	65.400	93.800	11.100
0500	.298	14.200	8.651	.846	13.990	42.450	71.000	493.400	12.500
1000	.225	8.640	7.378	.555	11.380	22.500	43.000	115.800	10.400
2000	.268	10.090	6.499	.716	11.760	12.340	31.100	57.200	
Station 16									
0100	.495	10.550	7.881	.696	8.900	19.450	50.400	179.800	
0500	.281	8.100	8.853	.510	8.190	23.020	44.500		10.600
1000	.217	11.040	8.278	.771	11.520	17.870	44.700	55.000	10.300
2000	.402	13.990	7.457	.724	11.420	20.110	71.400	50.600	
Station 17									
0100	.892	13.110	12.690	.583	9.250	136.020	192.900	245.400	9.600
*.823	12.090	12.173	.523	9.060	120.330	180.200	200.200	12.800	
0500	.235	14.460	7.905	.712	11.730	59.330	64.600	193.400	12.100
*.371	11.230	8.706	.663	11.180	54.150	73.400	203.800	10.800	
1000	.173	14.770	7.457	.775	13.690	41.700	60.700	99.600	11.100
2000	.201	10.740	6.153	.747	10.070	17.360	33.800	114.200	10.400
Station 18									
0100	.251	7.770	11.288	.703	10.600	14.650	50.300	88.800	8.200
*.398	12.780	8.402	.812	9.150	20.000	50.100			
0500	.300	9.700	7.536	.711	9.110	11.500	13.700	87.600	9.200
1000	.178	6.800	7.820	.478	7.520	15.700	40.900	86.200	9.000
2000	.144	9.360	6.582	.656	9.560	14.740	30.800		9.400
Station 19									
0100	.023	2.700	1.033	.185	4.820	2.090	19.300		1.400
0500	.008	3.240	.910	.253	5.650	2.110	16.300		2.100
1000	.041	3.470	.830	.229	5.510	.000	14.500		
2000	.014	2.320	1.107	.151	4.430	2.090	17.500		
Station 20									
0100	.273	8.580	8.682	.704	11.520	10.000	34.400		8.200
0500	.155	7.320	4.761	.505	9.230	7.900	31.200		
1000	.206	6.880	6.499	.625	7.950	5.230	29.400		6.900
2000	.232	6.260	9.739	.535	8.290	12.030	29.500	56.000	8.200

* Entry skewed four places to the left

APPENDIX C1

Graph of Metal Concentration vs. Fe Concentration for 5N HNO₃ Leach of Surficial Sediments

I: May 1978 Cruise (Primary Platforms)

II: August 1978 Cruise (Secondary Platforms and Control Sites)

For each graph:

1—indicates two data points

2—indicates three data points

3—indicates four data points

BA VERSUS FE FOR BLM2B1XC

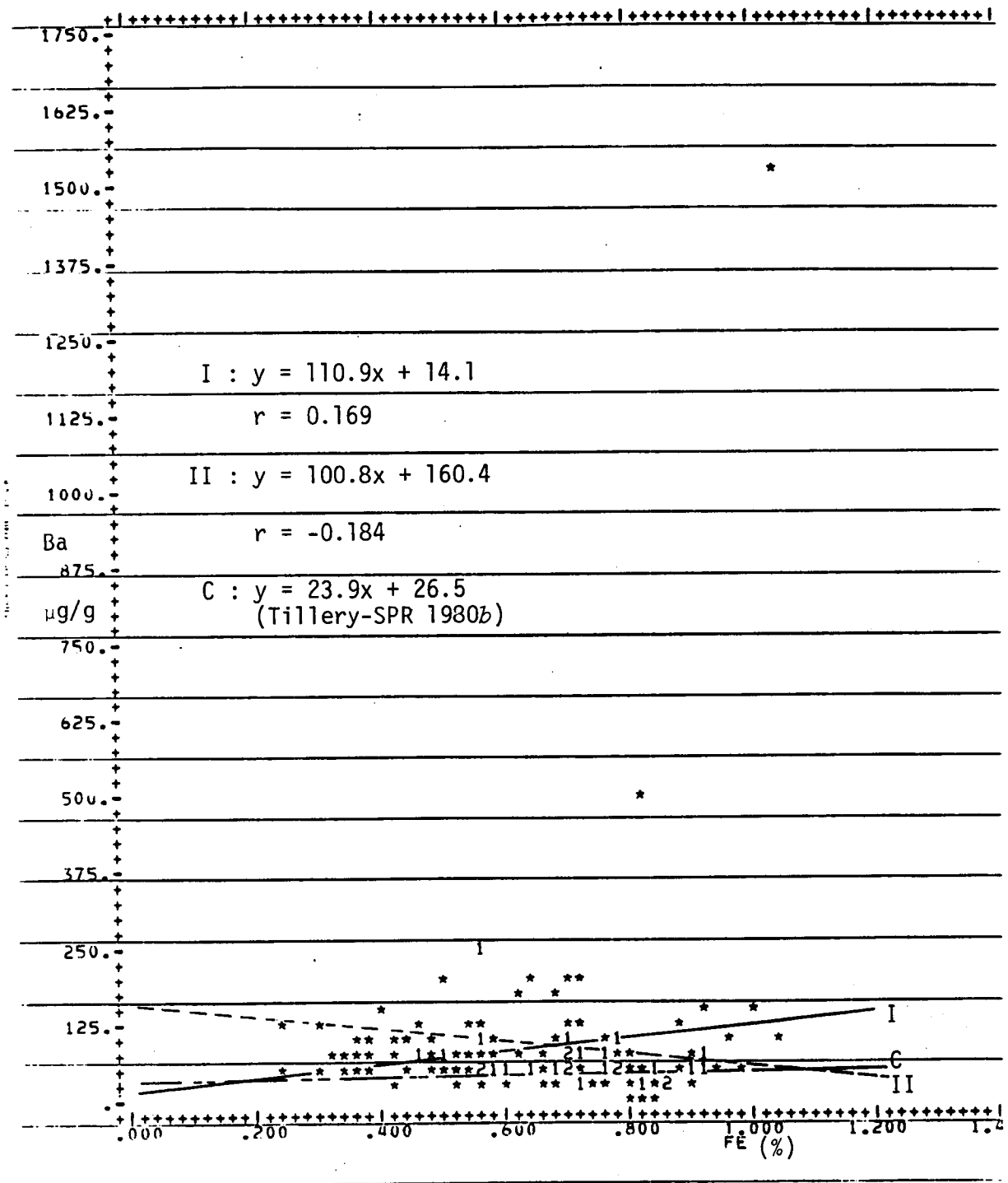


FIG. 1. Surficial sediment Ba concentration ($\mu\text{g/g}$) vs Fe concentration (%)

Cd VERSUS Fe FOR BLM251CX

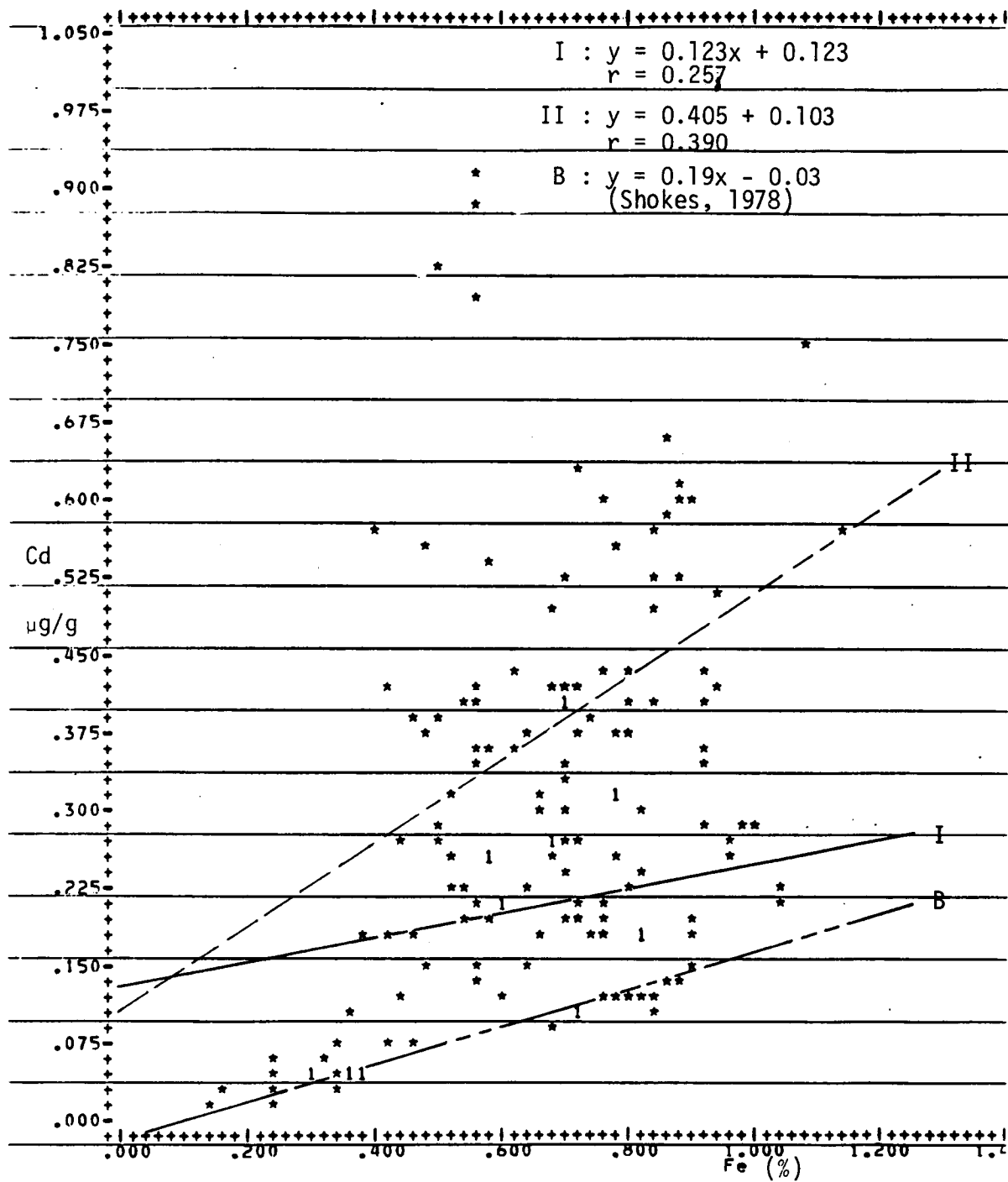


FIG. 2. Surficial sediment Cd concentration (µg/g) vs Fe concentration (%)

Cr VERSUS Fe FOR BLM2B1CX

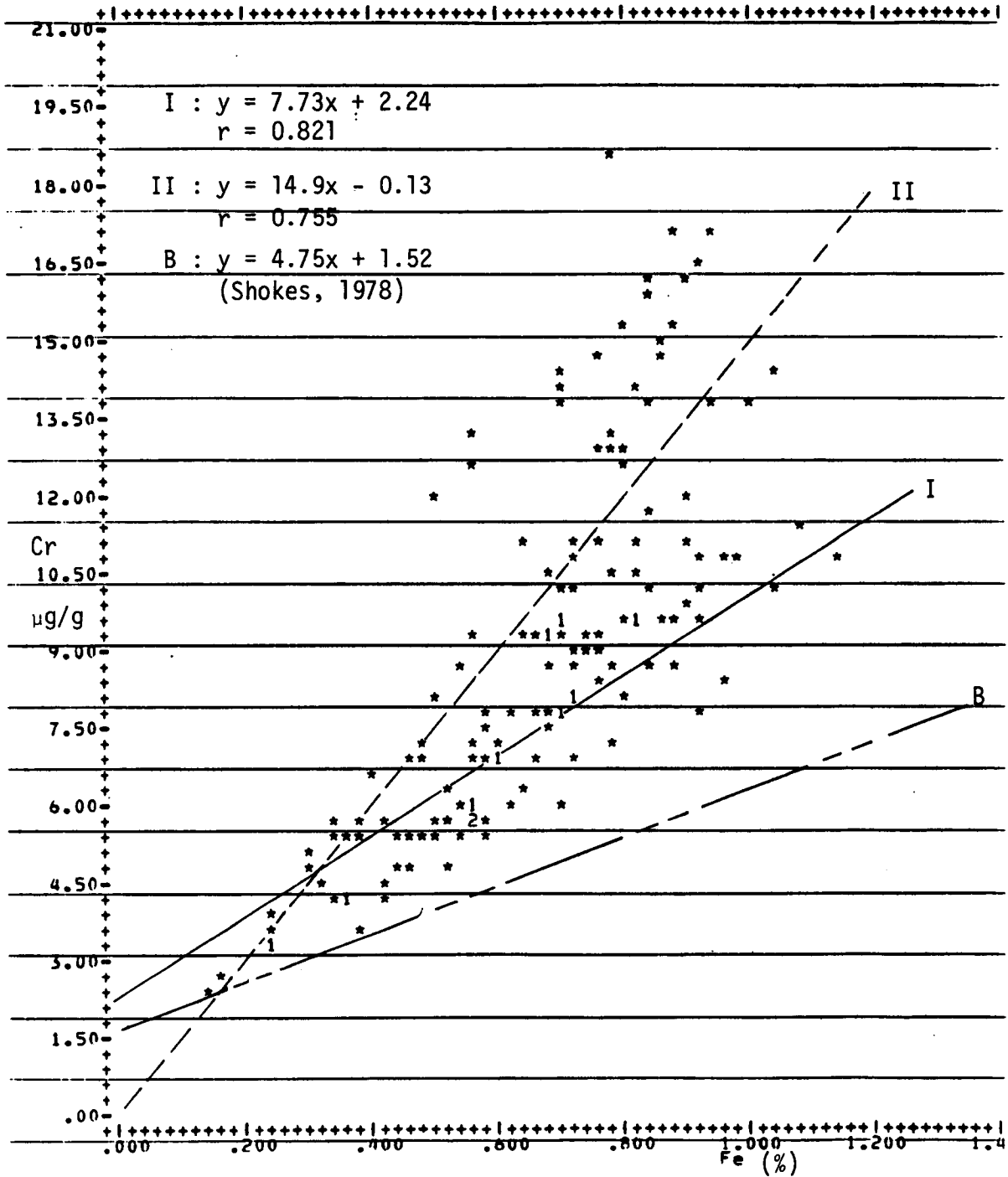


FIG. 3. Surficial sediment Cr concentration (µg/g) vs Fe concentration (%)

Cu VERSUS Fe FOR BLM2B1CX

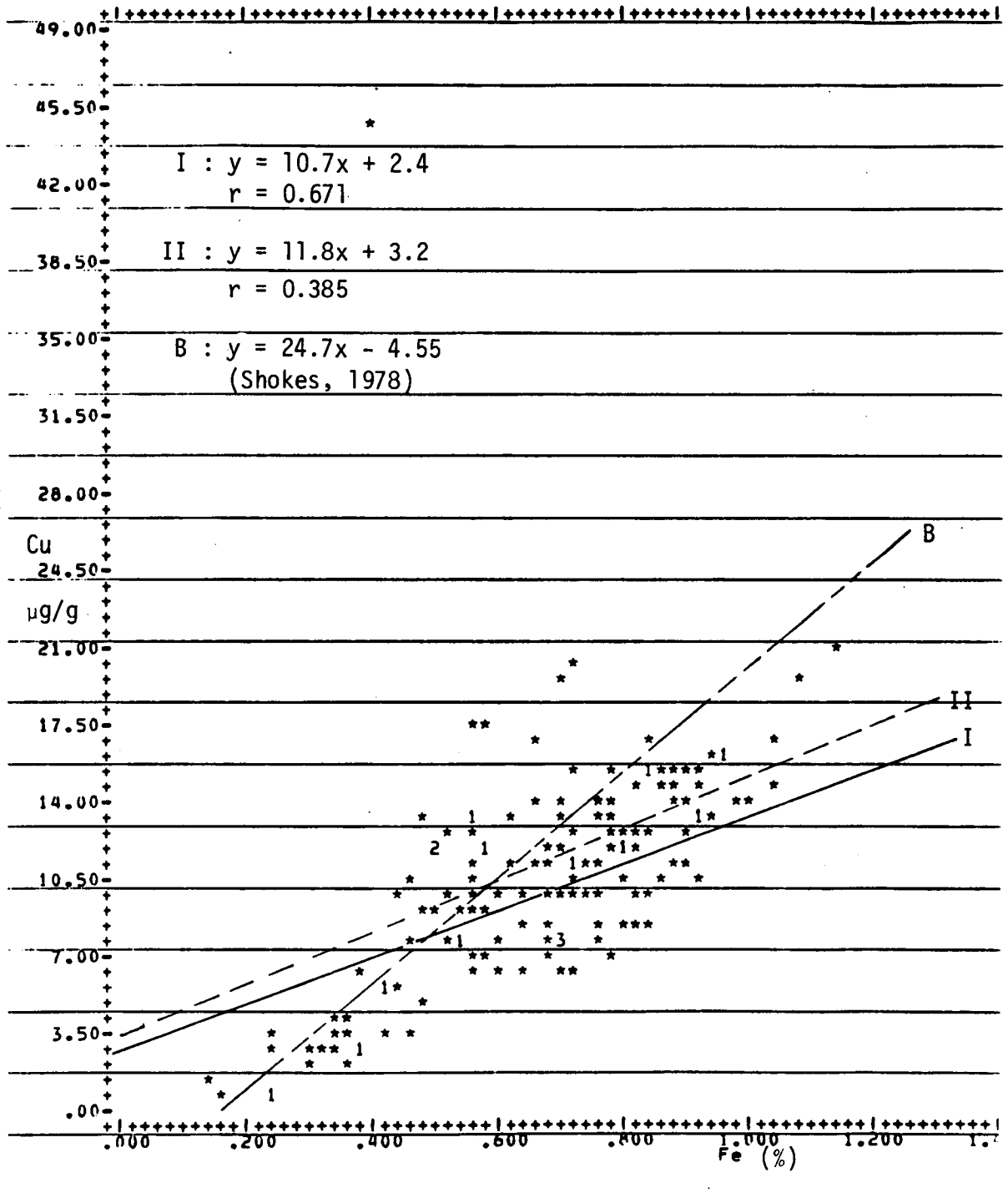


FIG. 4. Surficial sediment Cu concentration (µg/g) vs Fe concentration (%)

Pb VERSUS Fe FOR 6LM2B1CX

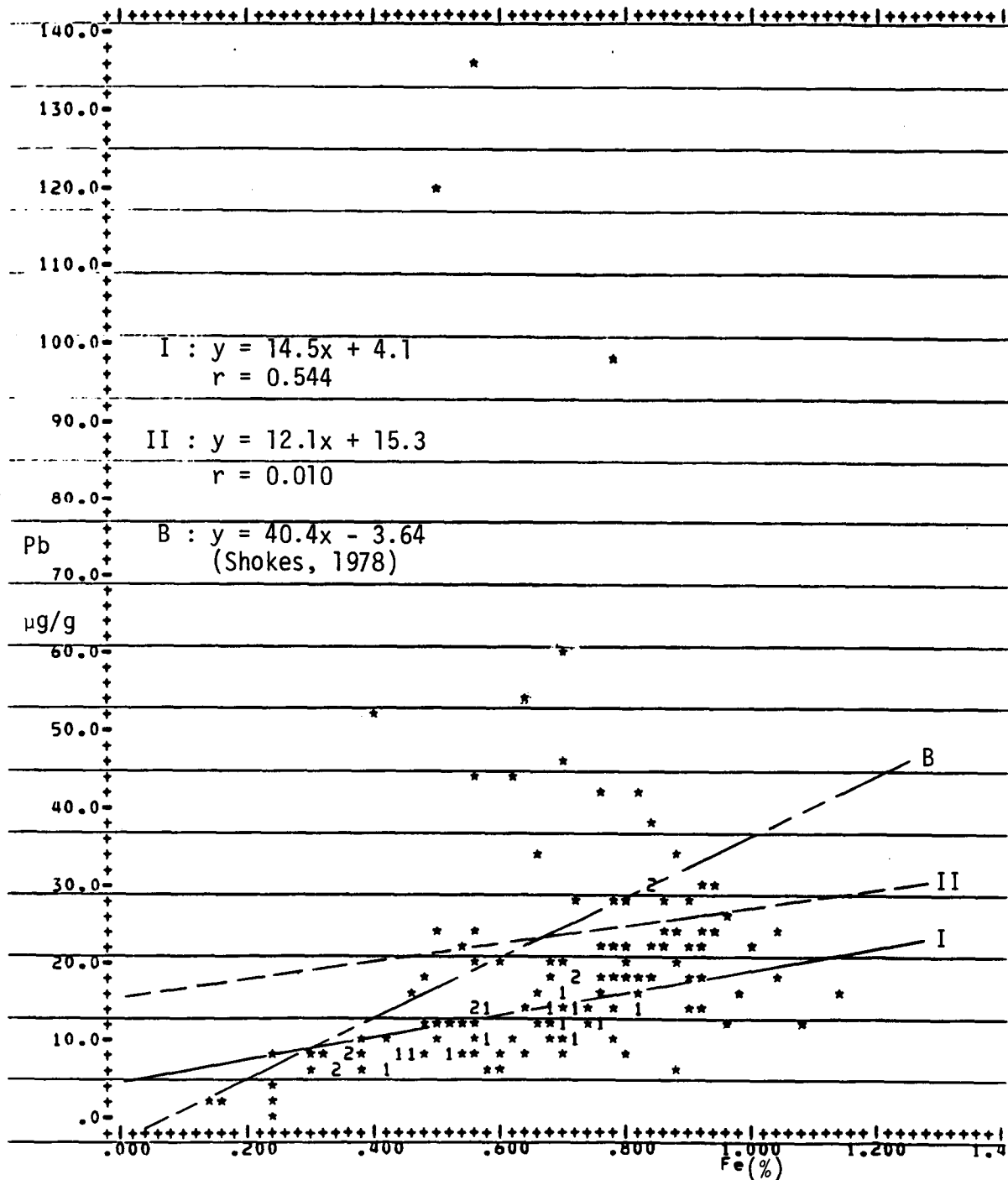


FIG. 5. Surficial sediment Pb concentration ($\mu\text{g/g}$) vs Fe concentration (%)

Ni VERSUS Fe FOR BLM2B1CX

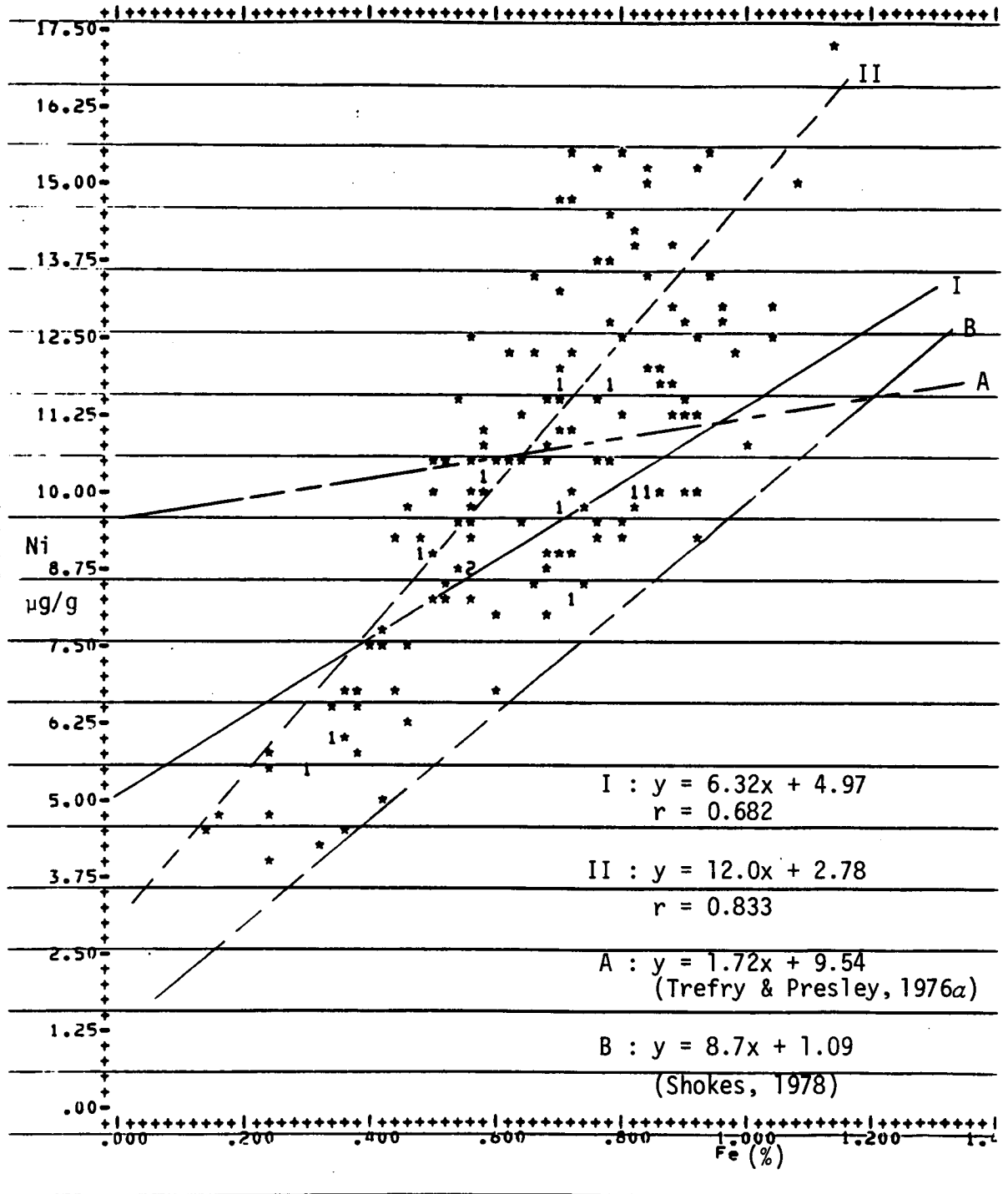


FIG. 6. Surficial sediment Ni concentration ($\mu\text{g/g}$) vs Fe concentration (%)

V VERSUS FE FOR BLM281XC

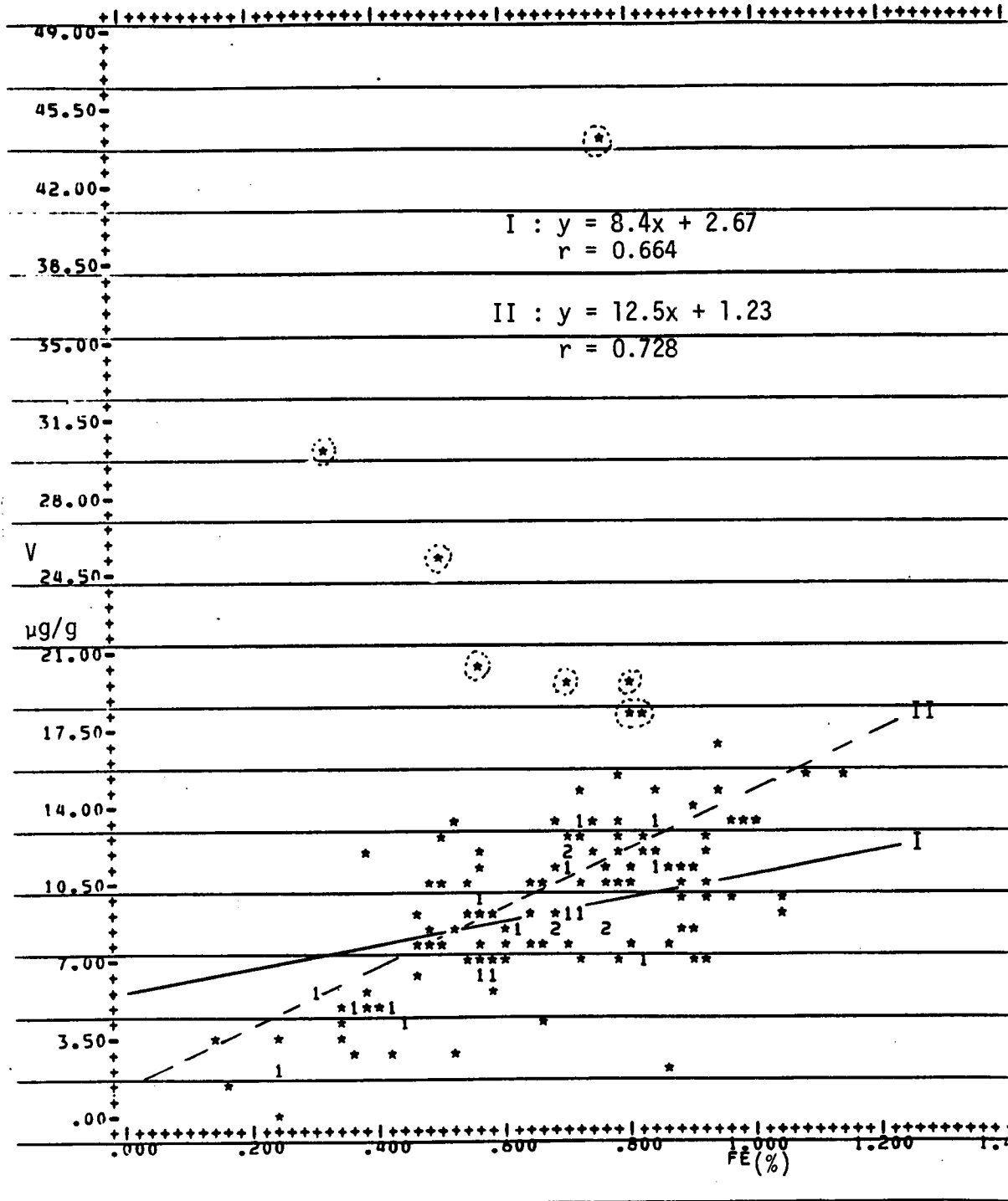


FIG. 7. Surficial sediment V concentration (µg/g) vs Fe concentration (%)
(Samples circled not included in regression)

Zn VERSUS Fe FOR 8LM2B1CX

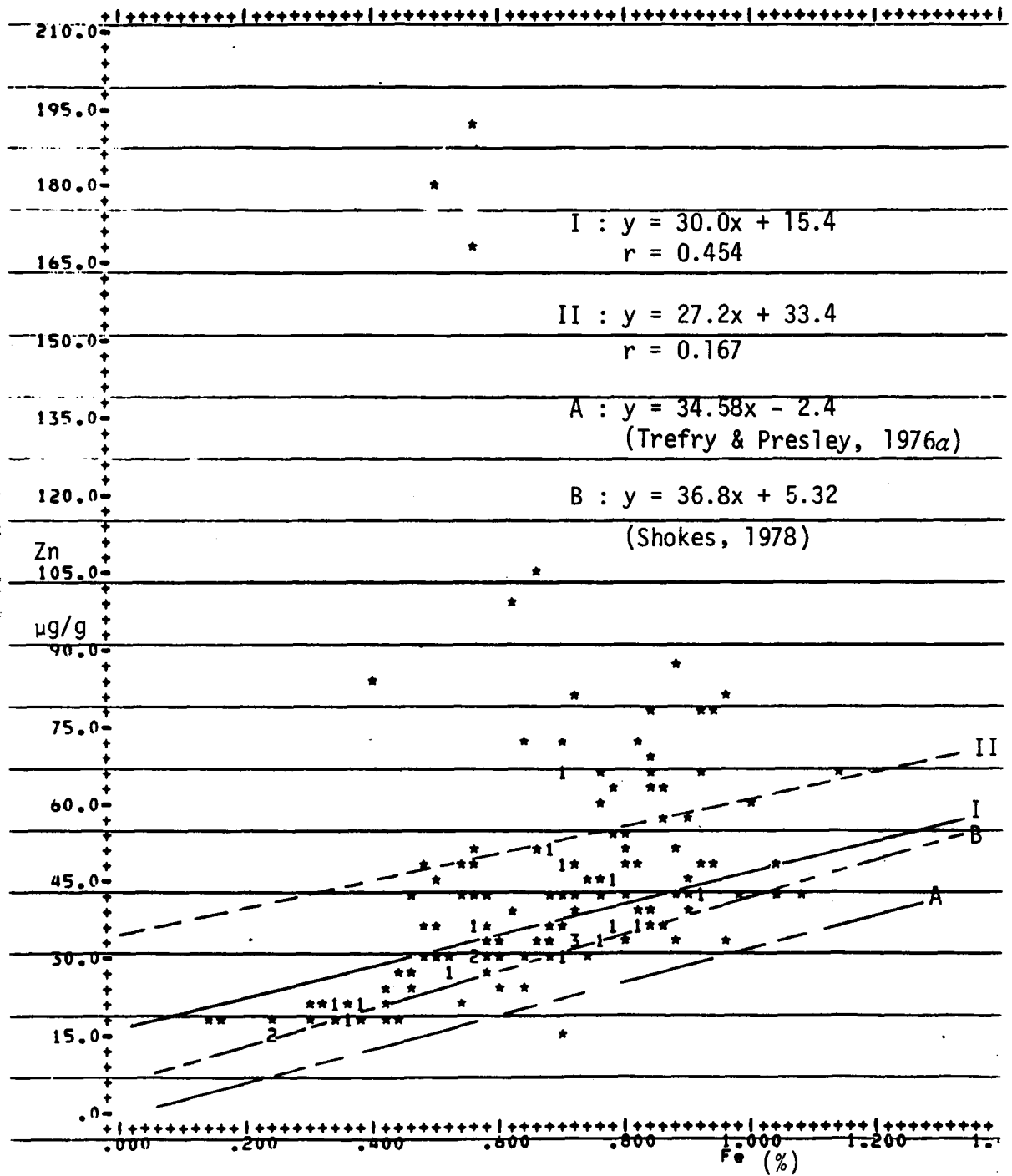


FIG. 8. Surficial sediment Zn concentration (µg/g) vs Fe concentration (%)

APPENDIX C2

Metal Concentrations vs Fe Concentrations from 5N HNO₃ Leach of Surficial Sediments by Platform and Station

		Distance				
		0100	0500	1000	2000	Mean
1	Transect					
2	Station 01					
3	E	.437	.223	.730	.277	.416
4	N	.213	.714	.614	.309	.462
5	S	.857	.568	.239	.281	.486
6	W	.218	.200	.209	.599	.306
7	Mean	.431	.426	.448	.366	.417
8	Station 02					
9	E	.451	.341	.292	.598	.420
10	N	.400	.478	.333	.823	.509
11	S	.441	.419	.241	.527	.407
12	W	.602	.350	.392	.282	.406
13	Mean	.473	.397	.315	.557	.435
14	Station 03					
15	E	.123	.113	.093	.126	.113
16	N	.190	.170	.232	.350	.232
17	S	.138	.121	.119	.184	.140
18	W	.141	.154	.219	.463	.244
19	Mean	.148	.139	.166	.277	.182
20	Station 04					
21	E	.442	.131	.134	.243	.237
22	N	.199	.147	.168	.153	.166
23	S	.580	.157	.141	.160	.310
24	W	.666	.143	.119	.051	.244
25	Mean	.471	.144	.140	.202	.239
26	Station 21					.469
27	Station 22					.284
28	Station 23					.187
29	Station 24					.154
30	Grand Mean	.380	.276	.267	.350	.295
31						
32						
33						
34						
35						
36						
37						
38						
39						
40						
41						
42						
43						
44						
45						
46						
47						
48						
49						
50						
51						
52						
53						
54						
55						
56						
57						

Transect	Distance				Mean
	0100	0500	1000	2000	
Station 01					
E	11.427	11.528	11.007	11.380	11.335
N	13.491	12.028	10.647	10.817	11.745
S	14.886	10.658	11.919	10.807	12.067
W	12.988	11.900	11.170	13.020	12.259
Mean	13.188	11.528	11.185	11.506	11.851
Station 02					
E	9.068	9.633	10.940	10.615	10.064
N	10.440	8.608	9.754	10.063	9.716
S	9.139	9.651	10.711	10.635	10.034
W	9.208	9.810	8.910	8.683	9.152
Mean	9.463	9.425	10.078	9.999	9.741
Station 03					
E	14.501	14.450	15.383	11.026	13.840
N	12.828	9.728	12.966	10.950	11.618
S	15.810	14.533	13.990	11.522	13.963
W	15.430	11.049	11.413	10.390	12.070
Mean	14.642	12.440	13.438	10.972	12.873
Station 04					
E	10.236	13.485	11.984	12.340	12.011
N	9.632	11.527	10.789	11.765	10.928
S	10.914	10.706	12.506	10.396	11.130
W	12.028	12.248	13.395	5.508	10.794
Mean	10.702	11.991	12.168	10.002	11.215
Station 21					
					8.998
Station 22					
					9.471
Station 23					
					10.338
Station 24					
					10.714
Grand Mean	11.998	11.346	11.717	10.619	10.650

Transect	Distance				Mean
	0100	0500	1000	2000	
Station 01					
E	15.988	17.406	13.982	16.361	15.934
N	13.719	16.130	16.833	14.296	15.244
S	20.681	18.074	12.926	13.810	16.372
W	15.165	13.776	13.818	20.056	15.703
Mean	16.388	16.346	14.389	16.130	15.813
Station 02					
E	15.821	15.477	10.000	21.418	15.679
N	13.475	19.558	15.813	21.519	17.591
S	19.544	15.549	14.655	22.531	18.069
W	23.208	10.962	15.756	16.574	16.625
Mean	18.012	15.386	14.056	20.510	16.991
Station 03					
E	6.744	10.941	10.172	6.250	8.526
N	7.146	7.295	9.574	16.300	10.078
S	7.847	7.049	7.199	12.766	8.715
W	12.867	7.407	11.222	14.586	11.520
Mean	8.651	8.173	9.541	12.475	9.710
Station 04					
E	24.386	14.398	11.379	17.395	16.889
N	15.602	12.355	12.255	12.215	13.106
S	27.536	12.194	11.584	11.317	15.647
W	17.006	15.023	14.362	5.266	12.914
Mean	21.132	13.492	12.385	11.548	14.639
Station 21					
.					17.409
Station 22					
.					14.758
Station 23					
.					10.929
Station 24					
.					12.159
Grand Mean	16.045	13.349	12.592	15.165	14.051

Distance					
Transect	0100	0500	1000	2000	Mean
Station 01					
E	9.787	12.022	16.871	12.537	12.804
N	11.726	16.482	15.330	12.036	13.893
S	14.791	15.254	11.261	12.442	13.437
W	11.026	12.248	11.856	16.964	13.023
Mean	11.832	14.001	13.829	13.494	13.289
Station 02					
E	14.484	15.979	17.441	20.330	17.058
N	17.616	16.990	15.458	20.208	17.568
S	17.365	16.722	13.261	20.019	16.841
W	19.625	15.103	15.663	13.117	15.881
Mean	17.276	16.198	15.455	18.418	16.836
Station 03					
E	16.829	16.032	16.339	11.711	15.227
N	12.566	11.406	18.099	16.828	14.754
S	16.606	17.802	16.894	10.929	15.557
W	15.315	12.716	16.371	15.762	15.040
Mean	15.328	14.489	16.925	13.807	15.137
Station 04					
E	18.201	10.912	11.772	16.707	14.398
N	12.226	11.648	12.422	12.020	12.079
S	21.214	12.724	11.608	10.685	14.057
W	16.316	11.226	11.361	5.462	11.091
Mean	16.989	11.627	11.790	11.218	12.906
Station 21					
					16.182
Station 22					
					12.076
Station 23					
					13.051
Station 24					
					11.236
Grand Mean	15.356	14.078	14.499	14.234	13.839

14.459

		Distance				
Transect	0100	0500	1000	2000	Mean	
Station 01						
N	25.154	16.787	22.950	16.424	20.328	
S	22.170	23.189	17.155	20.032	20.636	
W	36.568	19.204	14.455	15.641	21.467	
Mean	20.306	15.201	15.957	21.779	18.810	
Station 02						
N	23.804	12.232	18.512	18.088	18.159	
S	23.241	11.575	14.085	18.000	16.725	
W	16.689	17.687	14.594	21.365	17.583	
Mean	16.302	22.397	17.931	12.346	17.244	
Station 03						
N	21.739	20.777	18.142	18.658	19.829	
S	22.391	14.739	17.985	12.077	16.798	
W	21.407	16.484	16.414	31.234	21.384	
Mean	29.414	15.802	14.820	14.535	18.642	
Station 04						
N	49.779	24.343	21.714	34.246	32.520	
S	16.509	20.606	24.259	19.182	20.139	
W	17.967	23.173	24.277	23.073	31.472	
Mean	68.808	24.142	25.387	8.668	31.751	
Station 21						
					15.729	
Station 22						
					19.227	
Station 23						
					24.963	
Station 24						
					24.104	
Grand Mean						
	27.015	18.645	18.477	19.209	20.921	

Transect	Distance				Mean
	0100	0500	1000	2000	
Station 01					
E	83.600	42.359	84.892	48.516	64.841
N	45.943	89.428	59.770	51.220	61.590
S	108.479	57.750	40.736	43.088	62.513
W	46.179	42.780	44.799	71.122	51.220
Mean	71.050	58.079	57.549	53.486	60.041
Station 02					
F	51.385	38.226	50.131	56.488	49.056
N	49.769	43.319	37.676	57.292	47.014
S	51.182	44.592	42.005	59.231	49.252
W	53.208	50.172	43.247	34.568	45.298
Mean	51.386	44.077	43.264	51.893	47.655
Station 03					
F	52.174	53.619	54.645	50.526	52.741
N	56.851	42.177	62.357	38.687	50.008
S	60.245	50.000	48.990	51.282	52.629
W	71.094	51.440	59.834	47.770	57.534
Mean	60.091	49.309	56.456	47.056	53.228
Station 04					
F	155.900	42.627	41.667	62.218	75.603
N	38.962	40.727	49.081	42.967	42.938
S	65.757	48.206	40.898	44.580	49.850
W	153.251	46.185	43.266	18.593	65.323
Mean	103.467	44.436	43.728	42.079	58.427
Station 21					
					52.142
Station 22					
					45.726
Station 23					
					48.478
Station 24					
					42.177
Grand Mean					
	71.498	48.975	50.249	48.628	50.984

Trace Metals/FE in Surficial Sediments - Second Cruise

	Cd	Cr	Cu	Fe	Ni	Pb	Zn
Station 05							
0100	.954	12.883	12.023	1.000	17.941	11.968	53.547
0500	1.126	11.154	17.773	1.000	18.158	24.879	73.482
1000	.555	11.626	17.320	1.000	19.781	14.413	54.710
2000	1.353	10.086	29.864	1.000	21.492	23.328	72.727
Station 06							
0100	.578	18.208	17.587	1.000	17.364	33.861	90.751
0500	.470	18.989	18.315	1.000	17.897	34.712	76.381
1000	.615	16.150	19.397	1.000	14.026	35.610	80.282
2000	.593	17.157	16.339	1.000	13.110	27.046	56.410
Station 07							
0100	.391	23.144	17.720	1.000	17.067	121.040	76.485
0500	.317	16.345	16.986	1.000	18.160	34.180	65.957
1000	.281	18.619	15.499	1.000	18.863	33.386	66.870
2000	.462	16.139	14.973	1.000	13.119	27.438	56.683
Station 08							
0100	.571	12.701	16.086	1.000	13.352	23.463	64.820
0500	.461	13.929	20.929	1.000	19.911	22.363	73.708
1000	.748	13.820	26.264	1.000	18.120	35.060	98.200
2000	.282	13.389	15.417	1.000	17.067	20.288	56.135
Station 09							
0100	.509	12.375	14.879	1.000	12.795	17.848	58.266
0500	.606	12.387	23.047	1.000	18.168	42.007	84.642
1000	.457	15.109	14.361	1.000	13.563	23.519	51.812
2000	.435	14.345	14.044	1.000	14.094	24.754	49.738
Station 10							
0100	.678	10.245	17.597	1.000	13.682	10.264	39.455
0500	.672	9.622	15.226	1.000	14.494	7.442	35.929
1000	.628	10.000	19.235	1.000	15.214	15.712	63.636
2000	.493	10.964	13.277	1.000	16.533	12.289	53.681
Station 11							
0100	1.402✓	15.805	109.405✓	1.000	18.341	124.415✓	201.220✓
0500	.334	12.226	11.645	1.000	18.455	11.545	51.987
1000	.732	9.825	18.476	1.000	15.534	12.820	54.816
2000	.366	11.826	17.217	1.000	19.745	14.000	56.376
Station 12							
0100	.730	10.747	26.736	1.000	18.396	22.434	66.390
0500	.681	10.619	19.166	1.000	14.554	11.386	43.564
1000	.886	11.240	28.779	1.000	17.851	23.355	59.008
2000	.747	11.031	23.827	1.000	19.514	19.844	70.623

Trace Metals/FE in Surficial Sediments - Second Cruise

	Cd	Cr	Cu	Fe	Ni	Pb	Zn
Station 13							
0100	.760	16.383	17.547	1.000	11.827	27.069	82.614
0500	.657	16.873	16.564	1.000	13.588	27.289	64.229
1000	.648	17.492	16.816	1.000	13.751	29.395	61.514
2000	.453	17.250	16.345	1.000	16.154	31.654	69.547
Station 14							
0100	1.567	21.329	22.874	1.000	17.087	76.201	285.009
0500	.392	13.362	10.430	1.000	12.486	19.239	52.011
1000	.148	13.253	9.251	1.000	14.975	21.684	56.203
2000	.267	13.281	10.341	1.000	13.643	18.778	48.061
Station 15							
0100	.446	18.895	13.391	1.000	20.470	63.771	90.331
0500	.352	16.785	10.226	1.000	16.537	50.177	83.924
1000	.405	15.568	13.294	1.000	20.505	40.541	77.477
2000	.374	14.092	9.077	1.000	16.425	17.235	43.436
Station 16							
0100	.711	15.158	11.323	1.000	12.787	27.945	72.414
0500	.551	15.882	17.359	1.000	16.059	45.137	89.255
1000	.281	14.319	10.737	1.000	14.942	33.178	57.977
2000	.555	19.323	10.300	1.000	15.773	27.776	98.619
Station 17							
0100	1.530	22.487	21.767	1.000	15.866	233.310	330.875
0500	.330	20.309	11.103	1.000	16.475	83.329	90.730
1000	.223	19.058	9.622	1.000	17.665	53.806	78.323
2000	.269	14.378	8.237	1.000	13.481	23.240	45.248
Station 18							
0100	.357	11.053	16.057	1.000	15.078	20.839	71.550
0500	.422	13.643	10.599	1.000	12.813	16.174	19.269
1000	.372	14.226	16.360	1.000	15.732	32.845	85.565
2000	.220	14.268	10.034	1.000	16.573	22.470	48.951
Station 19							
0100	.124	14.595	5.584	1.000	26.054	11.297	104.324
0500	.032	12.806	3.597	1.000	22.332	8.380	64.427
1000	.158	13.398	3.205	1.000	21.274	0.000	55.985
2000	.093	15.364	7.331	1.000	29.338	13.841	115.894
Station 20							
0100	.388	12.188	12.332	1.000	16.364	14.205	48.664
0500	.307	14.495	9.428	1.000	10.277	15.644	61.782
1000	.330	11.008	10.398	1.000	10.720	8.368	47.040
2000	.434	11.701	18.204	1.000	15.495	22.486	55.180

APPENDIX D

Metal Concentrations vs Percent Clay Content of Surficial Sediments

		Distance				
Transect		0100	0500	1000	2000	Mean
Station 01						
E		.019	.006	.025	.015	.016
N		.013	.028	.031	.013	.021
S		.034	.055	.016	.013	.024
W		.011	.019	.018	.013	.020
Mean		.019	.022	.022	.018	.020
Station 02						
E		.019	.012	.019	.019	.017
N		.025	.015	.012	.026	.019
S		.025	.011	.008	.010	.013
W		.010	.018	.010	.007	.011
Mean		.019	.014	.012	.015	.015
Station 03						
E		.009	.006	.004	.006	.006
N		.030	.023	.007	.029	.022
S		.006	.008	.007	.013	.008
W		.012	.016	.011	.018	.014
Mean		.014	.013	.007	.016	.012
Station 04						
E		.027	.006	.009	.010	.013
N		.032	.013	.007	.006	.014
S		.036	.018	.015	.039	.027
W		.025	.015	.008	.009	.014
Mean		.030	.013	.009	.016	.017
Station 21						.021
Station 22						.029
Station 23						.010
Station 24						.007
Grand Mean		.020	.015	.012	.016	.016

		Distance				
Transect	0100	0500	1000	2000	Mean	
Station 01						
N	.509	.333	.380	.634	.464	
S	.808	.469	.517	.440	.563	
W	.582	.648	.810	.485	.591	
Mean	.645	.645	.670	.565	.631	
Station 02						
N	.387	.352	.710	.338	.446	
S	.644	.274	.446	.322	.396	
W	.515	.261	.350	.212	.318	
Mean	.423	.351	.410	.269	.367	
Station 03						
N	1.070	.719	.670	.493	.738	
S	2.000	1.341	.383	.944	1.167	
W	.718	.962	.803	.826	.827	
Mean	1.317	1.119	.597	.594	.836	
Station 04						
N	.614	.592	.779	.498	.620	
S	1.547	1.012	.454	.467	.870	
W	.678	1.209	1.374	1.116	1.094	
Mean	.823	1.019	.885	1.024	.919	
Station 21					.464	
Station 22					.956	
Station 23					.428	
Station 24					.480	
Grand Mean	.791	.762	.644	.568	.636	

Summary for CU/CLAY in Surficial Sediments

PAGE 3

Transect	Distance				Mean
	0100	0500	1000	2000	
Station 01					
E	.712	.502	.483	.911	.652
N	.822	.629	.849	.581	.720
S	.809	1.098	.878	.619	.851
W	.798	1.308	1.181	1.085	1.093
Mean	.785	.884	.847	.799	.828
Station 02					
E	.675	.565	.649	.681	.642
N	.832	.624	.561	.689	.676
S	1.102	.421	.479	.449	.612
W	.375	.578	.418	.395	.441
Mean	.786	.547	.526	.553	.593
Station 03					
E	.498	.544	.443	.279	.441
N	1.114	1.005	.283	1.406	.952
S	.356	.467	.413	.916	.538
W	1.098	.750	.587	.553	.747 *
Mean	.766	.691	.431	.788	.669
Station 04					
E	1.463	.632	.740	.702	.884
N	2.506	1.084	.515	.485	1.147 *
S	1.711	1.377	1.268	1.215	1.192 *
W	.642	1.553	1.003	.979	1.044
Mean	1.580	1.161	.881	.845	1.116
Station 21					
					.770
Station 22					
					1.490
Station 23					
					.503
Station 24					
					.544
Grand Mean					
	.969	.820	.671	.746	.814

Summary for FE/CLAY in Surficial Sediments

PAGE 4

Transect	Distance				Mean
	0100	0500	1000	2000	
Station 01					
FE	.045	.029	.035	.056	.041
N	.060	.039	.050	.041	.047
S	.039	.061	.068	.045	.053
W	.053	.095	.085	.054	.071
Mean	.049	.056	.059	.049	.053
Station 02					
FE	.043	.037	.065	.032	.044
N	.062	.032	.036	.032	.040
S	.056	.027	.033	.020	.034
W	.016	.053	.027	.024	.030
Mean	.044	.037	.040	.027	.037
Station 03					
FE	.074	.050	.044	.045	.053 *
N	.156	.138	.030	.086	.102
S	.045	.066	.057	.072	.060
W	.085	.101	.052	.038	.069
Mean	.090	.088	.045	.060	.070
Station 04					
FE	.060	.044	.065	.040	.052
N	.161	.088	.042	.040	.082
S	.062	.113	.110	.107	.098
W	.038	.103	.070	.186	.099
Mean	.080	.087	.071	.093	.082
Station 21					
					.055
Station 22					
					.101
Station 23					
					.040
Station 24					
					.045
Grand Mean	.065	.067	.053	.057	.060

		Distance				
Transect	0100	0500	1000	2000	Mean	
Station 01						
N	.436	.347	.583	.698	.516	
S	.702	.643	.773	.489	.651	
W	.578	.927	.765	.558	.707	
Mean	.580	1.163	1.013	.918	.918	
Mean	.574	.770	.783	.665	.698	
Station 02						
N	.618	.584	1.132	.647	.745	
S	1.087	.542	.549	.647	.706	
W	.979	.453	.434	.399	.566	
Mean	.317	.796	.416	.313	.460	
Mean	.750	.593	.632	.501	.619	
Station 03						
N	1.242	.797	.712	.524	.818	
S	1.959	1.572	.535	1.451	1.379	
W	.754	1.178	.970	.784	.921	
Mean	1.307	1.288	.857	.597	1.012	
Mean	1.315	1.208	.768	.839	1.032	
Station 04						
N	1.092	.479	.766	.674	.752	
S	1.964	1.022	.522	.477	.996	
W	1.318	1.457	1.292	1.147	1.294	
Mean	.616	1.161	.793	1.016	.896	
Mean	1.247	1.024	.839	.828	.984	
Station 21						
					.731	
Station 22						
					1.219	
Station 23						
					.584	
Station 24						
					.503	
Grand Mean	.971	.898	.755	.708	.796	

		Distance				
Transect	0100	0500	1000	2000	Mean	
Station 01						
N	1.119	.484	.793	.915	.827	
S	1.328	.904	.865	.814	.977	
W	1.430	1.167	.982	.701	1.070	
E	1.069	1.883	1.368	1.287	1.290	
Mean	1.236	.999	1.001	.929	1.041	
Station 02						
N	1.016	.447	1.202	.574	.810	
S	1.434	.369	.500	.576	.719	
W	.941	.479	.477	.426	.580	
E	.264	1.181	.476	.294	.553	
Mean	.913	.619	.663	.468	.665	
Station 03						
N	1.604	1.033	.790	.834	1.065	
S	3.491	2.031	.531	1.082	1.773	
W	.972	1.091	.942	2.240	1.311	
E	2.510	1.600	.775	.551	1.359	
Mean	2.144	1.438	.759	1.166	1.376	
Station 04						
N	2.987	1.068	1.412	1.382	1.712	
S	2.652	1.809	1.020	.761	1.560	
W	1.116	2.616	2.338	2.477	2.136	
E	2.599	2.496	1.772	1.612	2.119	
Mean	2.338	1.997	1.635	1.558	1.882	
Station 21						
					.829	
Station 22						
					1.941	
Station 23						
					1.022	
Station 24						
					1.079	
Grand Mean	1.657	1.263	1.014	1.030	1.229	

Transect	Distance				Mean
	0100	0500	1000	2000	
Station 01					
N	3.720	1.222	2.932	2.702	2.644
S	2.751	3.486	3.014	2.082	2.833
W	4.242	3.508	3.768	1.932	3.112
Mean	2.431	4.062	3.828	1.848	3.542
Mean	3.286	3.069	3.135	2.641	3.032
Station 02					
N	2.194	1.397	3.254	1.797	2.160
S	3.071	1.381	1.338	1.833	1.905
W	2.886	1.207	1.373	1.180	1.661
Mean	.860	2.645	1.148	.824	1.369
Mean	2.252	1.657	1.778	1.408	1.773
Station 03					
N	3.849	2.667	2.381	2.259	2.789
S	8.864	5.813	1.843	3.333	8.963
W	2.736	3.309	2.812	3.678	3.133
Mean	6.067	5.208	3.130	1.810	4.053
Mean	5.379	4.249	2.541	2.770	3.734
Station 04					
N	9.354	1.871	2.710	2.510	4.111
S	6.250	3.574	2.064	1.706	3.400
W	4.085	5.443	4.494	4.782	4.701
Mean	5.789	4.775	3.020	3.458	4.260
Mean	6.371	3.915	3.072	3.114	4.118
Station 21					
					2.515
Station 22					
					4.616
Station 23					
					2.133
Station 24					
					1.888
Grand Mean	4.322	3.222	2.631	2.483	2.976

Trace Metals/CLAY in Surficial Sediments - Second Cruise

	Cd	Cr	Cu	Fe	Ni	Pb	Zn
1							
2	Station 05						
3	0100	.035	.477	.445	.037	.664	.443
4	0500	.025	.244	.348	.022	.397	.544
5	1000	.046	.959	1.428	.082	1.631	1.188
6	2000	.107	.795	2.353	.079	1.693	1.838
7	Station 06						
8	0100	.017	.530	.512	.029	.506	.986
9	0500	.009	.345	.243	.018	.325	.631
10	1000	.018	.463	.356	.029	.408	1.025
11	2000	.012	.437	.321	.020	.257	.531
12	Station 07						
13	0100	.012	.703	.538	.030	.518	3.677
14	0500	.005	.232	.241	.014	.258	.486
15	1000	.007	.496	.413	.027	.503	.890
16	2000	.014	.476	.442	.029	.387	.809
17	Station 08						
18	0100	.010	.223	.283	.018	.235	.412
19	0500	.009	.285	.428	.020	.407	.457
20	1000	.031	.581	1.104	.042	.761	1.473
21	2000	.011	.513	.591	.038	.658	.778
22	Station 09						
23	0100	.049	1.179	1.417	.095	1.219	1.700
24	0500	.033	.886	1.246	.054	.982	2.271
25	1000	.046	1.526	1.450	.101	1.370	2.577
26	2000	.043	1.447	1.397	.099	1.402	2.463
27	Station 10						
28	0100	.097	1.464	2.514	.143	1.955	1.846
29	0500	.140	2.012	3.183	.209	3.030	1.556
30	1000	.061	.972	1.869	.097	1.478	1.527
31	2000	.088	1.950	2.361	.178	2.940	2.186
32	Station 11						
33	0100	.101	1.137	7.869	.072	1.319	8.949
34	0500	.034	1.227	1.168	.100	1.852	1.158
35	1000	.079	1.058	1.991	.108	1.674	1.381
36	2000	.045	1.444	2.103	.122	2.411	1.710
37	Station 12						
38	0100	.030	.439	1.092	.041	1.751	.916
39	0500	.054	.841	1.518	.079	1.553	.902
40	1000	.086	1.097	2.808	.098	1.743	2.279
41	2000	.053	.788	1.701	.071	1.393	1.417
42							
43							
44							
45							
46							
47							
48							
49							
50							
51							
52							
53							
54							
55							
56							
57							

Trace Metals/CLAY in Surficial Sediments - Second Cruise

	Cd	Cr	Cu	Fe	Ni	Pb	Zn
Station 13 ✓							
0100	.046	1.001	1.072	.061	.722	1.653	5.047
0500	.023	.600	.589	.036	.483	.970	2.284
1000	.019	.520	.500	.030	.409	.874	1.830
2000	.010	.399	.378	.023	.374	.733	1.610
Station 14 ✓							
0100	.161	2.196	2.356	.103	1.760	7.847	29.351
0500	.018	.596	.465	.045	.557	.858	2.521
1000	.012	1.079	.753	.081	1.220	1.766	2.577
2000	.013	.669	.522	.050	.689	.746	2.427
Station 15							
0100	.038	1.609	1.141	.085	1.744	5.432	7.694
0500	.032	1.543	.940	.092	1.521	4.614	7.717
1000	.023	.882	.753	.057	1.161	2.296	4.388
2000	.026	.970	.625	.069	1.131	1.187	2.990
Station 16 ✓							
0100	.062	1.319	.985	.087	1.113	2.431	6.300
0500	.016	.460	.503	.029	.465	1.308	2.528
1000	.016	.806	.604	.056	.841	1.304	3.263
2000	.021	.729	.388	.038	.595	1.047	3.719
Station 17 ✓							
0100	.093	1.366	1.322	.061	.964	14.169	20.094
0500	.014	.866	.473	.043	.702	3.553	3.868
1000	.011	.953	.481	.050	.883	2.690	3.916
2000	.015	.808	.463	.056	.757	1.305	2.541
Station 18							
0100	.016	.483	.701	.044	.658	.910	3.124
0500	.014	.451	.351	.033	.424	.535	3.637
1000	.007	.276	.318	.019	.306	.638	1.663
2000	.012	.787	.553	.055	.803	1.239	2.588
Station 19							
0100	.013	1.500	.574	.103	2.678	1.161	10.722
0500	.004	1.705	.479	.133	2.974	1.111	8.579
1000	.020	1.652	.395	.123	2.624	.000	6.905
2000	.005	.892	.426	.058	1.704	.804	6.731
Station 20 ✓							
0100	.029	.913	.924	.075	1.226	1.064	3.660
0500	.012	.559	.363	.039	.705	.603	2.382
1000	.012	.405	.382	.037	.468	.308	1.729
2000	.013	.352	.547	.030	.466	.676	1.657

APPENDIX E

Metal Concentration vs Hydrocarbon Concentration in Surficial Sediments by Platform and Station

Transect	Distance				Mean
	0100	0500	1000	2000	
Station 01					
N	.012	.010	.037	.006	.016
S	.002	.052	.013	.007	.018
W	.090	.021	.005	.011	.031
Mean	.003	.010	.018	.009	.010
Mean	.026	.023	.018	.008	.018
Station 02					
N	.009	.050	.011	.090	.040
S	.040	.099	.024	.219	.095
W	.013	.030	.011	.023	.019
Mean	.101	.009	.071	.142	.080
Mean	.040	.047	.029	.118	.058
Station 03					
N	.004	.007	.010	.006	.006
S	.014	.014	.020	.068	.029
W	.006	.004	.006	.021	.009
Mean	.004	.007	.034	.044	.022
Mean	.007	.008	.017	.034	.016
Station 04					
N	.012	.006	.007	.005	.012
S	.028	.013	.007	.008	.014
W	.101	.008	.004	.082	.049
Mean	.000	.002	.003	.004	.003
Mean	.054	.007	.005	.024	.022
Station 21					
.					.013
Station 22					
.					.012
Station 23					
.					.015
Station 24					
.					.003
Grand Mean	.031	.021	.017	.046	.019

Transect	Distance				Mean
	0100	0500	1000	2000	
Station 01					
N	.301	.516	.551	.260	.407
NE	.120	.878	.229	.237	.366
S	1.563	.403	.260	.442	.667
SW	.193	.602	.968	.206	.492
Mean	.544	.599	.502	.286	.482
Station 02					
N	.171	1.420	.419	1.605	.903
NE	1.043	1.789	.710	2.681	1.552
S	.274	.702	.508	.459	.485
SW	1.545	.258	1.622	4.369	1.948
Mean	.758	1.042	.814	2.278	1.223
Station 03					
N	.486	.858	1.689	.551	.896
NE	.935	.776	1.113	2.218	1.260
S	.713	.498	.682	1.321	.803
SW	.457	.498	1.763	.989	.926
Mean	.647	.657	1.311	1.269	.971
Station 04					
N	.744	.578	.609	.263	.548
NE	1.350	.992	.445	.603	.847
S	1.932	.561	.349	2.302	1.296
SW	.000	.150	.349	.407	.502
Mean	1.342	.570	.438	.903	.813
Station 21					
.					.288
Station 22					
.					.395
Station 23					
.					.607
Station 24					
.					.228
Grand Mean	.822	.717	.766	1.184	.625

Transect	Distance				Mean
	0100	0500	1000	2000	
Station 01					
N	.422	.779	.700	.374	.568
S	.123	1.178	.362	.313	.494
W	2.171	.684	.202	.565	.925
Mean	.227	.697	1.197	.318	.609
Mean	.735	.834	.635	.392	.649
Station 02					
N	.298	2.282	.383	3.239	1.550
S	1.346	4.065	1.151	5.734	3.074
W	.587	1.131	.695	.972	.846
Mean	3.894	.289	2.869	8.339	3.847
Mean	1.531	1.981	1.274	4.571	2.329
Station 03					
N	.226	.649	1.117	.312	.576
S	.521	.582	.822	3.302	1.306
W	.354	.241	.251	1.464	.602
Mean	.381	.334	1.733	1.388	.959
Mean	.370	.451	1.005	1.616	.860
Station 04					
N	1.772	.617	.578	.371	.834
S	2.187	1.063	.505	.626	1.095
W	4.875	.639	.323	2.580	2.096
Mean	.000	.183	.375	.389	.215
Mean	2.944	.625	.445	.984	1.249
Station 21					
.					.477
Station 22					
.					.615
Station 23					
.					.713
Station 24					
.					.258
Grand Mean	1.395	.962	.839	1.890	.893

Transect	Distance				Mean
	0100	0500	1000	2000	
Station 01					
N	.026	.045	.050	.023	.036
S	.009	.073	.021	.022	.031
W	.105	.038	.022	.041	.051
Mean	.015	.051	.087	.016	.042
Mean	.038	.051	.045	.025	.039
Station 02					
N	.019	.147	.038	.151	.088
S	.100	.208	.073	.266	.161
W	.030	.073	.047	.043	.048
Mean	.168	.026	.182	.503	.219
Mean	.079	.113	.085	.240	.129
Station 03					
N	.034	.059	.110	.050	.063
S	.073	.080	.086	.203	.110
W	.045	.034	.049	.115	.060
Mean	.030	.045	.154	.095	.081
Mean	.045	.054	.099	.115	.078
Station 04					
N	.073	.043	.051	.021	.047
S	.140	.086	.041	.051	.079
W	.177	.052	.028	.225	.120
Mean	.000	.012	.026	.074	.037
Mean	.130	.048	.036	.092	.076
Station 21					
.					.034
Station 22					
.					.042
Station 23					
.					.057
Station 24					
Grand Mean	.073	.066	.066	.118	.059

Transect	Distance				Mean
	0100	0500	1000	2000	
Station 01					
E	.258	.538	.845	.287	.482
N	.105	1.204	.329	.264	.475
S	1.553	.577	.246	.509	.721
W	.165	.620	1.027	.269	.520
Mean	.520	.734	.611	.332	.549
Station 02					
E	.273	2.356	.667	3.074	1.592
N	1.760	3.532	1.125	5.385	2.950
S	.321	1.216	.628	.863	.807
W	3.296	.398	2.852	6.600	3.286
Mean	1.862	1.875	1.318	3.980	2.158
Station 03					
E	.564	.952	1.794	.585	.973
N	.916	.910	1.553	3.409	1.697
S	.789	.610	.824	1.253	.859
W	.454	.573	2.528	1.500	1.263
Mean	.670	.761	1.674	1.686	1.197
Station 04					
E	1.323	.468	.598	.356	.686
N	1.714	1.003	.512	.616	.961
S	3.756	.667	.324	2.407	1.788
W	.000	.137	.296	.404	.279
Mean	2.264	.568	.432	.945	1.052
Station 21					
.					.453
Station 22					
.					.503
Station 23					
.					.828
Station 24					
.					.239
Grand Mean	1.229	.984	1.008	1.735	.872

Transect	Distance				Mean
	0100	0500	1000	2000	
Station 01					
F	.663	.752	1.150	.576	.735
N	.198	1.693	.368	.439	.674
S	3.839	.727	.315	.640	1.380
W	.303	.769	1.383	.377	.708
Mean	1.250	.985	.804	.458	.874
Station 02					
F	.449	1.804	.708	2.735	1.424
N	2.321	2.406	1.025	4.796	2.637
S	.501	1.287	.692	.921	.850
W	2.735	.590	3.265	6.212	3.200
Mean	1.501	1.521	1.422	3.666	2.027
Station 03					
F	.729	1.233	1.991	.932	1.221
N	1.633	1.176	1.544	2.447	1.700
S	.966	.565	.800	3.581	1.478
W	.872	.712	2.289	1.383	1.314
Mean	1.050	.921	1.656	2.085	1.428
Station 04					
F	3.618	1.043	1.103	.730	1.623
N	2.314	1.774	1.000	.983	1.517
S	3.181	1.214	.594	5.199	2.547
W	3.000	.295	.662	.641	.532
Mean	3.037	1.081	.839	1.888	1.711
Station 21					
.					.514
Station 22					
.					.801
Station 23					
.					1.449
Station 24					
.					.512
Grand Mean	1.709	1.127	1.180	2.024	1.164

Transect	Distance				Mean
	0100	0500	1000	2000	
Station 01					
mg					
ZN	2.205	1.897	4.253	1.110	2.366
WS	.410	6.530	1.284	1.123	2.336
NS	11.390	2.185	3.889	1.762	4.056
MS	.690	2.164	3.882	1.127	1.965
Mean	3.673	3.194	2.577	1.280	2.681
Station 02					
mg					
ZN	.969	5.636	1.918	8.541	4.266
WS	4.971	9.005	2.743	15.266	7.996
NS	1.536	3.244	1.991	2.554	2.331
MS	8.928	1.322	7.874	17.393	8.879
Mean	4.101	4.801	3.631	10.938	5.867
Station 03					
mg					
ZN	1.749	3.183	5.998	2.523	3.363
WS	4.145	3.364	5.352	7.830	5.172
NS	2.719	1.713	2.388	5.880	3.175
MS	2.107	2.319	9.241	4.547	4.553
Mean	2.680	2.644	5.744	5.195	4.065
Station 04					
mg					
ZN	11.330	1.827	2.117	1.326	4.150
WS	5.461	3.505	2.023	2.202	3.297
NS	11.642	2.526	1.142	10.035	6.336
MS	.000	.564	1.129	1.374	1.022
Mean	9.477	2.105	1.602	3.734	4.229
Station 21					
.					1.558
Station 22					
.					1.905
Station 23					
.					3.026
Station 24					
.					.896
Grand Mean	4.982	3.186	3.388	5.286	3.028

Trace Metals/HC in Surficial Sediments - Second Cruise

	Cd	Cr	Cu	Fe	Ni	Pb	Zn
Station 05							
0100	.031	.422	.394	.033	.588	.392	1.754
0500	.082	.810	1.291	.073	1.319	1.807	5.339
1000	.012	.250	.373	.022	.426	.311	1.179
2000	.202	1.506	4.460	.149	3.209	3.483	10.860
Station 06							
0100	.007	.227	.220	.012	.217	.423	1.133
0500	.011	.426	.411	.022	.401	.779	1.713
1000	.020	.513	.616	.032	.445	1.130	2.548
2000	.159	4.605	4.386	.268	3.519	7.260	15.142
Station 07							
0100	.013	.760	.582	.033	.560	3.974	2.511
0500	.008	.437	.454	.027	.486	.914	1.764
1000	.005	.321	.267	.017	.325	.575	1.151
2000	.013	.466	.432	.029	.379	.792	1.637
Station 08							
0100	.018	.409	.518	.032	.430	.755	2.086
0500	.021	.628	.943	.045	.897	1.008	3.322
1000	.012	.216	.410	.016	.283	.548	1.535
2000	.095	4.493	5.173	.336	5.727	6.808	19.844
Station 09							
0100	.049	1.187	1.427	.096	1.227	1.711	5.587
0500	.029	.781	1.098	.048	.866	2.002	4.033
1000	.014	.462	.439	.031	.415	.781	1.586
2000	.010	.337	.325	.023	.326	.573	1.151
Station 10							
0100	.097	1.470	2.525	.143	1.963	1.473	5.661
0500	.357	5.110	8.086	.531	7.697	3.952	19.080
1000	.046	.733	1.411	.073	1.116	1.152	4.667
2000	.019	.420	.509	.038	.634	.471	2.058
Station 11							
0100	.179	2.016	13.954	.128	2.339	15.869	25.665
0500	.028	1.009	.961	.083	1.523	.952	4.454
1000	.132	1.770	3.329	.180	2.799	2.310	9.877
2000	.052	1.686	2.454	.143	2.814	1.996	8.036
Station 12							
0100	.067	.983	2.444	.091	1.682	2.051	6.070
0500	.042	.656	1.185	.062	.900	.704	2.693
1000	.019	.235	.601	.021	.373	.488	1.233
2000	.055	.807	1.742	.073	1.427	1.451	5.164

Trace Metals/HC in Surficial Sediments - Second Cruise

	Cd	Cr	Cu	Fe	Ni	Pb	Zn
Station 13							
0100	.007	.162	.173	.010	.117	.267	.815
0500	.010	.249	.245	.015	.201	.403	.950
1000	.013	.349	.336	.020	.273	.587	1.229
2000	.017	.665	.630	.059	.623	1.220	2.680
Station 14							
0100	.034	.465	.498	.022	.372	1.660	6.210
0500	.009	.308	.240	.023	.287	.443	1.197
1000	.008	.720	.502	.054	.813	1.178	3.052
2000	.060	2.947	2.302	.223	3.037	3.290	10.698
Station 15							
0100	.011	.486	.345	.026	.527	1.641	2.324
0500	.018	.841	.513	.050	.829	2.515	4.206
1000	.010	.583	.327	.025	.504	.997	1.906
2000	.022	.833	.537	.059	.971	1.019	2.569
Station 16							
0100	.041	.872	.651	.058	.735	1.607	4.165
0500	.019	.558	.610	.035	.565	1.587	3.067
1000	.007	.373	.280	.026	.389	.604	1.510
2000	.011	.373	.199	.019	.304	.536	1.902
Station 17							
0100	.070	1.030	.997	.046	.727	10.684	15.152
0500	.005	.320	.175	.016	.260	1.313	1.430
1000	.013	1.072	.541	.059	.993	3.026	4.405
2000	.021	1.109	.635	.077	1.040	1.793	3.490
Station 18							
0100	.009	.275	.399	.025	.375	.518	1.780
0500	.005	.158	.123	.012	.149	.188	.224
1000	.059	2.236	2.571	.157	2.473	5.162	13.448
2000	.007	.485	.341	.034	.495	.764	1.596
Station 19							
0500	.004	1.604	.450	.125	2.796	1.044	8.067
1000	.008	.712	.170	.053	1.131	.000	2.976
2000	.006	.931	.444	.061	1.778	.839	7.026
Station 20							
0100	.010	.302	.306	.025	.405	.352	1.211
0500	.028	1.324	.861	.091	1.670	1.429	3.645
1000	.017	.582	.550	.053	.673	.443	2.489
2000	.112	3.021	4.700	.258	4.001	5.806	14.237

APPENDIX F1
Concentration of Trace Metals in Downcore Sediments

DOWNCORE SEDIMENTS - TRACE METALS

PAGE 1

Sample Label	Core Sea cm	Cadmium ua/a	Chromium ua/a	Copper ua/a	Iron %	Nickel ua/a	Lead ua/a	Zinc ua/a	VANADIUM ua/a	BARIUM ua/a
7A0501PN05002B3	0.0-10.0	.386	14.2	14.8	1.50	13.5	10.6	43.9	9.6	69.6
7A0501PN05002B3	10.0-20.0	.392	13.8	16.7	1.54	15.0	13.1	46.4	10.8	35.2
7A0501PN05002B3	20.0-30.0	.390	13.8	16.6	1.18	15.0	12.6	44.9	12.6	-- *
7A0501PN05002B3	30.0-40.0	.421	11.9	16.9	1.23	14.3	12.1	41.4	10.4	95.6
7A0501PN05002B3	40.0-50.0	.415	13.5	17.6	1.50	15.2	13.5	46.9	13.0	148.6
7A0502PN05002B3	0.0-10.0	.436	8.2	14.6	1.03	14.1	11.1	37.9	12.4	76.0
7A0502PN05002B3	10.0-13.5	.459	8.6	14.2	1.06	14.5	10.1	40.4	10.6	96.8
7A0502PN05002B3	13.5-16.0	.457	8.2	13.8	1.05	13.7	9.7	39.4	11.4	143.0
7A0502PN05002B3	16.0-26.0	.449	8.0	14.1	1.02	13.9	8.7	38.4	10.2	132.2
7A0503PN05002B3	0.0-10.0	.554	15.7	18.0	2.07	17.1	12.6	50.8	11.5	61.0
7A0503PN05002B3	10.0-20.0	.644	15.4	19.8	1.05	18.4	13.5	54.3	14.5	--
7A0503PN05002B3	20.0-30.0	.709	15.6	22.0	1.65	18.6	13.5	51.3	15.8	80.0
7A0503PN05002B3	30.0-40.0	.696	14.9	22.2	1.47	19.0	13.5	53.3	15.7	61.8
7A0503PN05002B3	40.0-50.0	.686	15.7	22.7	1.28	19.7	13.1	56.8	15.1	--
7A0504PN05002B3	0.0-10.0	.411	10.7	16.8	1.24	15.0	11.6	49.4	7.4	--
7A0504PN05002B3	10.0-12.0	.388	13.5	18.1	1.72	15.6	11.6	51.8	9.6	--
7A0504PN05002B3	12.0-14.0	.395	11.4	18.1	1.39	17.1	14.5	53.8	11.3	43.8
7A0504PN05002B3	14.0-22.0	.464	10.0	18.1	1.17	18.0	13.5	55.3	10.6	140.6
7A0521C 2B3	0.0-10.0	.561	10.1	18.5	1.19	15.0	13.5	46.4	10.8	78.0
7A0521C 2B3	10.0-20.0	.557	10.4	18.9	1.45	16.1	12.6	49.9	11.6	86.8
7A0521C 2B3	20.0-30.0	.535	9.5	18.9	1.23	15.2	13.5	48.9	12.2	100.2
7A0521C 2B3	30.0-40.0	.561	10.6	19.6	1.66	16.3	14.0	49.4	12.8	83.2
7A0521C 2B3	40.0-50.0	.605	9.7	19.8	1.63	14.8	14.0	47.4	12.6	143.4
7A0522C 2B3	0.0-10.0	.391	8.8	12.1	.93	12.3	9.7	39.9	9.9	40.0
7A0522C 2B3	10.0-20.0	.650	10.1	17.3	1.08	15.2	11.6	47.4	10.2	120.0
7A0522C 2B3	20.0-30.0	.641	8.6	16.6	.98	15.4	9.2	45.4	9.5	178.6
7A0522C 2B3	30.0-40.0	.632	7.4	11.7	.84	13.7	9.2	38.4	8.2	118.6
7A0522C 2B3	40.0-50.0	.623	9.7	16.6	1.59	15.3	12.6	44.9	8.7	187.0
7A0523C 2B3	0.0-10.0	.377	14.9	14.9	.54	14.5	14.0	48.4	10.4	96.6
7A0523C 2B3	10.0-20.0	.271	11.1	14.9	1.26	12.8	12.6	43.4	7.6	--
7A0523C 2B3	20.0-30.0	.260	11.7	15.4	1.30	13.6	12.6	47.4	9.0	--
7A0523C 2B3	30.0-40.0	.260	11.9	15.0	1.31	14.7	13.1	46.9	9.4	88.8
7A0523C 2B3	40.0-50.0	.309	12.4	13.8	1.27	13.7	12.1	43.4	10.8	114.4
7A0524C 2B3	0.0-10.0	.236	9.0	6.8	.92	9.0	11.1	32.4	7.8	--
7A0524C 2B3	10.0-20.0	.216	10.1	9.3	1.04	11.9	8.7	39.9	11.1	50.6
7A0524C 2B3	20.0-30.0	.535	9.9	18.7	1.29	17.2	12.1	53.3	9.3	87.6
7A0524C 2B3	30.0-40.0	.583	12.2	19.6	1.66	18.1	13.1	54.8	9.6	55.8

* Below detection limit

APPENDIX F2

Concentration of Trace Metals in Downcore Sediments

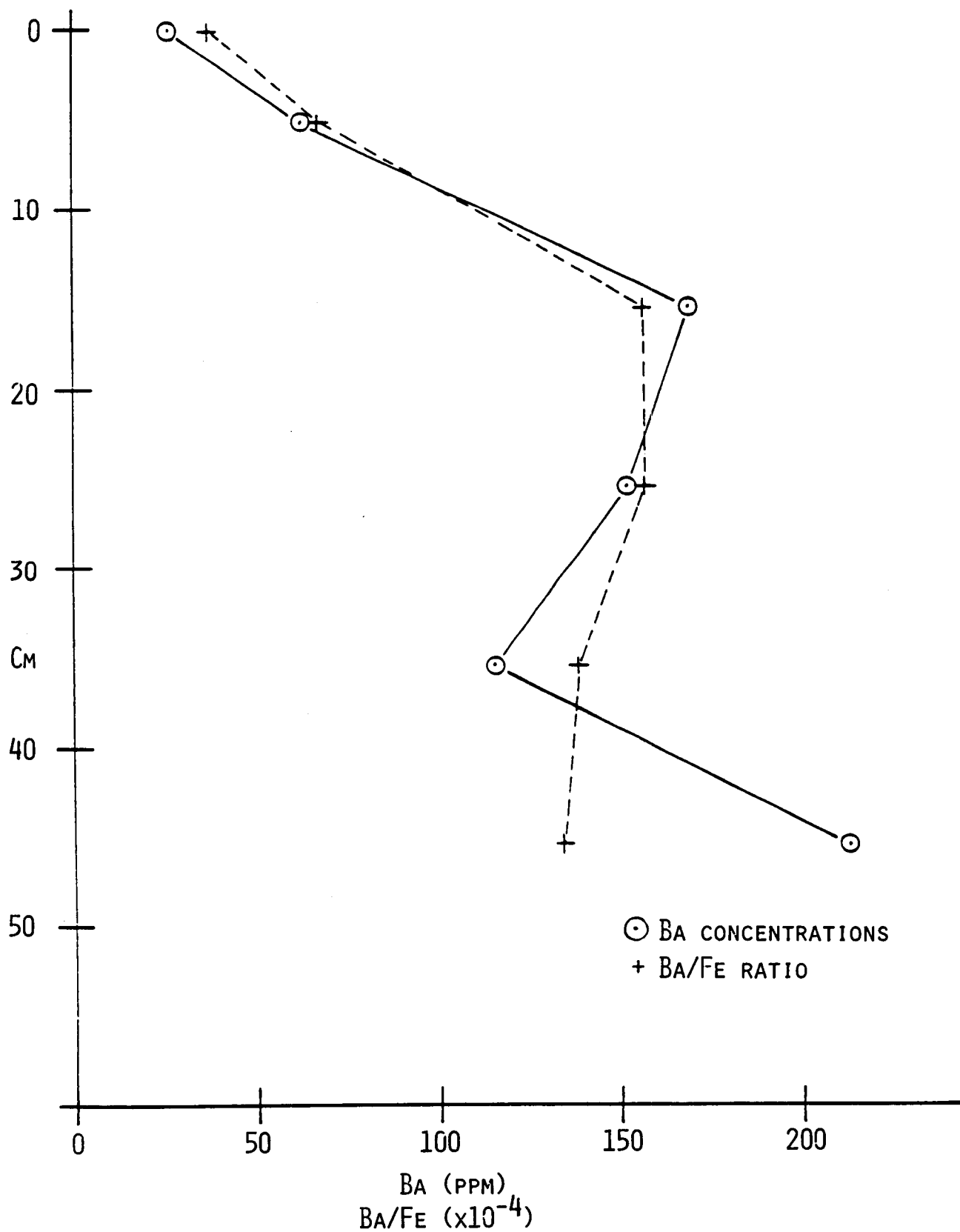


FIG. 1. Barium concentration (ppm) and ratio of Ba/Fe concentrations with depth (cm) in downcore sediment sample from C22.

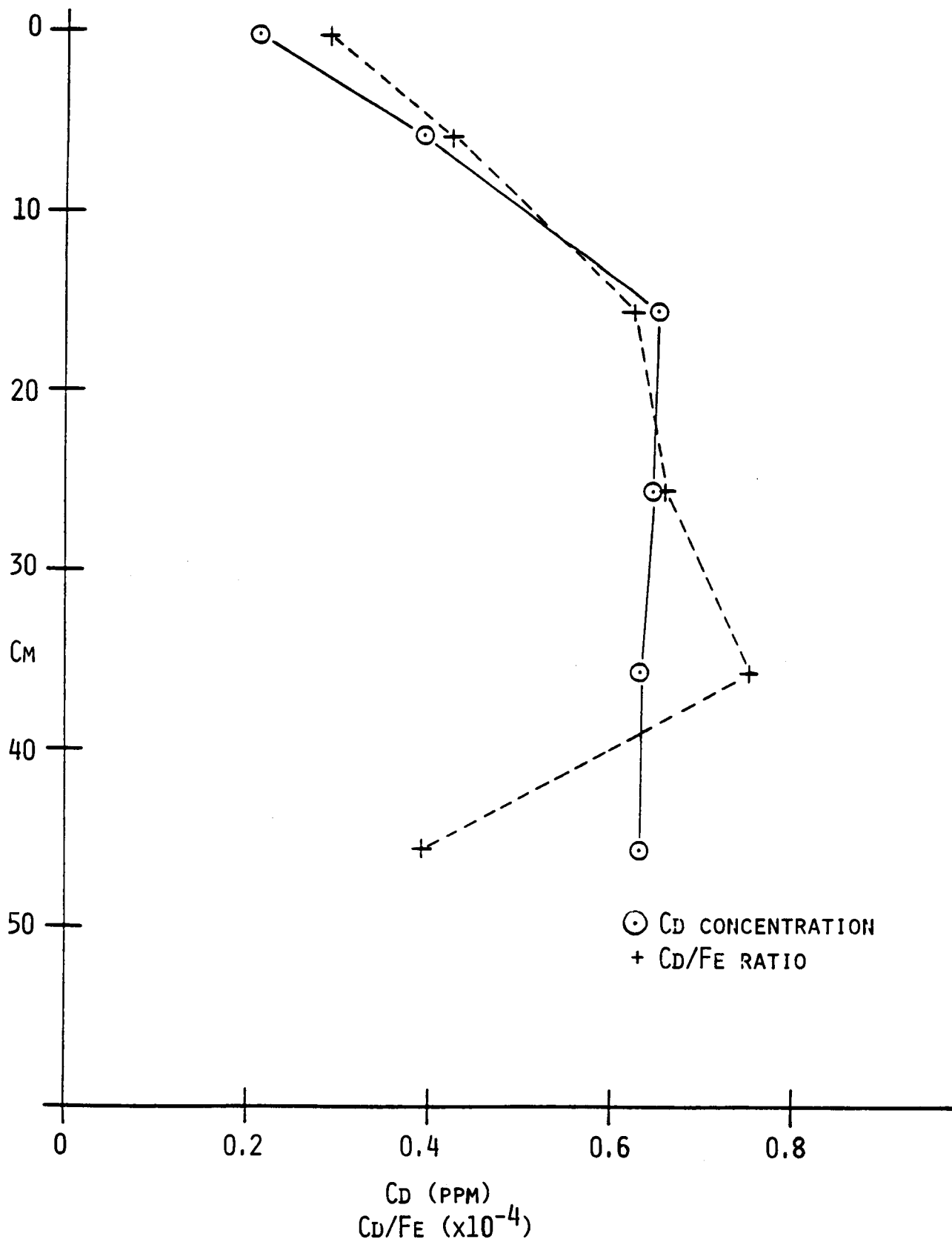


FIG. 2. Cadmium concentration (ppm) and ratio of Cd/Fe concentrations with depth (cm) in downcore sediment sample from C22.

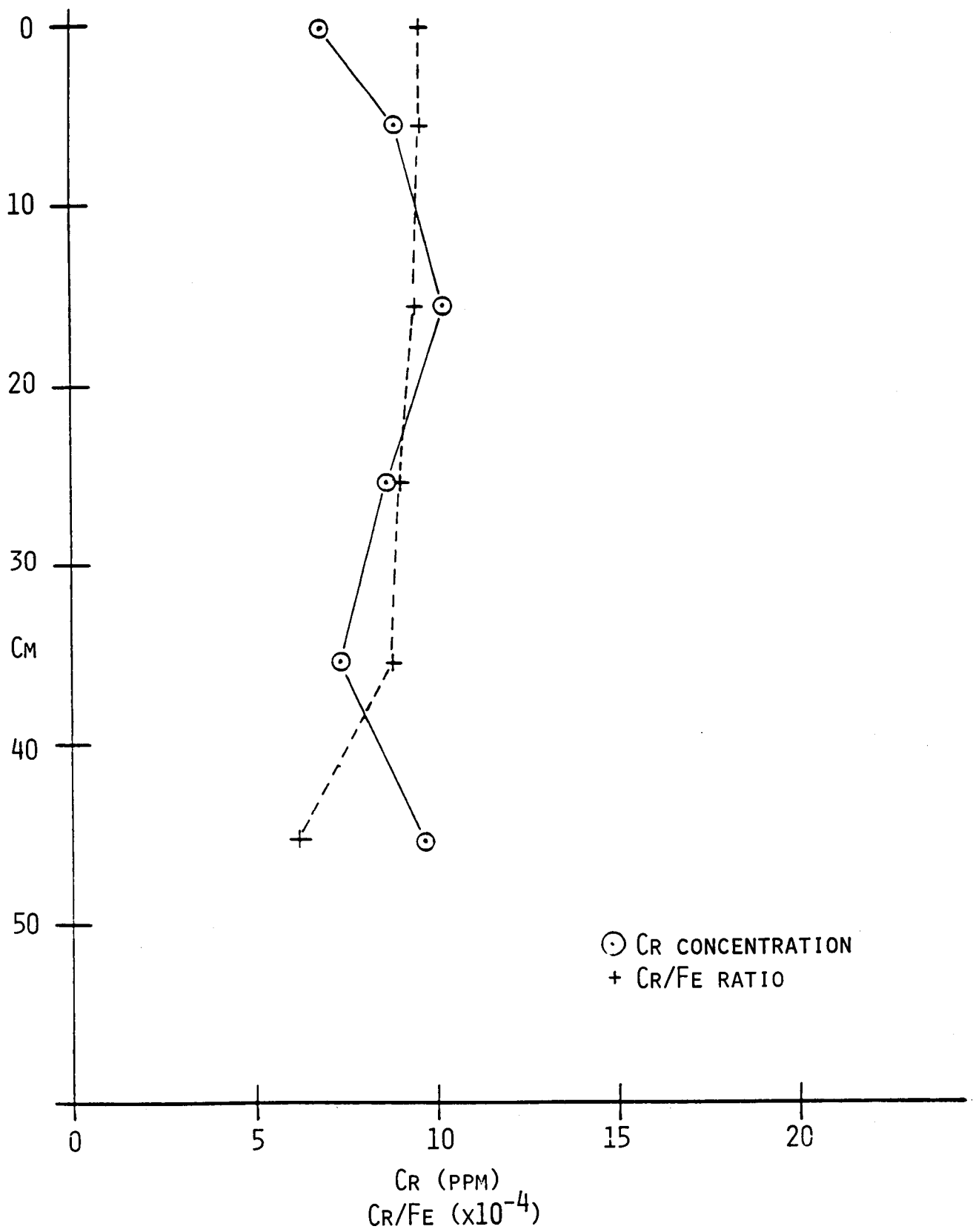


FIG. 3. Chromium concentration (ppm) and ratio of Cr/Fe concentrations with depth (cm) in downcore sediment sample from C22.

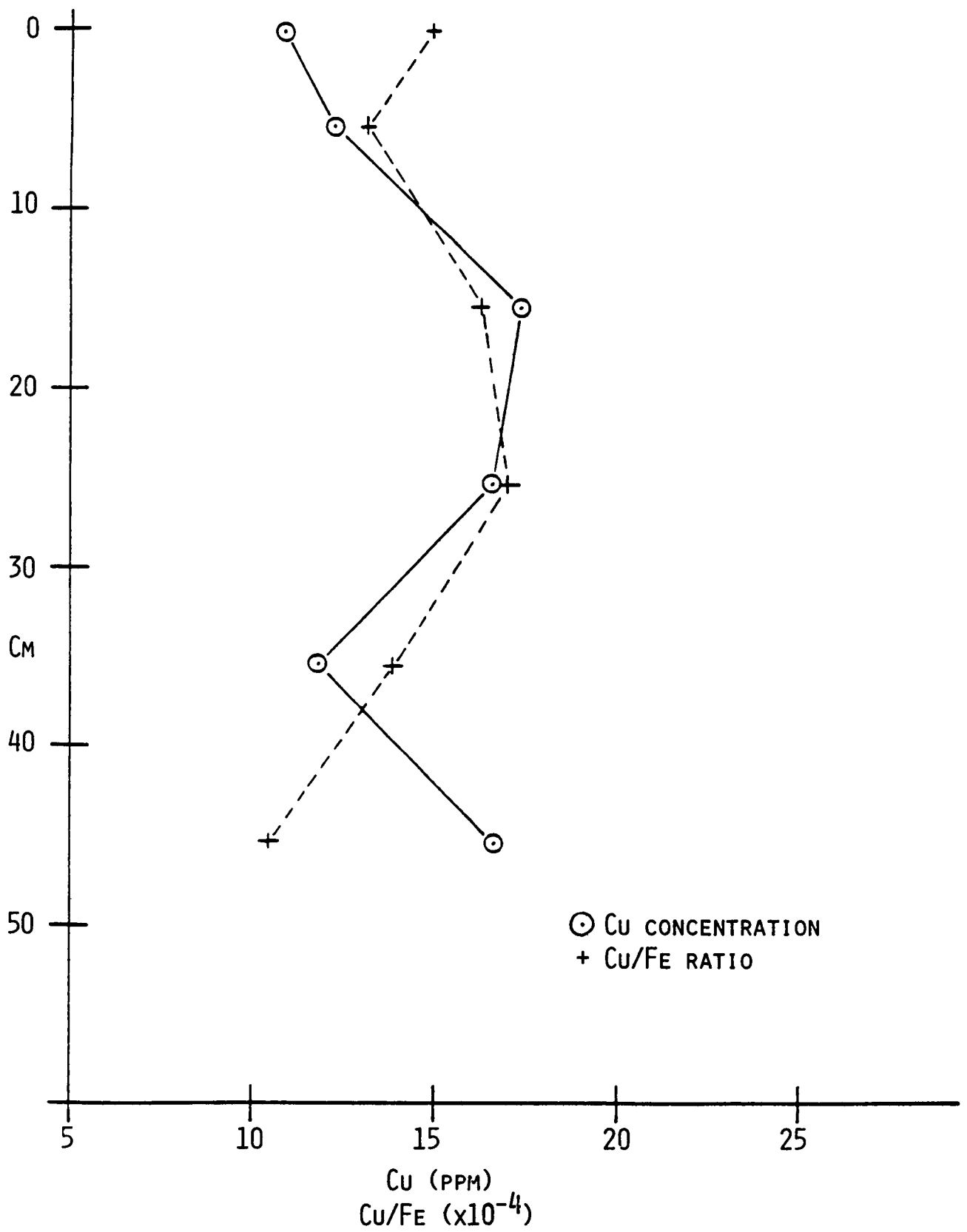


FIG. 4. Copper concentration (ppm) and ratio of Cu/Fe concentrations with depth (cm) in downcore sediment sample from C22.

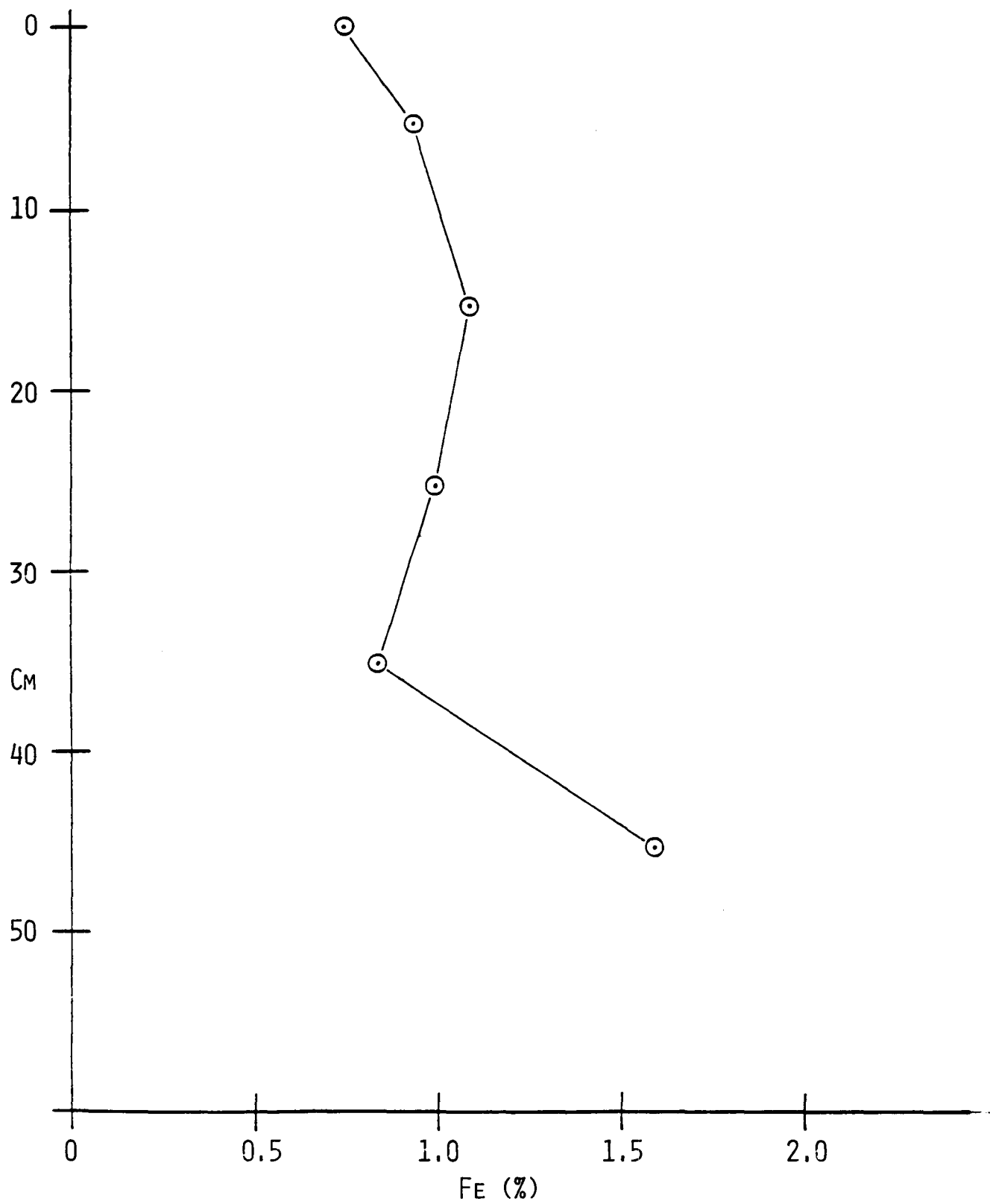


FIG. 5. Iron concentration (ppm) with depth (cm) in downcore sediment sample from C22.

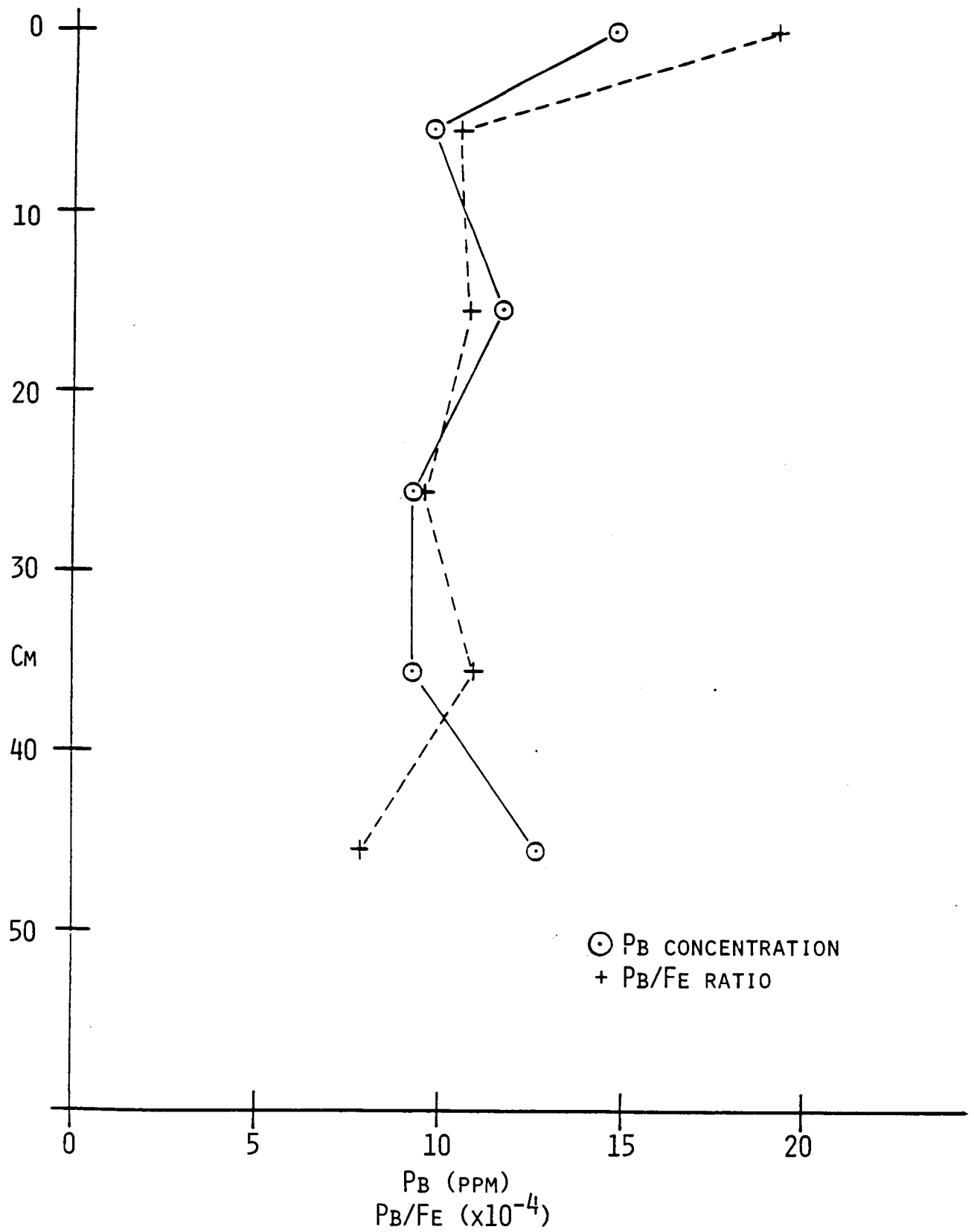


FIG. 6. Lead concentration (ppm) with ratio of Pb/Fe concentrations with depth (cm) in downcore sediment sample from C22.

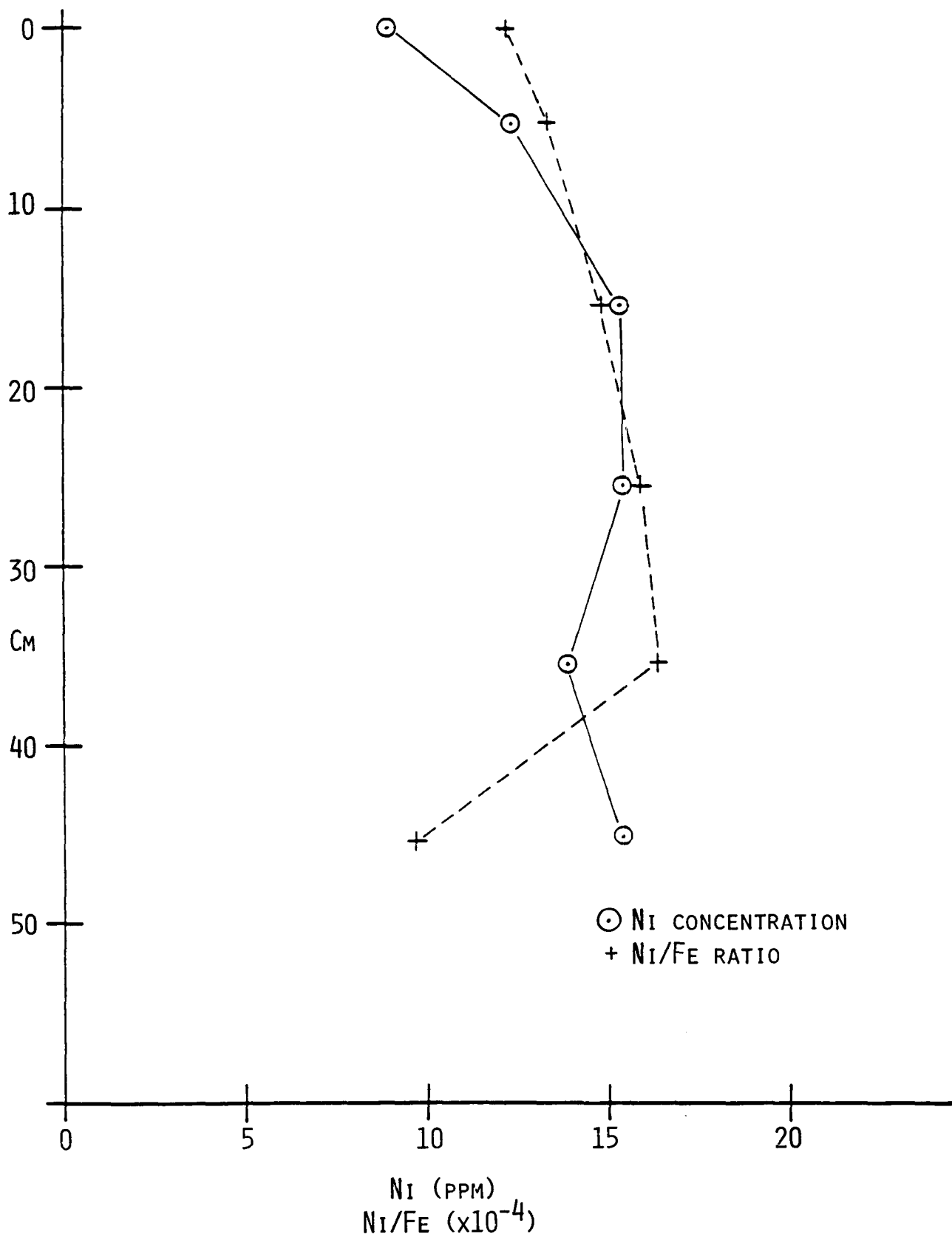


FIG. 7. Nickel concentration (ppm) and ratio of Ni/Fe concentrations with depth (cm) in downcore sediment sample from C22.

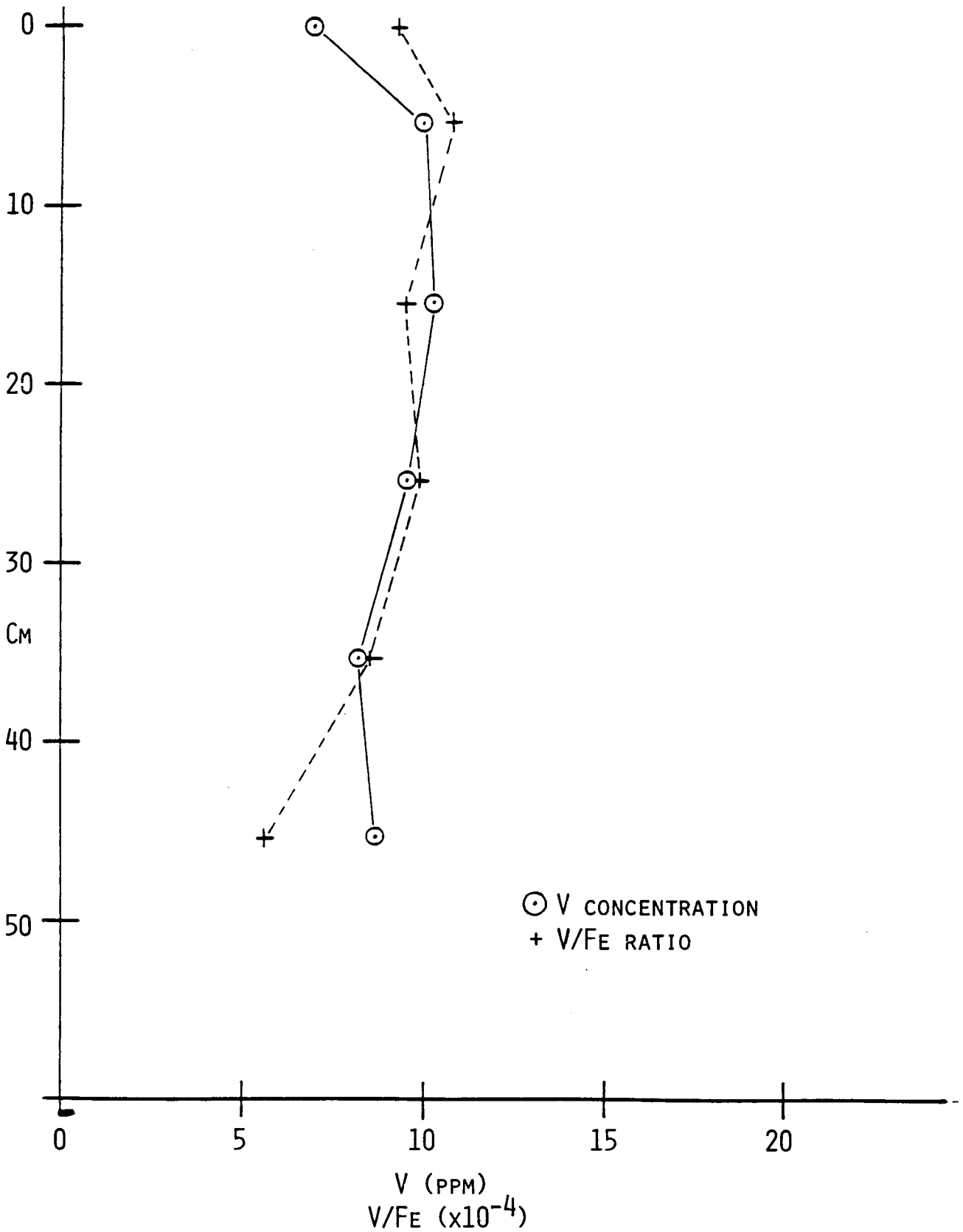


FIG. 8. Vanadium concentration (ppm) and ratio of V/Fe concentrations with depth (cm) in downcore sediment sample from C22.

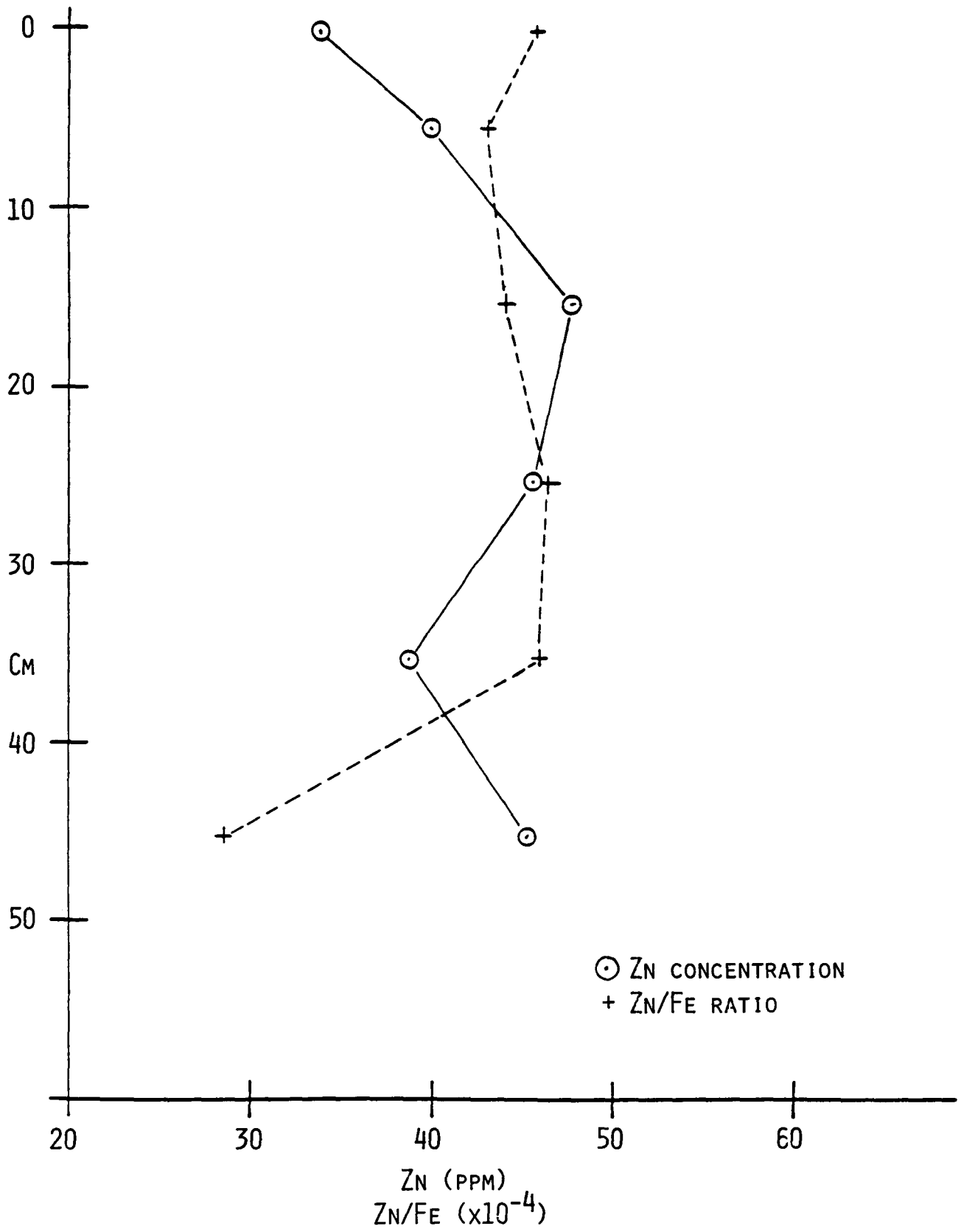


FIG. 9. Zinc concentration (ppm) and ratio of Zn/Fe concentrations with depth (cm) in downcore sediment sample from C22.

APPENDIX G

Downcore Sediment Pb-210 Data from Control Site 22

<u>Sample Identification</u>	<u>Moisture %</u>	<u>Total Weight of Sample (dry)(grams)</u>	<u>pCl/g (dry)</u>
<u>Down Sed:</u>			
Cut #1 0-0.4cm	73.1	2.15	1.2+0.7
Cut #2 0.4-0.8cm	62.5	4.20	2.0+0.7
Cut #3 0.8-1.2cm	44.2	4.30	0.6+1.0
Cut #4 1.2-1.6cm	41.9	4.30	1.1+0.7
Cut #5 1.6-2.0cm	44.8	4.80	0.35+0.04
Cut #6 2.0-2.4cm	42.5	7.50	0.18+0.04
Cut #7 2.4-2.8cm	40.1	5.15	2.2+1.9
Cut #8 2.8-3.2cm	42.5	7.30	1.9+0.8
Cut #9 3.2-3.6cm	41.1	8.30	1.7+0.5
Cut #10 3.6-4.0cm	41.6	8.65	0.46+0.08
Cut #11 4.0-4.4cm	35.2	9.20	0.15+0.09
Cut #12 4.4-4.8cm	36.4	9.80	0.20+0.09
Cut #13 4.8-5.2cm	36.4	13.80	0.54+0.12
Cut #14 5.2-5.6cm	34.7	12.80	0.61+0.13
Cut #15 5.6-6.0cm	31.2	20.30	0.38+0.12
Cut #16 6.0-6.4cm	32.6	13.35	1.7+0.5
Cut #17 6.4-6.8cm	25.0	11.10	1.2+0.5
Cut #18 6.8-7.2cm	27.8	15.50	1.2+0.4
Cut #19 7.2-7.6cm	22.2	14.00	1.2+0.5
Cut #20 7.6-8.0cm	13.3	12.05	0.70+0.29
Cut #21 8.0-8.4cm	18.8	13.00	0.41+0.28
Cut #22 8.4-8.8cm	23.8	13.45	0.41+0.28
Cut #23 8.8-9.2cm	24.1	15.40	1.78+0.14
Cut #24 9.2-9.6cm	28.2	13.75	0.85+0.51
Cut #25 9.6-10.0cm	28.1	12.80	2.9+0.7
Cut #26 10.0-10.4cm	28.1	11.50	2.0+0.2
Cut #27 10.4-10.8cm	29.8	12.00	5.5+0.7
Cut #28 10.8-11.2cm	27.2	12.30	2.9+0.6
Cut #29 11.2-11.6cm	28.4	9.20	1.7+0.5
Cut #30 11.6-12.0cm	26.2	10.20	1.3+0.7
Cut #31 12.0-12.4cm	27.2	11.10	1.1+0.6
Cut #32 12.4-12.8cm	22.5	13.40	1.0+0.6
Cut #33 12.8-13.2cm	23.7	12.40	1.6+0.6
Cut #34 13.2-13.6cm	32.0	12.10	1.3+0.6
Cut #35 13.6-14.0cm	26.0	11.80	1.9+1.1
Cut #36 14.0-14.4cm	32.7	10.70	1.6+0.7
Cut #37 14.4-14.8cm	30.6	13.60	1.6+0.6

<u>Sample Identification</u>	<u>Moisture %</u>	<u>Total Weight of Sample (dry) (grams)</u>	<u>pCi/g (dry)</u>
<u>Down Sed:</u>			
Cut #38 14.8-15.2cm	33.0	10.45	2.1+0.4
Cut #39 15.2-15.6cm	27.8	9.60	1.3+0.3
Cut #40 15.6-16.0cm	21.5	10.20	1.4+0.4
Cut #41 16.0-16.4cm	18.7	13.50	1.2+0.3
Cut #42 16.4-16.8cm	16.3	10.80	2.0+0.4
Cut #43 16.8-17.2cm	20.7	9.60	3.2+0.5
Cut #44 17.2-17.6cm	32.1	13.10	5.5+0.9
Cut #45 17.6-18.0cm	23.1	17.60	7.1+0.7
Cut #46 18.0-18.4cm	27.1	15.20	2.1+0.5
Cut #47 18.4-18.8cm	24.9	13.75	1.2+0.5
Cut #48 18.8-19.2cm	14.9	12.85	0.9+0.4
Cut #49 19.2-19.6cm	25.8	18.40	0.9+0.4
Cut #50 19.6-20.0cm	18.0	19.10	1.0+0.6
Cut #51 20.0-20.4cm	20.4	26.35	1.1+0.5
Cut #52 20.4-20.8cm	28.3	18.00	1.0+0.5
Cut #53 20.8-21.2cm	31.2	11.90	3.6+0.6
Cut #54 21.2-21.6cm	33.9	17.00	9.1+1.0
Cut #55 21.6-22.0cm	36.8	18.55	1.8+0.6
Cut #56 22.0-22.4cm	25.5	12.00	1.8+0.6
Cut #57 22.4-22.8cm	31.8	16.50	1.0+0.5
Cut #58 22.8-23.2cm	28.4	11.50	1.6+0.6
Cut #59 23.2-23.6cm	31.1	6.20	1.9+0.6
Cut #60 23.6-24.0cm	30.9	11.05	0.63+0.14
Cut #61 24.0-24.4cm	34.6	10.10	0.69+0.26
Cut #62 24.4-24.8cm	27.7	12.00	1.5+0.3
Cut #63 24.8-25.2cm	16.4	9.20	1.3+0.4
Cut #64 25.2-25.6cm	17.1	13.80	0.73+0.16
Cut #65 25.6-26.0cm	27.8	11.70	1.4+0.2
Cut #66 26.0-26.4cm	18.9	12.90	0.66+0.14
Cut #67 26.4-26.8cm	22.1	12.00	0.78+0.22
Cut #68 26.8-27.2cm	28.8	9.90	1.0+0.6

VOLUME I—POLLUTANT FATE AND EFFECTS STUDIES
Part 5—Microbiology and Microbiological Processes

by

Lewis R. Brown
Gary W. Childers
Robert W. Landers, Jr.

John D. Walker

Mississippi State University
Mississippi State, Mississippi 39762

Environmental Protection Agency
Washington, DC 20204

Editor: C. A. Bedinger, Jr.
Editorial Assistant: Laura Z. Kirby

TABLE OF CONTENTS

	Page
I. INTRODUCTION	123
II. METHODS AND MATERIALS	125
A. Study Area and Sampling Design	125
B. Sample Collection and Preparation	127
1. <i>Primary Platforms</i>	127
2. <i>Control Sites</i>	127
3. <i>Secondary Platforms</i>	127
C. Materials	128
D. Sample Analysis	128
1. <i>Aerobic Heterotrophic Bacteria</i>	128
2. <i>Oil-Degrading Bacteria</i>	128
3. <i>Yeasts and Fungi</i>	128
4. <i>Nitrate-Reducing, Hydrocarbon-Utilizing Microorganisms</i>	128
5. <i>Sulfate-Reducing, Hydrocarbon-Utilizing Microorganisms</i>	128
6. <i>Nitrogen, Ammonium, Nitrate, Nitrite, Phosphate and Phosphorus</i>	130
7. <i>Oil-Degradation</i>	130
a. <i>Oxygen Consumption</i>	130
b. <i>Respirometry</i>	130
c. <i>Hydrocarbon Analyses</i>	130
8. <i>Nitrification</i>	131
9. <i>Nitrogen Fixation</i>	131
10. <i>Denitrification</i>	131
11. <i>Sulfate Reduction</i>	131
12. <i>Sulfur Oxidation</i>	131
13. <i>Photosynthesis</i>	131
14. <i>Heterotrophic Activity</i>	131
15. <i>Phosphorus Uptake</i>	132
16. <i>Lipolysis</i>	132
17. <i>Chitinolysis</i>	132
18. <i>Cellulolysis</i>	132
19. <i>Proteolysis</i>	132
E. Pure Culture Studies	132
F. Statistical Analyses	133
III. RESULTS	135
A. Sample Analysis	135
1. <i>Aerobic Heterotrophic Bacteria</i>	135
2. <i>Aerobic Bacteria on Oil Agar</i>	135
3. <i>Yeasts and Fungi</i>	143
4. <i>Nitrate-Reducing, Hydrocarbon-Utilizing Microorganisms</i>	143
5. <i>Sulfate-Reducing, Hydrocarbon-Utilizing Microorganisms</i>	143
6. <i>Chemical Nutrients</i>	150
7. <i>Oil Degradation</i>	155
a. <i>Sediments</i>	155
b. <i>Mixed and Pure Cultures</i>	161
8. <i>Nitrification</i>	170
9. <i>Nitrogen Fixation</i>	170
10. <i>Denitrification</i>	170
11. <i>Sulfate Reduction</i>	170
12. <i>Sulfur Oxidation</i>	170
13. <i>Photosynthesis</i>	170
14. <i>Heterotrophic Activity</i>	170
15. <i>Phosphorus Uptake</i>	182
16. <i>Lipolysis</i>	182
17. <i>Chitinolysis</i>	182
18. <i>Cellulolysis</i>	182
19. <i>Proteolysis</i>	182
B. Pure Culture Studies	182
IV. DISCUSSION	203
A. Analysis of Microbial Populations	203
1. <i>Aerobic Heterotrophic Bacteria</i>	203
2. <i>Aerobic Bacteria on Oil Agar</i>	205

TABLE OF CONTENTS (cont'd)

	Page
3. <i>Yeasts and Fungi</i>	206
4. <i>Microorganisms from Rosenfeld's Nitrate-Hydrocarbon Medium (MRNHM)</i>	207
5. <i>Microorganisms from Rosenfeld's Sulfate-Hydrocarbon Medium (MRSHM)</i>	208
B. Sample Analysis of Chemical Nutrients	208
C. Oil Degradation	211
D. Magnitude of and Impact of Oil upon Microbial Processes	211
1. <i>Nitrification</i>	211
2. <i>Nitrogen Fixation</i>	211
3. <i>Denitrification</i>	212
4. <i>Sulfate Reduction</i>	212
5. <i>Sulfur Oxidation</i>	212
6. <i>Photosynthesis</i>	212
7. <i>Heterotrophic Activity</i>	212
8. <i>Phosphorus Uptake</i>	212
9. <i>Lipolysis</i>	212
10. <i>Chitinolysis</i>	213
11. <i>Cellulolysis</i>	213
12. <i>Proteolysis</i>	213
E. Recommendations for Further Studies	213
V. CONCLUSIONS	215
VI. ACKNOWLEDGEMENTS	217
VII. LITERATURE CITED	219
VIII. PUBLICATIONS RESULTING FROM THIS STUDY	223

LIST OF FIGURES

Figure	Page
1. Maps of the study area - (Top) Location of study area. (Bottom) Study area showing sampling sites	126
2. Counts of aerobic heterotrophs obtained at control sites during Cruises I, II, and III	136
3. Counts of aerobic heterotrophs obtained during Cruise II	138
4. Total aerobic plate-based on correlated t-test	139
5. Effect of freezing on total aerobic heterotrophic bacteria in marine sediments	140
6. Counts of bacteria on oil agar obtained at control sites during Curises I, II, and III	142
7. Aerobic hydrocarbonoclasts based on Duncan's Multiple Range Test (SPSS)	144
8. Distribution of bacteria from Cruise I cultured on oil agar expressed as percentage of the bacteria cultured on marine agar	146
9. Distribution of bacteria from Cruise II cultured on oil agar expressed as a percentage of the bacteria cultured on marine agar	147
10. Subsets of sulfate-reducing, hydrocarbon-utilizing bacteria based on Duncan's Multiple Range Test (SPSS)	152
11. Oxygen utilization by sediments during incubation with oil at 27 C in BOD bottles	159
12. Effect of temperature on oxygen utilization by sediments taken from platform areas (Cruise II) during incubation with oil in BOD bottles	160
13. Oxygen consumption and carbon dioxide production by a sediment sample from the Gulf of Mexico	162
14. Percent alkanes (heptadecane through eicosane, and pristane and phytane) as a percent of the total oil during the microbial degradation of Empire Mix crude oil	163
15. Changes in the ratios of heptadecane (<i>n</i> -C ₁₇) to pristane and octadecane (<i>n</i> -C ₁₈) to phytane (straight-to branched-chains) during the microbial degradation of Empire Mix crude oil	164
16. Oxygen consumption and carbon dioxide production during the microbial metabolism of Empire Mix crude oil by enrichments prepared from samples taken on Cruise I	165
17. Gas chromatography tracings of the aliphatics before and after microbial degradation by a control enrichment culture and a platform enrichment culture	166
18. Liquid chromatography tracings of the aromatics in Empire Mix crude oil before and after microbial degradation by a control enrichment culture and a platform enrichment culture	167
19. Oxygen consumption and carbon dioxide production during the microbial metabolism of Empire Mix crude oil by enrichments prepared from samples taken on Cruise II	168
20. Oxygen consumption and carbon dioxide production by <i>Pseudomonas</i> sp 1 during the metabolism of Empire Mix crude oil	169
21. Oxygen consumption and carbon dioxide production by <i>Flavobacterium</i> sp 1 during the metabolism of Empire Mix crude oil	171
22. Oxygen consumption and carbon dioxide production by <i>Pseudomonas</i> sp 2 during the metabolism of Empire Mix crude oil	172
23. Oxygen consumption and carbon dioxide production by <i>Flavobacterium</i> sp 2 during the metabolism of Empire Mix crude oil	173
24. Concentrations of water-soluble ³⁵ S at weeks 0, 1, 2, and 3 in Test (Δ), Control (□), 1×Oil (o), and 10×Oil (*) inoculated with sediment microorganisms from platforms and controls	177
25. Percent ¹⁴ CO ₂ produced from ¹⁴ C-glucose by Platform 2 sediment microorganisms collected during Cruises I, II, and III	181
26. Production of CO ₂ from chitin in Test (o), Control (□), 1×Oil (Δ), and 10×Oil (+) systems	189
27. Impact of oil on the rate of CO ₂ produced from proteolytic activity by <i>Pseudomonas</i> sp 3	197
28. Impact of oil on the heterotrophic activity of <i>Enterobacter</i> sp 1 as determined by measuring CO ₂ using GLC	198
29. Impact of oil on cellulose utilization by measuring ¹⁴ CO ₂ produced by <i>Cellulomonas</i> sp 1	199
30. Impact of oil on the rate of ¹⁴ CO ₂ produced during lipolysis by <i>Pseudomonas</i> sp 5	200
31. Impact of oil on chitin utilization by <i>Pseudomonas</i> sp 6	201
32. Nitrate-reducing, hydrocarbon-utilizing bacteria	209
33. Subsets of ammonia-nitrogen concentration based on Duncan's Multiple Range Test	210

LIST OF TABLES

Table	Page
1. List of primary platforms (P), secondary platforms (S), and control sites (C) selected for this study	125
2. Medium for nitrate-reducing, hydrocarbon-utilizing microorganisms.....	128
3. Composition of synthetic seawater (400 percent)	129
4. Composition of crude oil/water emulsion used in test media	129
5. Rosenfeld's sulfate-reducing medium for hydrocarbon-utilizing microorganisms	129
6. Test systems for determining oil-degrading potential of sediment microorganisms	130
7. Number of sediment samples analyzed	135
8. Total colony-forming units (CFU) of aerobic heterotrophic bacteria cultured on marine agar per ml of sediment collected at the control sites, expressed as \bar{x} CFU $\times 10^{-3} \pm S\bar{x}$	135
9. Total aerobic heterotrophic bacterial CFU ($\times 10^{-3}$) per ml of sediment collected at the primary platforms ...	137
10. Total aerobic heterotrophic bacterial CFU ($\times 10^{-3}$) per ml of sediment collected at the secondary platforms during Cruise II	137
11. Counts of aerobic heterotrophic bacteria in fresh and frozen sediments expressed as CFU ($\times 10^{-4}$) per ml of sediment	141
12. Average number of aerobic bacterial CFU ($\times 10^{-3}$) cultured on oil agar per ml of sediment collected at the control sites	141
13. Average number of aerobic bacterial CFU ($\times 10^{-3}$) cultured on oil agar per ml of sediment collected at the primary platforms	143
14. Average number of aerobic bacterial CFU ($\times 10^{-3}$) cultured on oil agar per ml sediment collected at the secondary platform during Cruise II	145
15. Distribution of bacteria cultured on oil agar expressed as a percentage of the aerobic heterotrophic bacteria cultured on marine agar	145
16. Total number of yeast and fungal CFU cultured on Cooke's Rose Bengal Agar per ml of sediment collected at the control sites	148
17. Total number of yeast and fungal CFU cultured on Cooke's Rose Bengal Agar per ml of sediment collected at the primary platforms	148
18. Total number of yeast and fungal CFU cultured on Cooke's Rose Bengal Agar per ml of sediment collected at the secondary platforms during Cruise II	148
19. Total number of microorganisms cultured in Rosenfeld's nitrate-hydrocarbon medium per ml of sediment collected at the control sites	149
20. Total number of microorganisms cultured in Rosenfeld's nitrate-hydrocarbon medium per ml of sediment collected at the primary platforms	149
21. Total number of microorganisms cultured in Rosenfeld's nitrate-hydrocarbon medium per ml of sediment collected at the secondary platforms during Cruise II	149
22. Total number of microorganisms cultured on Rosenfeld's sulfate-hydrocarbon medium per ml of sediment collected at the control sites	150
23. Total number of microorganisms cultured in Rosenfeld's sulfate-hydrocarbon medium per ml of sediment collected at the primary platforms	150
24. Total number of microorganisms cultured in Rosenfeld's sulfate-hydrocarbon medium per ml of sediment collected at the secondary platforms during Cruise II	151
25. Concentrations of inorganic nutrients ($\mu\text{g atom/kg}$ dry weight sediment) in sediments collected from control sites during Cruise I.....	151
26. Concentrations of inorganic nutrients ($\mu\text{g atom/kg}$ dry weight sediment) in sediments collected from control sites during Cruise II.....	151
27. Concentrations of inorganic nutrients ($\mu\text{g atom/kg}$ dry weight sediment) in sediments collected from control sites during Cruise III	153
28. Concentrations of inorganic nutrients ($\mu\text{g atom/kg}$ dry weight sediment) in sediments collected from primary platforms during Cruise I.....	153
29. Concentrations of inorganic nutrients ($\mu\text{g atom/kg}$ dry weight sediment) in sediments collected from primary platforms during Cruise II	154
30. Concentrations of inorganic nutrients ($\mu\text{g atom/kg}$ dry weight sediment) in sediments collected from primary platforms during Cruise III	154
31. Concentrations of inorganic nutrients ($\mu\text{g atom/kg}$ dry weight sediment) in sediments collected from secondary platforms during Cruise II.....	155
32. Purpose of test systems shown in TABLE 6	155
33. Oxygen consumption ($\bar{x} \pm S$) by microorganisms from Cruise I control site sediments in test systems 1-8 (see TABLE 6).....	156
34. Oxygen consumption ($\bar{x} \pm S$) by microorganisms from Cruise I platform site sediments in test systems 1-8 (see TABLE 6).....	156
35. Oxygen consumption ($\bar{x} \pm S$) by microorganisms from Cruise II control site sediments in test systems 1-8 (see TABLE 6).....	157

LIST OF TABLES (cont'd)

	Page
36. Oxygen consumption ($\bar{x} \pm S$) by microorganisms from Cruise II platform site sediments in test systems 1-8 (see TABLE 6).....	157
37. Oxygen consumption ($\bar{x} \pm S$) by microorganisms from Cruise III control site sediments in test systems 1-8 (see TABLE 6).....	158
38. Oxygen consumption ($\bar{x} \pm S$) by microorganisms from Cruise III platform site sediments in test systems 1-8 (see TABLE 6).....	158
39. Respiration rates for enrichment cultures of primary platform and control site sediment microorganisms during oxidation of Empire Mix crude oil.....	161
40. Respiration rate for pure cultures during oxidation of Empire Mix crude oil.....	170
41. Concentrations of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, and $\text{NO}_2\text{-N}$ in systems inoculated with sediment from Primary Platform 1 collected during Cruise I and tested for nitrification.....	174
42. Concentration of nitrogen (N_2) in test, control, and oil-supplemented systems inoculated with sediment from Primary Platform 1 collected during Cruise I and tested for nitrogen fixation.....	174
43. Concentration of nitrogen (N_2) in systems inoculated with sediment from Primary Platform 1 collected during Cruise I and tested for denitrification.....	174
44. Concentrations of insoluble (I) and soluble (S) ^{35}S in test, control, and oil-supplemented systems inoculated with sediment and tested for sulfate reduction.....	175
45. Concentrations of insoluble (I) and soluble (S) ^{35}S in test, control, and oil-supplemented systems inoculated with sediment from replicate 1, Primary Platform 1, and tested for sulfur oxidation.....	176
46. Change in dissolved oxygen concentration (DO) in systems inoculated with sediment collected during Cruise I and tested for photosynthesis (PP) and metabolic activity (MA).....	176
47. CO_2 produced from glucose (expressed as percent $\text{CO}_2\text{-C}/\text{Total C}$) in systems inoculated with sediment collected during Cruise I and tested for heterotrophic activity.....	178
48. CO_2 produced from glucose (expressed as percent $\text{CO}_2\text{-C}/\text{Total C}$) in systems inoculated with sediment collected during Cruise II and tested for heterotrophic activity.....	179
49. CO_2 produced from glucose (expressed as percent $\text{CO}_2\text{-C}/\text{Total C}$) in systems inoculated with sediment collected during Cruise III and tested for heterotrophic activity.....	180
50. Concentrations of insoluble (I) and soluble (S) ^{32}P in systems inoculated with sediment collected from Primary Platform 1 during Cruise I and tested for phosphate uptake.....	182
51. CO_2 produced from tripalmitin (expressed as percent $\text{CO}_2\text{-C}/\text{Total C}$) in systems inoculated with sediment collected during Cruise I and tested for lipolysis.....	183
52. CO_2 produced from tripalmitin (expressed as percent $\text{CO}_2\text{-C}/\text{Total C}$) in systems inoculated with sediment collected during Cruise II and tested for lipolysis.....	184
53. CO_2 produced from tripalmitin (expressed as percent $\text{CO}_2\text{-C}/\text{Total C}$) in systems inoculated with sediment collected during Cruise III and tested for lipolysis.....	185
54. Quantity of CO_2 produced from chitin in systems inoculated with sediment from Cruise I.....	186
55. Quantity of CO_2 produced from chitin in systems inoculated with sediment from Cruise II.....	187
56. Quantity of CO_2 produced from chitin in systems inoculated with sediment from Cruise III.....	188
57. CO_2 produced from cellulose (expressed as percent $\text{CO}_2\text{-C}/\text{Total C}$) in systems inoculated with sediment collected during Cruise I.....	190
58. CO_2 produced from cellulose (expressed as percent $\text{CO}_2\text{-C}/\text{Total C}$) in systems inoculated with sediment collected during Cruise II.....	191
59. CO_2 produced from cellulose (expressed as percent $\text{CO}_2\text{-C}/\text{Total C}$) in systems inoculated with sediment collected during Cruise III.....	192
60. CO_2 produced from bovine serum albumin (expressed as percent $\text{CO}_2\text{-C}/\text{Total C}$) in systems inoculated with sediment collected during Cruise I and tested for proteolysis.....	193
61. CO_2 produced from bovine serum albumin (expressed as percent $\text{CO}_2\text{-C}/\text{Total C}$) in systems inoculated with sediment collected during Cruise II and tested for proteolysis.....	194
62. CO_2 produced from bovine serum albumin (expressed as percent $\text{CO}_2\text{-C}/\text{Total C}$) in systems inoculated with sediment collected during Cruise III and tested for proteolysis.....	195
63. Impact of oil on metabolic activity and photosynthesis of <i>Nostoc muscorum</i> ATCC # 27347.....	196
64. Rank of primary platform (P1-P4), secondary platforms (S5-S20) and control sites (C21-C24) sampled during Cruise II.....	204
65. Rank and Duncan subsets of sites as established by microbial enumerations.....	204
66. Rank of primary platform and control sites for Cruises I, II, and III from highest to lowest based on ABOA as a percentage of the total aerobic heterotrophic bacterial population.....	205

ABSTRACT

The objectives of this investigation were (1) to make comparisons between platform areas and control sites in terms of various microbial populations and nutrient chemicals, (2) to estimate the hydrocarbon-oxidizing potential of sediments from the sampling sites, and (3) to obtain information on the occurrence and magnitude of microbial processes in the sediments, and to determine the impact of oil on these processes.

While at times there were statistically significant differences between platform sites and control sites in terms of some parameters, it was concluded that nature's activities masked man's activities to such an extent that meaningful cause and effect relationships were obscured.

The sediments demonstrated cellulolytic, chitinolytic, heterotrophic, lipolytic, and proteolytic processes, as well as sulfur oxidation, which were not adversely affected by low levels of crude oil. The following processes did not occur at demonstrable levels: denitrification, nitrification, nitrogen fixation, phosphorus uptake, photosynthesis, and sulfate reduction.

The maximum oil-degrading potential of the sediments was 56 μg hydrocarbon-carbon oxidized to carbon dioxide per ml of sediment per twenty-four hours.

Sediment samples could be frozen at 20 C and then analyzed within seven days without causing a statistically significant difference in microbial counts.

Overall, it was concluded that:

- (1) The transient nature of the surficial sediments and the impact of nature's activity precluded drawing any meaningful conclusions in regard to cause and effect relationships between microflora and chemical nutrients in the sediments and the presence of oil production platforms,
- (2) those microbial processes in the sediments which demonstrated activity were not adversely affected by low levels of oil, and
- (3) the surficial sediments in the entire area have similar oil-degrading potentials.

I. INTRODUCTION

Microorganisms are responsible for the degradation of much of the organic matter that enters estuarine and marine environments. Pollutants entering the marine environment may cause several changes in the microbial community including: (1) inhibiting desirable microbes, (2) enhancing the growth of undesirable microbes, (3) shifting the protozoan populations (Coler and Gunner, 1969), (4) producing toxic metabolic by-products (Brown et al., 1969), and (5) promoting biological magnification of materials toxic to higher members of the food chain.

Since the earliest recorded oil spill in 1907 (Bourne, 1968), the number of reports concerning oil pollution has increased significantly to the point where it is no longer possible for one individual to be an expert on all aspects of oil pollution. Published information concerning oil pollution includes at least 2500 scientific manuscripts (Moulder and Varley, 1971, 1975; Samson et al., 1980) as well as popular articles and books (Marx, 1971; Nelson-Smith, 1973; Boesch, Hershner, and Milgram, 1974). A number of symposia directed at the problem of oil pollution have been organized (Carthy and Arthur, 1968; Anon., 1969, 1971, 1973, 1974, 1975, 1976, 1977, 1979, 1981; Hout, 1969; Holmes and Dewitt, 1970; Cowell, 1971; Hepple, 1971; D'Emidio, 1972). Textbooks on petroleum microbiology have been published (Sharpley, 1966; Davis, 1967) and several reviews on the subject are available (ZoBell, 1969; Friede et al., 1972; Atlas and Bartha, 1973; Crow, Meyers, and Ahearn, 1974; Colwell and Walker, 1977). Workshops have been conducted on the general (National Academy of Sciences, 1975) and microbiological aspects of oil pollution (Ahearn and Meyers, 1973).

Despite the plethora of information that has been written on oil pollution, the long-term (chronic) effects of oil pollution resulting from seeps, spills, and especially from exploratory and developmental drilling are uncertain. In order to provide information on the chronic effects of oil pollution, studies are being and have been performed by Woods Hole Oceanographic Institute (WHOI) to examine the chronic effects of oil spilled from the barge *Florida* (Blumer et al., 1972; Blumer and Sass, 1972a,b; Zafiriou, Blumer and Myers, 1972). One of the earliest and reportedly exhaustive studies of the effects of chronic exposure to oil was the two-year offshore Ecology Investigation (OEI), sponsored by the Gulf Universities Research Consortium (GURC). This research, funded by 80 petroleum and petroleum-related industries, was initiated to assess the environmental impact of petroleum drilling and production off the coast of Louisiana. Based on OEI data, it was concluded that 79% of the investigations demonstrated either a beneficial or at least no harmful impact from exploratory and developmental drilling. The other 21% of the investigations required further study but did not demonstrate harmful effects. Recently, evaluations of the OEI data (Ward, Bender, and Reish, 1979) and other studies suggest that chronic exposure to petroleum may indeed be harmful to certain marine organisms. Thus, after many years of exploratory and developmental drilling for petroleum, we still may not be able to identify harmful

effects to marine organisms resulting from chronic exposure to oil.

In an attempt to correct this deficiency, federal agencies—in particular the Department of Commerce's National Oceanic and Atmospheric Administration (NOAA) and the Department of Interior's Bureau of Land Management (BLM) have funded studies to: (1) collect baseline data at potential offshore drilling sites and (2) determine the impact of exploratory and developmental drilling.

Even before the first of these studies was contemplated, investigations were done specifically to collect baseline data in the South Atlantic Bight (Colwell et al., 1976; Conrad, Walker, and Colwell, 1976; Seesman, Walker, and Colwell, 1976). After these studies were done, the federal agencies mentioned above initiated studies to collect baseline data in northeastern U. S. waters, George's Bank; in central eastern waters, Baltimore Canyon; in southeastern waters, South Atlantic Bight (Texas Instruments, 1979); in south central waters, South Texas Outer Continental Shelf (OCS) (University of Texas Marine Science Institute, 1979); and in northwestern waters [Alaskan Continental Shelf (OCS Environmental Assessment Program, 1979)].

In the absence of baseline data, research to determine the impact of exploratory and developmental petroleum drilling has focused on the need to compare the biological, chemical, and physical properties of petroleum-drilling environments with similar properties of non-petroleum-drilling environments (control sites). In addition to the present study, at least three other studies have been done to assess the ecological impact of exploratory and developmental drilling. Templeton et al. (1975) examined several sites in Lake Maracaibo, Venezuela, in an attempt to evaluate the effects of 60 years of exploratory and developmental drilling. Although they did not designate any of the sites as controls and did not report any microbiology studies, they concluded that the drilling operation did not cause discernible ecological damage, but that discharge of domestic and industrial waste into the lake may contribute to the deterioration of water quality and reduce the biological resources of the lake. The OEI (briefly described above) has been the subject of some controversy, since many of the studies managed to evade the process of peer-review publication; and when papers describing these studies were finally published, many of the conclusions appeared to disqualify statements made in earlier reports (Walton, 1981). The third study focused on one active production platform and its satellites in the Buccaneer oil and gas field, and a nearby control site. Sizemore and Olsen (1980) examined the bacterial communities of the oil field and control area. They concluded that similar numbers and types of bacteria existed at both the Buccaneer field and the control site, except for some seasonal differences and increases in oil-degrading and sulfur-oxidizing bacteria in the producing field. Recently, Hada and Sizemore (1981) reported an increase in plasmid-bearing strains of marine *Vibrio* spp. at the oil and gas field, and concluded that the increase in plasmid incidence and diversity may have resulted from platform discharges. In a rather extensive Environmental Protection

Agency (EPA) sponsored project, a number of chronic effects of low level oil pollution were reported, including an increased incidence of fin rot in fish caused by a *Vibrio* spp. (Giles, Brown, and Minchew, 1978; Brown, 1980).

The present study was designed, using appropriate controls, to assess the ecological impact resulting from a number of offshore production operations. It appears to be the first study designed to obtain detailed information on the microbial communities at several production platforms and control sites during different seasons.

The objectives of this investigation were to obtain: (1) a sufficient amount of information on the cell densities of several types of microorganisms and the quantities of certain chemical nutrients in order to make comparisons between Platform areas and Control Sites, (2) a sufficient amount of information to approximate the hydrocarbon-oxidizing potential of sediments from the sampling areas, and (3) information on the occurrence and magnitude of various microbial processes in the sediments and to determine the effect of oil on these processes.

Toward achieving these objectives, five groups of sediment microorganisms were enumerated: aerobic heterotrophic bacteria; aerobic molds and yeasts; nitrate-reducing, hydrocarbon-utilizing microorganisms; and sulfate-reducing, hydrocarbon-utilizing microorganisms. Twelve selected sediment microbial processes were examined: nitrification, nitrogen fixation, denitrification, sulfate reduction, sulfur oxidation, photosynthesis, heterotrophic activity, phosphorus uptake, lipolysis, chitinolysis, cellulolysis, and proteolysis, and studies concurrently performed to examine the effects of crude oil on these twelve processes. Concentrations of six sediment nutrients were determined: total nitrogen, ammonium, nitrate, nitrite, total phosphorus and phosphate. During studies to evaluate rates of

petroleum degradation, variations in temperature and concentrations of nitrogen, phosphorus, and crude oil were made. In addition to the studies prescribed by the original contract, studies were also performed to determine the effect of freezing and thawing on microbial populations and on the magnitude of selected microbial metabolic processes.

At this time, some of the rationale employed in designing the overall microbiological aspects of this project should be explained. The first issue addressed in the overall project was the experimental design of the sampling program. Obviously, the greater the number of replications, the greater the probability of statistical validation of the results. Cost effectiveness, however, dictated that the number of replications should be the minimum number required for statistical analysis. The microbiological sampling program was designed to achieve scientifically meaningful data at a minimal cost.

The next issue considered was whether to conduct the analyses on-board ship or in a shore facility. The magnitude of the microbiological analyses required, coupled with the uncertainties of weather, argued strongly against on-board analyses, particularly when costs were considered. Another possibility was to refrigerate the samples on-board ship and carry out the analyses at a shore laboratory. In order to ensure comparability of results between cruises, the length of time of refrigeration had to be both minimal and constant. Once again, the uncertainties of weather and the cost of this method tended to rule it out as a viable option. All things considered, the only practical method of handling the sediment samples was to freeze them immediately after collection and maintain them in the frozen state until analyzed. It is noteworthy to point out that Stewart and Marks (1978) have shown that freezing for a period of seven days does not significantly alter the microbial counts of marine sediments.

II. METHODS AND MATERIALS

A. Study Area and Sampling Design

Samples for this program were collected at 20 platforms and four control sites located on the Louisiana OCS. These sites are contained in a roughly rectangular area lying west of the Mississippi Delta and extending from 5 km (3 miles) to 120 km (75 miles) offshore and about 320 km (200 miles) west. Location of the study area is shown in Fig. 1 and characteristics of the study sites are given in Table 1. The criteria used in the selection process included:

- geographical location—only platforms in the north central Gulf of Mexico were considered;
- type of petroleum produced—gas and oil fields were considered, and designated control sites were selected because they had never been exposed to any exploratory, developmental, or production activities (Table 1);
- age of platforms—platform age ranged from 4-24 years (Table 1);
- water depth and distance from shore—platforms and controls ranged in water depth from 4-98 meters and in distance from shore from 5-160 km (Table 1);
- benthic sediment type—sediment was assigned a low priority because of the non-specific

nature of the sediment texture, but most of the platforms exist in areas which are clayey-silt, sandy-silt, or silty-sand;

- high production of commercial or recreational finfish or shellfish;
- previous examination by other studies;
- influence by documented oil spills.

Many of these criteria are described in Table 1. However, there are a few facts which distinguish some of these platforms and controls that were considered during the sampling design and which may influence populations or activities of sediment microbes.

Primary Platform 1—Dissolved oxygen (DO) depletion occurs during the late summer; conditions appear to exist which would favor the accumulation of petroleum hydrocarbons

Primary Platform 2—DO depletion and brine production

Primary Platform 3—Influenced by oceanic currents

Secondary Platform 7—Conditions do not favor accumulation of hydrocarbons

Secondary Platform 9—Relatively clean

Secondary Platforms 10 and 11—DO depletion; included in GURC's OEI

Secondary Platform 12—1971 spill of 53,000 barrels of crude oil

TABLE 1. List of primary platforms (P), secondary platforms (S), and control sites (C) selected for this study

Study ^a Site	LOCATION		Water Depth (m)	Year Installed	No. of Wells	Distance from Shore (km)	Lease Area
	Lat. N	Long. W					
P1 (O)	29°07'42"	89°41'25"	18	1961	15	19	West Delta
P2 (O)	29°02'50"	90°09'46"	12	1954	24	5	Bay Marchand
P3 (O/G)	28°39'25"	90°14'08"	35	1968	11	42	South Timbalier
P4 (O/G)	28°34'09"	90°24'32"	46	1964	9	53	South Timbalier
S5 (O/G)	29°12'32"	89°32'23"	9	1962	1	6	West Delta
S6 (O/G)	28°57'08"	89°41'02"	52	1965	24	42	West Delta
S7 (O/G)	28°48'34"	89°47'17"	65	1965	12	56	West Delta
S8 (O/G)	28°57'37"	90°01'25"	27	1957	10	27	Grand Isle
S9 (O)	28°44'04"	89°44'07"	85	1965	7	64	West Delta
S10 (O/G)	28°49'53"	90°23'18"	20	1955	16	20	South Timbalier
S11 (O/G)	28°49'3"	92°22'36"	20	1957	12	21	South Timbalier
S12 (O)	28°59'07"	90°09'41"	17	1965	17	11	South Timbalier
S13 (O)	28°56'48"	89°42'23"	51	1968	24	41	West Delta
S14 (G)	28°41'51"	91°37'21"	29	1973	12	68	Eugene Island
S15 (G)	28°10'02"	91°29'39"	98	1974	21	115	Eugene Island
S16 (G)	28°28'28"	91°16'45"	45	1971	18	97	Ship Shoal
S17 (O)	28°13'35"	91°41'05"	75	1972	18	120	Eugene Island
S18 (O)	28°48'50"	91°44'20"	25	1970	13	52	Eugene Island
S19 (O)	28°51'34"	91°07'52"	6	1960	9	27	Ship Shoal
S20 (G)	28°48'19"	90°36'29"	18	1969	9	21	Eugene Island
C21	29°12'	89°44'	3	—	—	9	West Delta
C22	28°53'	90°16'	21	—	—	10	South Timbalier
C23	28°27'	90°38'	37	—	—	32	South Timbalier
C24	28°50'	91°27'	18	—	—	39	Eugene Island

^aO = oil production, G = gas production

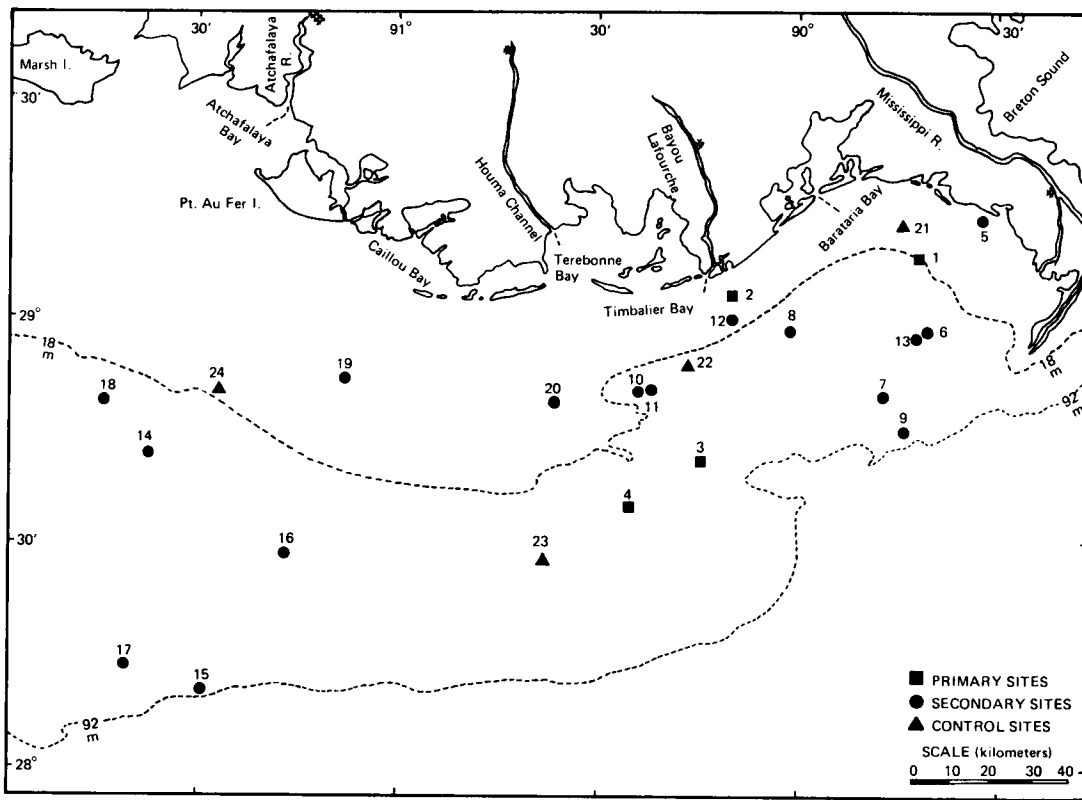
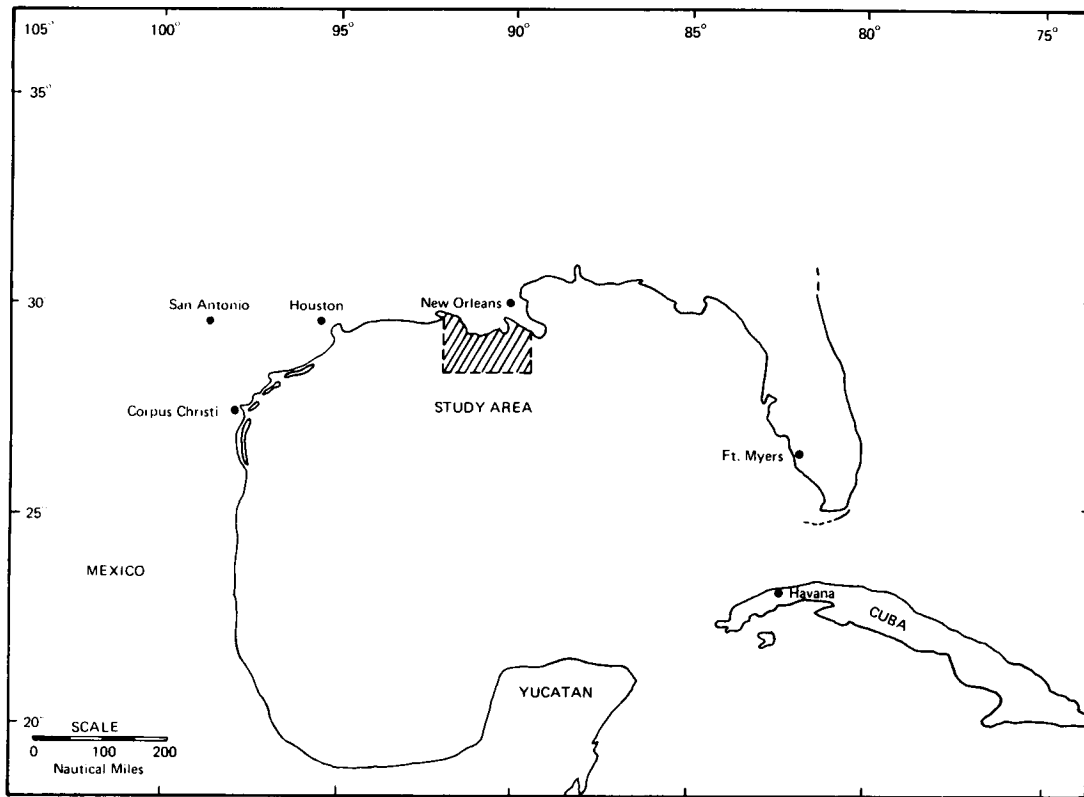


FIG. 1. Maps of the study area - (Top) Location of study area. (Bottom) Study area showing sampling sites.

Secondary Platform 13—1967 spill of 160,000 barrels of crude oil

Secondary Platform 14—Gas field

Secondary Platform 15—Insignificant pollution

Secondary Platform 19—Sandy sediment

Control Site 21—Adjacent to Primary Platform 1 and similar depth to Primary Platform 2 and Secondary Platform 5

Control Site 22—Similar depth to Primary Platform 2, and Secondary Platforms 8, 10, 11, and 12

Control Site 23—Similar depth to Primary Platforms 3 and 4, and Secondary Platform 16

Control Site 24—Similar depth to Secondary Platforms 14, 16, 18, 19, and 20.

Sediment samples were collected during three sampling cruises: Cruise I, May 1978; Cruise II, August-September 1978; and Cruise III, January 1979. Four Primary Platforms (P1-P4) and four Control Sites (C21-C24) were sampled during each Cruise. During Cruise II, an additional 16 Secondary Platforms (S5-S20) were sampled. Four transects, one along each compass heading, were established at each primary site, a north transect was established at each secondary site, and a single sampling station was established at each control site. Samples collected at primary platforms were obtained at a distance of 500 m from the platform along each transect. Samples collected at secondary platforms were obtained at a distance of 500 m from the platform along the north transect.

B. Sample Collection and Preparation

Sampling methodology was maintained constant throughout the study, utilizing a modified Kahlsico® stainless steel Smith-McIntyre grab. All samples (at least 200 ml) were scraped from the top 2 cm of each grab using a sterile wooden tongue depressor and placed in autoclaved sampling jars. All sample jars were contained in styrofoam trays which were color-coded to expedite sample collection and identification. Additionally, each jar lid was color-coded, with the sample number painted on top. Appropriate labels preprinted and coded were placed on each jar immediately after the sample was collected. Sediments not used during ship-board sample processing were frozen at -20 C until processed. Samples were processed after 7-14 days of frozen storage.

1. Primary Platforms

Four grabs were obtained at each 500-m sampling location N, S, E and W of the platforms. A total of 192 samples were collected (4 sediment samples × 4 compass points × 4 platforms × 3 seasons). Each of the 192 samples was analyzed in triplicate for microbial populations using 6 different types of media (1,152 analyses) and for 6 chemical nutrients (1,152 analyses).

For the on-board photosynthesis experiments, two 160-ml composites were prepared for each primary platform by combining 20-ml samples from two grabs at each of the four compass points and repeating the procedure for the remaining eight grabs. Twenty-four samples were analyzed for photosynthesis (2 composites × 4 platforms × 3 seasons).

For the remaining microbial processes two 600-ml composite samples were prepared for each platform by combining 75-ml samples from two grabs at each compass point, and repeating the procedure for the

remaining eight grabs (2 composites × 4 platforms × 2 seasons = 16 samples × 11 microbial processes = 176 analyses for Cruises I and II). However, results from Cruises I and II indicated that nitrogen fixation, denitrification, nitrification, sulfate reduction and phosphate uptake were not detected by the methods employed. An experiment was designed to determine if freezing and thawing of sediments might affect these processes. Ten 150-ml sediment samples from Primary Platform P2 were collected and composited during Cruise III. Subsamples were removed, held at 0-4 C for 8-36 hours, and tested for the microbial processes indicated as well as for sulfur oxidation since the results on this process from Cruises I and II were not available at the time the change was initiated. The composite sample was frozen for 7 days at -20 C, thawed and re-tested. Samples for the processes that demonstrated activity were collected during Cruise III as described for Cruises I and II (2 composites × 4 platforms = 8 samples × 5 processes = 40 analyses).

To estimate the oil-degrading potential of sediment microorganisms, 7.5 ml from each of the 64 grab samples (4 grabs × 4 compass points × 4 primary platforms) were used to prepare one composite sample for each cruise. A suspension of 3.5 ml sediment/10 ml artificial seawater was made for each composite.

2. Control Sites

Eight grabs were obtained at each control site (8 sediment samples × 4 controls × 3 seasons = 96 samples). Four of the eight samples from each site were analyzed for microbial population using 6 different types of media (288 analyses) and for 6 nutrients (288 analyses).

For the on-board photosynthesis experiments, two 40-ml composites were prepared for each control site by combining 20-ml samples from the first two grabs and repeating the procedure for the next two grabs. Twenty-four samples were analyzed for photosynthesis (2 composites × 4 controls × 3 seasons).

For the remaining microbial processes two 600-ml composites were prepared for each control site (2 composites × 4 controls × 2 seasons = 16 samples × 11 microbial processes = 176 analyses for Cruises I and II). During Cruise III, ten 150-ml sediment samples from Control Site C22 were composited and subsamples were removed, held at 0-4 C for 18-36 hours and tested for the microbial processes described above that may have been susceptible to freezing and thawing (also including sulfur oxidation). Samples for the carbonaceous processes were collected as described for Cruises I and II (2 composites × 4 platforms = 8 samples × 5 processes = 40 analyses).

To estimate the oil-degrading potential of sediment microorganisms, 15 ml from each of the 32 grab samples (8 grabs × 4 controls) were used to prepare one composite sample for each cruise. A suspension of 3.5 ml sediment/10 ml artificial seawater was made for each composite.

3. Secondary Platforms

Four grabs were obtained at the N500 station of each secondary platform and the resulting sediment samples were analyzed for microbial populations and inorganic nutrients (4 sediment samples × 16 platforms = 64 samples). Each of the 64 samples was enumerated in triplicate for microorganisms using 6 different types

of media (384 analyses) and for 6 chemical nutrients (384 analyses). Microorganisms from secondary platform samples were not evaluated for their contribution to the nitrogen, sulfur, phosphorus or carbon cycles, or for their oil-degrading potential.

C. Materials

All water used in this project was glass distilled. All inorganic chemicals were reagent grade, and all organic solvents were analytical grade.

Except where specifically noted in the text, all artificial seawater was made with Rila Marine Mix® (Rila Products, Teaneck, NJ) to a final measured salinity of 30 parts per thousand (ppt).

All of the oil produced in the Gulf Coastal Area is typified by Empire Mix crude oil (parafinic 70-75%, API 30-35⁰) which was kindly supplied by Standard Oil Co. (KY), Pascagoula Refinery, Pascagoula, MS.

Radioisotopes (elemental ³⁵S, Na₂³⁵SO₄, K₂H³²PO₄, ¹⁴C-glucose, ¹⁴C-tripalmitin, ¹⁴C-cellulose, and ¹⁴C-bovine serum albumin) were obtained from ICN, Irvine, CA.

The counting cocktail used for the water-soluble fractions of the radioisotopic processes was ScintiVerse® Universal Cocktail obtained from Fisher Scientific Co., Atlanta, GA, while the cocktail used for the gas phase and insoluble (oxidized) fractions was Permafluor V® obtained from Packard Instrument Company, Downers Grove, IL.

D. Sample Analysis

1. Aerobic Heterotrophic Bacteria

Total numbers of marine aerobic heterotrophic bacteria were enumerated on spread plates of Bacto-Marine Agar (Difco®). Plates were incubated for 5-7 days at 20 C. An additional 40 samples (20 from a platform and 20 from a control site) taken during Cruise III, analyzed immediately for aerobic heterotrophic bacteria, and then frozen. After 7, 45, and 90 days of freezing, an aliquot of each sample was thawed and replated as above.

2. Oil-Degrading Bacteria

Oil-degrading bacteria were enumerated using the spread plate technique and modified ZoBell Marine Agar (ZoBell, 1946) with 0.58% Empire Mix crude oil substituted for the carbon sources (peptones, yeast extract, and ferric citrate) and supplemented with

1% (w/v) ammonium nitrate as a nitrogen source. Previous experience has demonstrated that the oil adequately disperses without emulsifying agents in the reconstituted marine agar plates when the sterilized medium is overlaid prior to use with the same medium prepared using oil-saturated water but without oil. Modified ZoBell Marine Agar without oil was used as a control medium to monitor the growth of non-oil-degrading bacteria which could utilize agar and/or any organic contaminants as a carbon source. Numbers of non-oil-degrading bacteria were subtracted from the number of bacteria appearing on the oil-agar plates to provide an indication of the total number of oil-degrading bacteria. Plates were incubated for 5-7 days at 20 C.

3. Yeasts and Fungi

Enumeration of yeasts and fungi was achieved by plating appropriate dilutions of each sample onto two different selective media (Difco® Potato Dextrose Agar and Difco® Cooke Rose Bengal Agar). Both media were prepared with Rila® Sea Salts at a salinity of 30 ppt. The spread-plate technique was used, and the plates were incubated for 5-7 days at 20 C.

4. Nitrate-Reducing, Hydrocarbon-Utilizing Microorganisms

Most Probable Number (MPN) tubes (3 per inoculum) containing nitrate-reducing medium (Rosenfeld's Patent No. 2,921,007, Tables 2, 3, and 4) were inoculated with 1/10, 1/100, and 1/1000-ml sediment and incubated for 3 weeks at 20 C. After 21 days, positive tubes were determined by spot tests for nitrite employing sulfanilic acid and α -naphthylamine reagents using the procedure described in *Standard Methods for the Examination of Water and Wastewater*, 14th edition, hereinafter referred to as *Standard Methods* (American Public Health Association, 1975). Negative tubes were retested after 120 days incubation.

5. Sulfate-Reducing, Hydrocarbon-Utilizing Microorganisms

MPN tubes (3 per inoculum) containing Rosenfeld's sulfate-reducing medium (Table 5) were inoculated with 1/10, 1/100, and 1/1000-ml sediment and incubated for 3 weeks at 20 C. Blackening of the agar was indicative of sulfate reduction. Negative tubes were re-examined after 120 days.

TABLE 2. Medium for nitrate-reducing, hydrocarbon-utilizing microorganisms^a

Component	Amount
FeSO ₄	0.1 g
K ₂ HPO ₄	0.5 g
KNO ₃	1.0 g
Synthetic seawater (400 percent) ^b	25.0 ml
Distilled water	975.0 ml
pH Adj. to 7.8 with KOH Bacto-agar	2.0 g
Crude oil/water emulsion ^c	50.0 ml

^aTubed and sterilized in an autoclave at 121 C for 15 min.

^bSee TABLE 3

^cSee TABLE 4

TABLE 3. Composition of synthetic seawater (400 percent)

<u>Component</u>	<u>Amount</u>
NaCl	778.00 g
Na ₂ SO ₄	130.00 g
MgCl ₂ ·6H ₂ O	352.00 g
CaCl ₂ ·2H ₂ O	36.00 g
KCl	11.00 g
Na ₂ HCO ₃	3.20 g
KBr	1.60 g
SnCl ₂ ·6H ₂ O	0.67 g
H ₃ BO ₃	0.41 g
Na ₂ SiO ₃ ·9H ₂ O	0.08 g
NaF	0.05 g
NH ₄ NO ₃	0.03 g
FePO ₄ ·4H ₂ O	0.02 g
Distilled water	8000 ml

TABLE 4. Composition of crude oil/water emulsion used in test media

<u>Component</u>	<u>Amount</u>
Empire Mix crude oil	5.0 ml
Distilled water	45.0 ml
Gum arabic	0.1 g

TABLE 5. Rosenfeld's sulfate-reducing medium for hydrocarbon-utilizing microorganisms^a

<u>Component</u>	<u>Amount</u>
FeSO ₄ ·(NH ₄) ₂ SO ₄ ·6H ₂ O	0.1 g
Ascorbic acid	0.2 g
K ₂ HPO ₄	0.5 g
(NH ₄) ₂ SO ₄	1.0 g
Crude oil emulsion ^b	50 ml
Synthetic seawater (400 percent) ^c	25 ml
Distilled water	975 ml

^aTubed and sterilized in an autoclave at 121 C for 15 min

^bSee Table 3

^cSee Table 2

6. Nitrogen, Ammonium, Nitrate, Nitrite, Phosphate and Phosphorus

Total nitrogen, ammonium, nitrate, nitrite, ortho-phosphate and total phosphorus analyses were done as described in the following sections of *Standard Methods*: 421 (total Kjeldahl nitrogen), 418B ammonia, nesslerization), 419D (nitrate, brucine), 420 (nitrite-nitrogen), 425E (phosphate), and 425C.I. and E (total phosphorus).

7. Oil-Degradation

a. Oxygen Consumption

Eight test systems were employed to assess the oil-degrading potential of sediment microorganisms (Table 6). Empire Mix crude oil was thoroughly mixed with the sediment sample, and 7-ml subsamples were placed in 300-ml Biochemical Oxygen Demand (BOD) bottles. Twelve bottles were prepared for each test system. Appropriate amounts of inorganic nutrients were placed in the BOD bottles (Table 6). Bottles were filled with air-saturated synthetic sea salts (30 ppt salinity), stoppered, tested immediately for dissolved oxygen using a YSI model No. 51B oxygen meter, and plated on oil agar.

composites from Cruises I and II by incubating 5 ml of the composite sample in 100 ml of synthetic sea salts solution supplemented with 1% (w/v) Empire Mix crude oil (10,000 ppm), 0.1% (w/v) KNO_3 , and 0.038% (w/v) K_2HPO_4 at 20 C for 50 days. The synthetic sea salts consisted of the following inorganic salts: sodium chloride, 19.45 g; magnesium chloride, 8.8 g; sodium sulfate, 3.24 g; calcium chloride, 1.8 g; potassium chloride, 0.55 g; sodium bicarbonate, 0.16 g; potassium bromide, 0.08 g; strontium chloride, 0.034 g; boric acid, 0.022 g; sodium silicate, 0.004 g; sodium fluoride, 0.0024 g; ammonium nitrate, 0.0016 g; disodium hydrogen phosphate, 0.008 g; and distilled water, 1 liter.

Warburg respirometers with 125-ml BOD flasks were employed to assess the potential of sediment enrichment cultures and pure cultures to oxidize oil. Flasks containing 25 ml synthetic sea salts supplemented with 0.1% (w/v) potassium nitrate and 0.038% (w/v) dipotassium hydrogen phosphate, a 10% inoculum of a sediment sample, an enrichment culture or a pure culture, and 20 μl of Empire Mix crude oil were shaken at 20 C. Oxygen consumption and carbon dioxide production were monitored manometrically, and respiratory quotients were calculated. At the termination of the experiment the contents of the flasks were analyzed by

TABLE 6. Test systems for determining oil-degrading potential of sediment microorganisms

Test System No.	NH_4Cl (ppm)	KNO_3 (ppm)	K_2HPO_4 (ppm)	Empire Mix Crude Oil (ppm)	Incubation Temperature (C)
1	X	X	X	0.58	15
2	X	X	X	0.58	20
3	X	X	X	0.58	27
4	0.7 ^a	X	0.7 ^a	0.58	27
5	6.6	X	4.0	0.58	27
6	X	6.6	4.0	0.58	27
7	6.6	X	4.0	5.80	27
8	X	6.6	4.0	5.80	27

X = Concentration found in sediment sample being employed in the experiment.

a = Maximum amount found in any sample.

After incubation, the streak plates were examined for the number of colonial types. Representative, predominant colonial types were purified for axenic cultures studies. The contents of three bottles from each test system were analyzed for hydrocarbons by both gas chromatography (GC) and high pressure liquid chromatography (HPLC) as described below. Dissolved oxygen (DO) was routinely monitored on all of the bottles from each test system. After 20 days incubation, three bottles from each test system were removed, aliquots were plated on oil agar, and the remaining contents of the bottles were analyzed for hydrocarbons. Similarly, three bottles from each test system were removed after 40 and 60 days of incubation and analyzed as described above. Thus, DO readings for the 20, 40, and 60 day readings were made on 9, 6, and 3 bottles, respectively. To insure an adequate supply of DO in the bottles, each bottle was re-aerated after each DO reading.

b. Respirometry

Four pure cultures were selected from the oxygen consumption studies. Four enrichment cultures were prepared from the two platform and two control

GC and HPLC for alkanes and aromatics, respectively. Ten samples were sent to Southwest Research Institute for gas chromatography-mass spectrometry (GC-MS) analysis.

c. Hydrocarbon Analyses

The 300-ml mixture of water and sediment from each BOD bottle was placed in a 500-ml separatory funnel and extracted three times with 50 ml of *n*-hexane. The *n*-hexane was evaporated *in vacuo* to near dryness, and the sample was then transferred to a storage vial with 5 ml of ethyl ether. The sample was dried under a stream of nitrogen to remove the ether, and frozen at -20 C until analyzed.

The samples were analyzed on a 0.32 cm \times 183 cm OD stainless steel column containing 3% SE-30 on 80-100 mesh Chromasorb W using a Beckman GC-45 equipped with a flame ionization detector. Injector and detector temperatures were 300 C and the column temperature was programmed from 100 to 300 C at a rate of 3 C per min. Qualitative identification of the components was achieved by comparing the retention times of oil components with known standards. Quantitation of peaks was accomplished with a 3380A Hewlett-Packard

recording integrator. Total oil was quantified from the area of the $n\text{-C}_{16}$ peak on the gas chromatograms by comparison to a standard curve prepared with fresh Empire Mix crude oil.

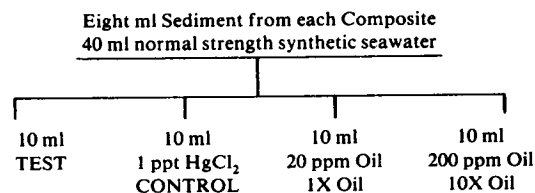
Each sample was also analyzed using a Waters Associates Model 200/401 HPLC with UV detector (wavelength, 277 nm). A $0.63 \times 25.4 \mu$ Bondapak C_{18} /corasil column was employed with a methanol:water (70:30) solvent system at a flow rate of 2.0 ml/min.

Analyses for total hydrocarbons were conducted using a Waters Associates ALC/GPC-502 HPLC fitted with an FS-770 Schoeffel fluorometer, with an excitation wavelength of 274 nm and an emission wavelength of 370 nm. The analyses were carried out using a methanol:water solvent system on samples dissolved in a known quantity of n -hexane (Brown and Minchew, 1978).

Petrogenic hydrocarbons were analyzed using the HPLC described above but with an excitation wavelength of 403 nm and an emission wavelength of 418 nm (Miles, Coign, and Brown, 1977). For these analyses the samples were dissolved in chloroform, and a chloroform solvent system was employed.

8. Nitrification

For every set of four bottles that was used to monitor the activity of sediment microbial processes (except photosynthesis) and assess the impact of oil on these processes, the following scheme was used.



In all cases, the TEST, 1X OIL, and 10X OIL bottles received HgCl_2 (1 ppt) prior to terminal analysis in order to insure sample uniformity.

For nitrification 16 ten-ml samples were placed in 8-oz prescription bottles containing 65-ml synthetic seawater supplemented with NH_4Cl (approximate final concentration of 1 g/liter). Immediately after preparation, the contents of one set of bottles (TEST, CONTROL, 1X OIL, and 10X OIL) were subjected to analysis for substrate (ammonia) and products (nitrite and nitrate) using colorimetric procedures in *Standard Methods*. Suspensions were incubated quiescently at 20 C. After one, two, and three weeks of incubation, subsequent sets of bottles were analyzed for ammonia, nitrite, and nitrate.

9. Nitrogen Fixation

Four ten-ml samples were placed in 6-oz prescription bottles and closed with rubber serum stoppers. The atmosphere of each bottle was replaced with a gas mixture composed of 65% argon, 30% N_2 , and 5% CO_2 . Samples were injected into a Fisher Gas Partitioner Model 1200 GC to detect decreases in N_2 . Samples were incubated quiescently at 20 C. All systems were monitored at time zero, one week, two weeks, and three weeks. Total Kjeldahl nitrogen content of the 3-week samples was determined as described in *Standard Methods*.

10. Denitrification

Four ten-ml samples were placed in 6-oz prescription bottles. Forty ml of synthetic seawater supplemented with KNO_3 (5 g/liter) was added to each bottle. A serum stopper was placed in each bottle and the atmosphere was replaced with argon. Incubation was at 20 C for three weeks without agitation. Gas composition was monitored weekly using a Fisher Gas Partitioner Model 1200 GC to determine the amount of N_2 produced. Analyses for nitrite using colorimetric procedures in *Standard Methods* were done at the beginning and at the termination of the experiment.

11. Sulfate Reduction

Sixteen ten-ml samples were placed in 2-oz prescription bottles and supplemented with 50 mg $\text{Na}_2^{35}\text{SO}_4$ (0.02 $\mu\text{Ci}/\text{mg}$). Immediately after preparation, the contents of one set of bottles (TEST, CONTROL, 1X OIL, and 10X OIL) were filtered through 0.45 μm pore size membrane filters, and washed twice. Increases in water-insoluble ^{35}S (material on the filter) were monitored using a Tennelec Model TC-545A counter-timer. Decreases in water-soluble ^{35}S were determined on the filtrate using a Packard Model 2650 Liquid Scintillation Spectrometer. The remaining suspensions were incubated quiescently at 20 C. After one, two, and three weeks of incubation, subsequent sets of bottles were analyzed as described above.

12. Sulfur Oxidation

Sixteen ten-ml samples were placed in 2-oz prescription bottles and supplemented with 50 mg elemental S containing ^{35}S (0.02 $\mu\text{Ci}/\text{mg}$). Immediately after preparation, the contents of one set of bottles (TEST, CONTROL, 1X OIL, and 10X OIL) were filtered through 0.45- μm pore size membrane filters, and rinsed twice. Decreases in water-insoluble ^{35}S (material on the filter) and increases in water-soluble ^{35}S were monitored as described in the sulfate reduction section. The remaining bottles were incubated quiescently at 20 C. After one, two, and three weeks of incubation, subsequent sets of bottles were analyzed as described above.

13. Photosynthesis

The test for photosynthesis was always done aboard ship on fresh samples. Five-ml subsamples from each sample were transferred to each of eight BOD bottles, four of which were painted black for determining the dark reaction. One pair of bottles (1 light reaction, 1 dark reaction) was treated with HgCl_2 and designated as CONTROL BOTTLES. Concurrently, tests were conducted in the presence of Empire Mix crude oil at the 1X and 10X concentrations. The bottles were then filled with a solution of 30 ppt Rila Sea Salts (pre-cooled to 20 C) and incubated at 20 C for 8-24 hours beneath three 20-watt fluorescent light bulbs. Dissolved oxygen was determined both initially and after incubation, using a model YSI-54B oxygen meter.

14. Heterotrophic Activity

Sixteen ten-ml subsamples were placed in 2-oz prescription bottles and supplemented with 50 mg UL ^{14}C -glucose (0.01 $\mu\text{Ci}/\text{mg}$). A serum stopper was placed in each bottle. Immediately after preparation the contents of one set of bottles (TEST, CONTROL, 1X OIL, and 10X OIL) were analyzed for $^{14}\text{CO}_2$ by acidifying the

medium, flushing the bottle with air, and trapping the $^{14}\text{CO}_2$ in Carbosorb® (obtained from Packard Instrument Company). The contents of each bottle were then filtered through a 0.45 μm pore size membrane filter and rinsed twice, and the insoluble portion remaining on the filter was oxidized in a Packard Model B306 sample oxidizer. Radioactivity of the three fractions was determined using a Packard Model 2650 Liquid Scintillation Spectrometer. The remaining bottles were incubated quiescently at 20 C. For Cruise I, subsequent sets of bottles were analyzed as described above after two, four, and six days of incubation. Since these data indicated the cultures had entered stationary phase, the protocol was changed for Cruises II and III to analyze after one, three, and five days of incubation.

15. Phosphorus Uptake

Sixteen ten-ml samples were placed in 2-oz prescription bottles and supplemented with 50 mg $\text{K}_2\text{H}^{32}\text{PO}_4$ (0.02 $\mu\text{Ci}/\text{mg}$). Immediately after preparation the contents of one set of bottles (TEST, CONTROL, 1X OIL, and 10X OIL) were analyzed for $^{32}\text{PO}_4^{3-}$ by filtering the contents of each bottle through a 0.45 μm pore size membrane filter with two rinses. Water-soluble ^{32}P was determined using a Beckman Model LS 100 liquid scintillation counter. Water-insoluble ^{32}P on the filter was also determined using a Tennelec model TC-545A counter-timer. The remaining bottles were incubated quiescently at 20 C. After five, ten, and fifteen days of incubation, subsequent sets of bottles were analyzed as described above.

16. Lipolysis

Sixteen ten-ml samples were placed in 2-oz prescription bottles and supplemented with 50 mg uniformly labeled ^{14}C -tripalmitin (0.01 $\mu\text{Ci}/\text{mg}$). Samples were analyzed as described in the heterotrophic activity section at zero time and after one, two, and three weeks of incubation.

17. Chitinolysis

Sixteen ten-ml samples were placed in 2-oz prescription bottles and supplemented with 0.1 g chitin. A serum stopper was placed in each bottle, and the atmosphere was replaced with a mixture of 5% argon, 20% oxygen, and 75% nitrogen. Immediately after preparation the contents of one set of bottles (TEST, CONTROL, 1X OIL, and 10X OIL) were analyzed for CO_2 using a Fisher Gas Partitioner Model 1200 GC. The remaining bottles were incubated quiescently at 20 C. After one, two, and three weeks of incubation, subsequent sets of bottles were analyzed as described above.

18. Cellulolysis

Sixteen ten-ml samples were placed in 2-oz prescription bottles and supplemented with 50 mg uniformly labeled ^{14}C -cellulose (0.01 $\mu\text{Ci}/\text{mg}$). Samples were analyzed weekly as described in the heterotrophic activity section.

19. Proteolysis

Sixteen ten-ml samples were placed in 2-oz prescription bottles and supplemented with 50 mg UL ^{14}C -acetylated bovine serum albumin (0.6 $\mu\text{Ci}/\text{g}$). Samples were analyzed weekly as described in the heterotrophic activity section.

E. Pure Culture Studies

The experimental design for assessing the impact of oil on pure cultures was essentially identical to that employed for the sediment processes, except that a pure culture was added (to the test bottles) instead of sediment. All cultures except those obtained commercially were isolated from sediments collected during Cruise II at Platforms P1 or P2, and identified by cellular morphology, colonial morphology, gram reaction, and substrate utilization, according to Skerman (1967).

Two cultures were employed to determine the effect of Empire Mix crude oil on the process of nitrification. *Nitrosomonas europaea* [American Type Culture Collection (ATCC) #19718, non-marine] and a nitrifying enrichment culture (non-marine, prepared from a nitrifying soil sample) were tested for the ability to grow in synthetic seawater. Satisfactory growth was not observed, so the tests were conducted using distilled water instead of seawater.

The activity of ammonia-oxidizing bacteria was determined by monitoring the disappearance of ammonia and appearance of nitrite or nitrate. The composition of the atmosphere of each culture was monitored weekly for a period of 30 days to correlate O_2 and CO_2 consumption with the rate of nitrification.

Studies to assess the impact of oil on photosynthesis were done with *Nostoc muscorum* (ATCC #27347). The alga was grown in 1-liter culture flasks containing CHU #10 medium (ATCC Catalogue #241) amended to 10 ppt salinity with Rila Sea Salts. This inoculum was incubated in a lighted BOD incubator (Precision 31214) at 20 C for 1 week. The cells were then added to 20 l of aerated medium and dispensed into 300-ml BOD bottles. The final cell concentration was 42.6 μg dry weight of cells/ml. The experimental design was the same as in the sediment studies except that there were five replications of each test. Metabolic activity and photosynthesis were determined after 12 hours of incubation by measuring DO using a YSI Model 51B oxygen meter.

Activity of proteolytic bacteria was monitored by measuring the amount of CO_2 produced using a Fisher Model 1200 GC as described in the section on chitinolysis. The two pure cultures (*Pseudomonas* sp. 3 and sp. 4) employed were isolated on Difco-Nutrient Gelatin Agar prepared with Rila Sea Salts.

One culture capable of utilizing glucose (*Enterobacter* sp. 1) was isolated from sediments on Rila Sea Salts amended with 1% glucose. Two methods (gas chromatographic and radioisotopic) of monitoring CO_2 production were employed to determine the impact of crude oil on glucose utilization.

Two pure cultures of cellulose-utilizing bacteria (*Cellulomonas* sp. 1 and sp. 2) were isolated from sediments by streaking enrichments onto Dubos Cellulose Agar (Dubos, 1928) amended with Rila Sea Salts. The impact of oil on cellulose utilization was determined by monitoring CO_2 production using UL ^{14}C -cellulose.

The impact of oil on two pure cultures (*Pseudomonas* sp. 5 and sp. 7) capable of lipolytic activity was determined by monitoring the rate of CO_2 production from ^{14}C -tripalmitin. These cultures were isolated on Difco Spirit Blue Agar prepared with Rila Sea Salts mixture.

Two pure cultures of chitin-utilizing bacteria (*Pseudomonas* sp. 6 and sp. 8) were isolated by incubating the sediment in 30 ppt Rila Sea Salts amended

with 1% finely ground chitin for 3 weeks. Pure cultures were selected after streaking the enrichments onto Rila Sea Salts agar containing 1% chitin.

F. Statistical Analyses

All statistical procedures were carried out on a Univac 1180 computer using the SPSS integrated system of

computer programs. Analyses of data for sediment chemistry and microbial enumerations included one-way analysis of variance, Duncan's multiple range test, and Student's t-test. Analysis of microbial processes and oil degradation were carried out using multiple regression, while comparisons between fresh and frozen samples were made using a correlated t-test.

III. RESULTS

A. Sample Analysis

All of the samples for microbiological analysis were collected aseptically from each grab sample. All of the samples were accounted for using the color-coding system described in II. METHODS AND MATERIALS. A total of 304 sediment samples were analyzed during Cruises I, II, and III (Table 7).

1. Aerobic Heterotrophic Bacteria

A total of 304 sediment samples from Cruises I, II, and III were plated in triplicate, resulting in 912 plates of marine agar that were enumerated for aerobic heterotrophic bacteria. Insignificant differences between counts of aerobic heterotrophs at control sites were observed for each cruise (Table 8). However, the counts for aerobic heterotrophs obtained during Cruise II were significantly lower than those obtained for Cruises I or III (Table 8, Fig. 2). Similar results were obtained for counts of aerobic heterotrophs at the primary platforms, except that the counts for Cruise I were higher at the primary platforms than at the control sites (Table 9). Counts of aerobic heterotrophs at the secondary platforms were similar to those obtained at the primary platforms and control sites during Cruise II (Table 10, Fig. 3), but appeared to decrease along an east-west transect (Fig. 4).

The effect of the freezing of sediments and its relationship to total bacterial counts can be seen in Table 11. There was no statistically significant effect (at the 0.01 probability level) of freezing in sediments that were frozen for 7 days ($60.5 \pm 14.8 \times 10^4$ vs $(54.1 \pm 14.0 \times 10^4$ cfu/ml). However, after 45 days of freezing, the total bacterial population was reduced 50% (to $30.2 \pm 6.7 \times 10^4$) from that of fresh sediments. This reduction was increased to 81% (to $9.8 \pm 1.5 \times 10^4$) after 90 days of freezing (Fig. 5). These results indicate, therefore, that sediments can be frozen without affecting the total plate count if analysis is initiated within one week of freezing.

2. Aerobic Bacteria on Oil Agar

A total of 304 sediment samples from Cruises I, II, and III were plated in triplicate resulting in 912 plates of oil agar that were enumerated for aerobic oil-degrading bacteria. Insignificant differences were observed between counts of bacteria from different control sites and different cruises except for counts from Control Site C22 during Cruise III, which were significantly higher than all other counts, (Table 12, Fig. 6). Differences were observed between counts of bacteria on oil agar from primary platforms for Cruises I, II, and III (Table 13). Most counts were higher for Cruises I and III than

TABLE 7. Number of sediment samples analyzed

Analysis (P = Primary, C = Control, S = Secondary)	Cruise I		Cruise II			Cruise III		Total
	P	C	P	C	S	P	C	
Microbial enumeration and sediment chemistry	64	16	64	16	64	64	16	304
Photosynthesis	8	8	8	8	0	8	8	48
Sediment processes ^a	8	8	8	8	0	8 ^b	8 ^b	48
Oil degradation ^a	1	1	1	1	0	1	1	6
Total	81	33	81	33	64	81	33	406

^aComposite samples

^bOnly 4 sample composites for nitrification, nitrogen fixation, denitrification, sulfur oxidation, sulfate reduction, and phosphate uptake

TABLE 8. Total colony-forming units (CFU) of aerobic heterotrophic bacteria cultured on marine agar per ml of sediment collected at the control sites, expressed as \bar{x} CFU $\times 10^{-3} \pm S\bar{x}$

Control Site	Cruise I	Cruise II	Cruise III
C21	770 \pm 440 (990 \pm 100) ^a	4100 \pm 8000 (85 \pm 34) ^a	380 \pm 150
C22	880 \pm 64	51 \pm 3	680 \pm 430 (970 \pm 140) ^a
C23	810 \pm 75	40 \pm 13	540 \pm 90
C24	2000 \pm 1900	44 \pm 30 (57 \pm 17) ^a	600 \pm 110
\bar{x}	1100 \pm 590	1000 \pm 4000 (50 \pm 30) ^a	550 \pm 240

^a $\bar{x} \pm S\bar{x}$ calculated after eliminating outliers

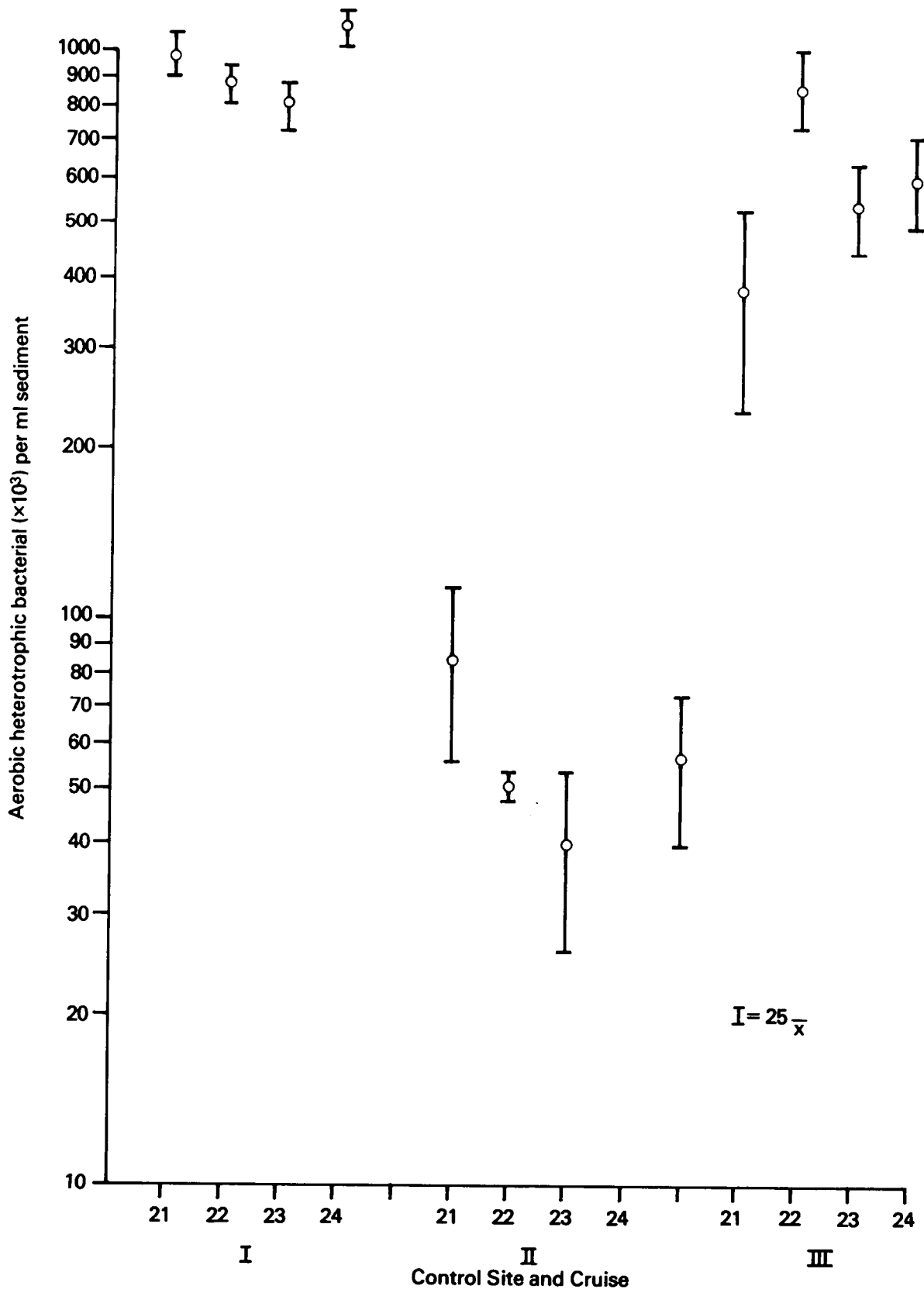


FIG 2. Counts of aerobic heterotrophs obtained at control sites during Cruises I, H, and III.

TABLE 9. Total aerobic heterotrophic bacterial CFU ($\times 10^{-3}$) per ml of sediment collected at the primary platforms

Primary Site	Compass Point	Cruise I	Cruise II	Cruise III
P1	N	2700 \pm 1500	66 \pm 14	260 \pm 50
	E	3775 \pm 380	74 \pm 19	520 \pm 340
	S	4000 \pm 1200	350 \pm 560 (72 \pm 19) ^a	850 \pm 130
	W	4100 \pm 346	77 \pm 8	800 \pm 260
	\bar{x}	3600 \pm 1100	140 \pm 280	600 \pm 320
P2	N	2100 \pm 2400 (4100 \pm 710) ^a	93 \pm 15	890 \pm 30
	E	3300 \pm 2000 (4300 \pm 120) ^a	84 \pm 23	680 \pm 440
	S	1800 \pm 2100 (3600 \pm 140) ^a	66 \pm 12	1200 \pm 470
	W	4000 \pm 1400	85 \pm 31	1600 \pm 640
	\bar{x}	2800 \pm 2000	82 \pm 22	1100 \pm 560
P3	N	6100 \pm 3300	140 \pm 120	820 \pm 480 (1100 \pm 75) ^a
	E	3800 \pm 500	43 \pm 84	1800 \pm 170
	S	4800 \pm 860	52 \pm 33	1200 \pm 560
	W	4600 \pm 1200	61 \pm 14	550 \pm 45
	\bar{x}	4800 \pm 1800	74 \pm 70	1100 \pm 610
P4	N	3600 \pm 280	42 \pm 9	530 \pm 130
	E	4000 \pm 580	42 \pm 13	360 \pm 230 (480 \pm 26) ^a
	S	3800 \pm 260	30 \pm 4	480 \pm 93
	W	3200 \pm 410	36 \pm 6	730 \pm 200
	\bar{x}	3700 \pm 480	37 \pm 9	530 \pm 210
\bar{x}		3700 \pm 1600	84 \pm 150 (66 \pm 41) ^a	840 \pm 520

^a $\bar{x} \pm S\bar{x}$ calculated after eliminating outliers

TABLE 10. Total aerobic heterotrophic bacterial CFU ($\times 10^{-3}$) per ml of sediment collected at the secondary platforms during Cruise II

Secondary Site	CFU \pm $S\bar{x}$	Secondary Site	CFU \pm $S\bar{x}$
S5	320 \pm 150	S13	86 \pm 13
S6	200 \pm 95	S14	18 \pm 11
S7	100 \pm 17	S15	12 \pm 4
S8	71 \pm 33	S16	42 \pm 8
S9	74 \pm 8	S17	25 \pm 6
S10	34 \pm 26	S18	70 \pm 100 (20 \pm 14) ^a
S11	73 \pm 27	S19	5 \pm 2
S12	52 \pm 4	S20	76 \pm 64
$\bar{x} \pm S\bar{x} = 78 \pm 90$			

^a $\bar{x} \pm S\bar{x}$ calculated after eliminating outliers

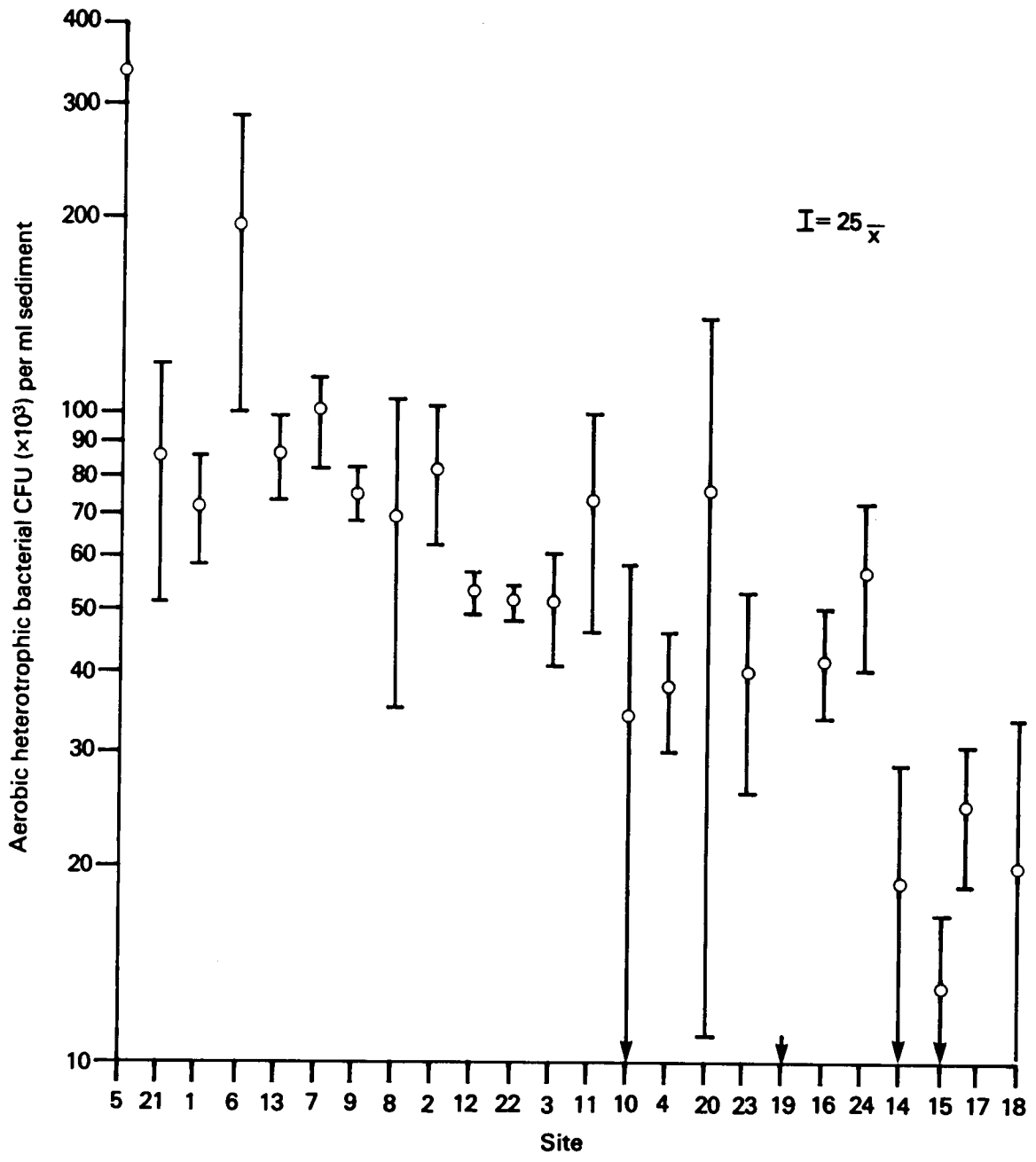


FIG. 3. Counts of aerobic heterotrophs obtained during Cruise II.

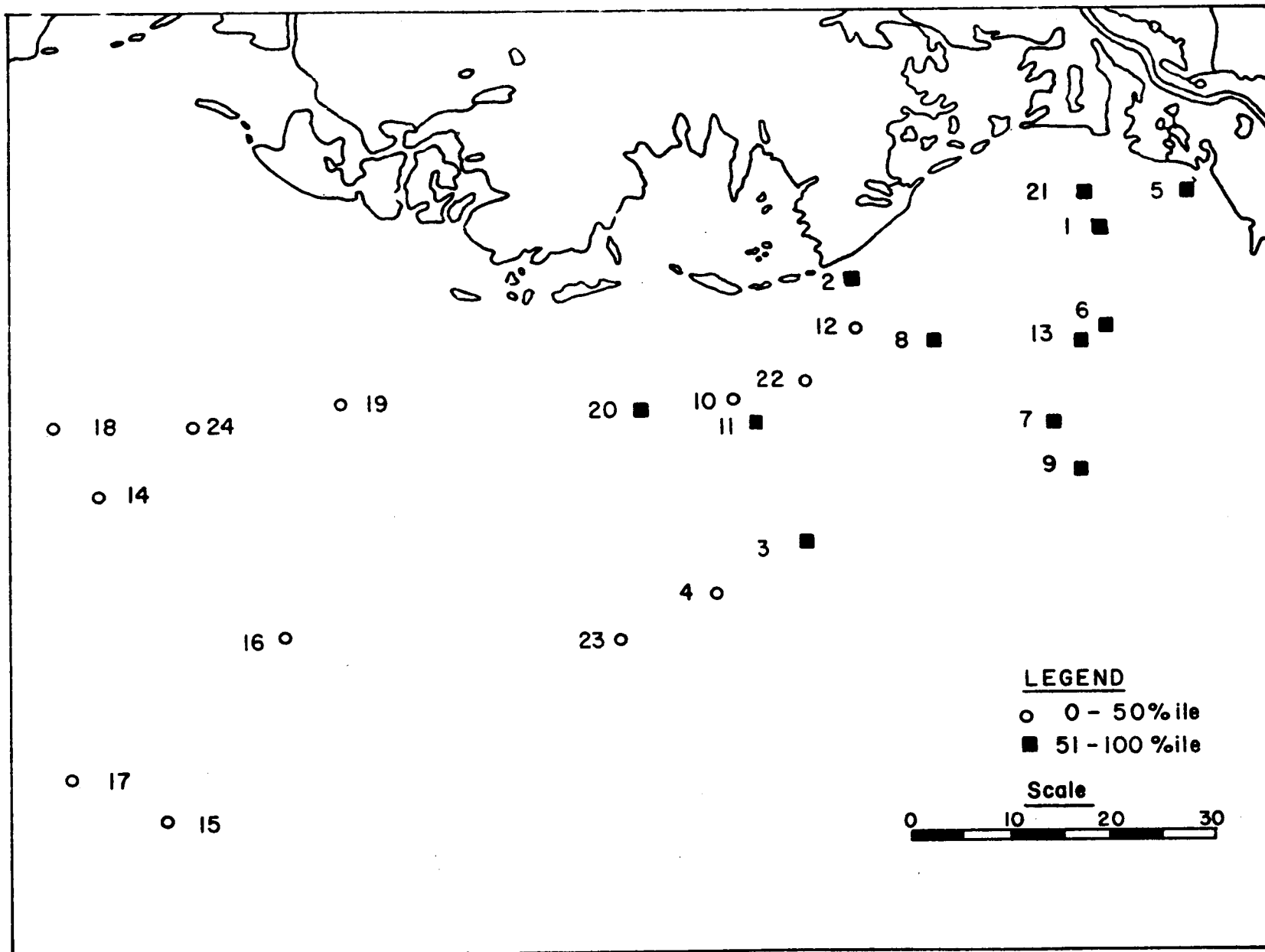


FIG. 4. Total aerobic plate - based on correlated t-test.

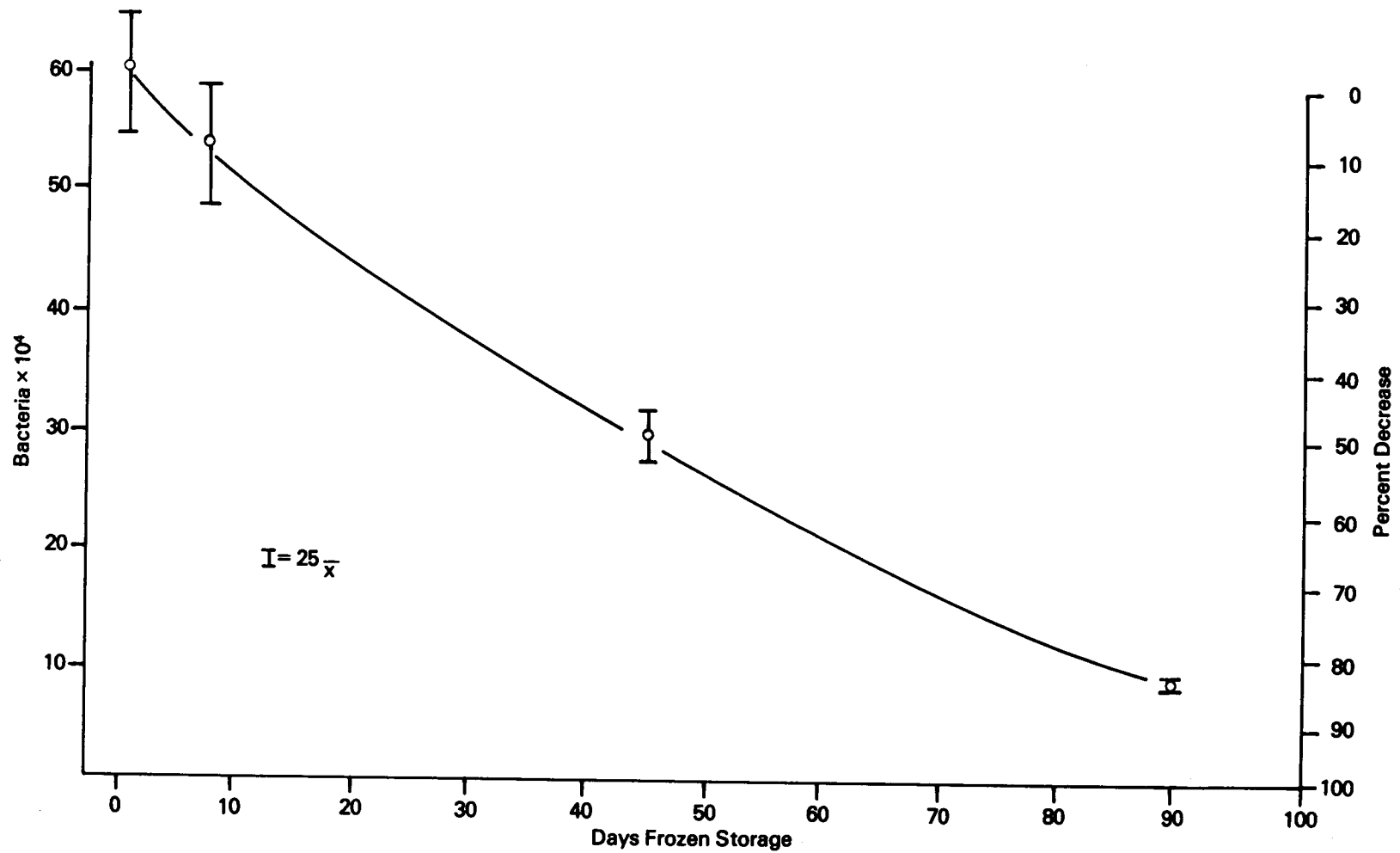


FIG. 5. Effect of freezing on total aerobic heterotrophic bacteria in marine sediments.

TABLE 11. Counts of aerobic heterotrophic bacteria in fresh and frozen sediments expressed as CFU ($\times 10^{-4}$) per ml of sediment

Sample	Fresh Sediments	Frozen Sediments		
		7 days	45 days	90 days
P1a	70	69	33	—
P1b	56	61	33	—
P2a	81	70	29	12.0
P2b	79	53	33	11.0
P3a	31	43	25	12.0
P3b	40	62	25	12.0
P4a	58	39	24	9.6
P4b	76	53	13	7.0
P5a	59	64	36	11.0
P5b	64	65	31	11.0
P6a	79	61	26	11.0
P6b	63	64	22	9.1
P7a	58	66	21	7.7
P7b	65	59	27	8.1
P8a	55	36	35	9.1
P8b	55	46	24	8.8
P9a	49	38	22	9.7
P9b	50	85	31	8.7
P10a	37	47	21	9.1
P10b	40	61	24	9.0
C1a	73	58	39	9.7
C1b	59	42	39	8.2
C2a	52	49	37	11.0
C2b	86	40	31	10.0
C3a	73	46	39	10.0
C3b	65	47	27	11.0
C5a	51	51	34	11.0
C5b	55	52	41	10.0
C6a	73	63	35	8.1
C6b	64	54	29	7.5
C7a	54	63	44	13.0
C7b	42	63	35	12.0
C8a	62	57	28	9.0
C8b	65	59	22	8.4
C9a	60	52	32	8.7
C9b	57	43	36	9.1
C10a	71	54	29	9.2
C10b	70	61	35	9.6
\bar{x}	60.45 ± 14.78	54.08 ± 14.02	30.18 ± 6.72	9.76 ± 1.46

TABLE 12. Average number of aerobic bacterial CFU ($\times 10^{-3}$) cultured on oil agar per ml of sediment collected at the control sites

Control Site	Cruise I	Cruise II	Cruise III
C21	34 ± 20	15 ± 13	220 ± 240
C22	8 ± 12 (2 ± 1) ^a	3 ± 4	500 ± 220
C23	1 ± 0.5	3 ± 1	11 ± 7
C24	1 ± 0.8	5 ± 7	19 ± 4
\bar{x}	11 ± 17	6 ± 9	190 ± 260

^a $\bar{x} \pm S\bar{x}$ calculated after eliminating outliers

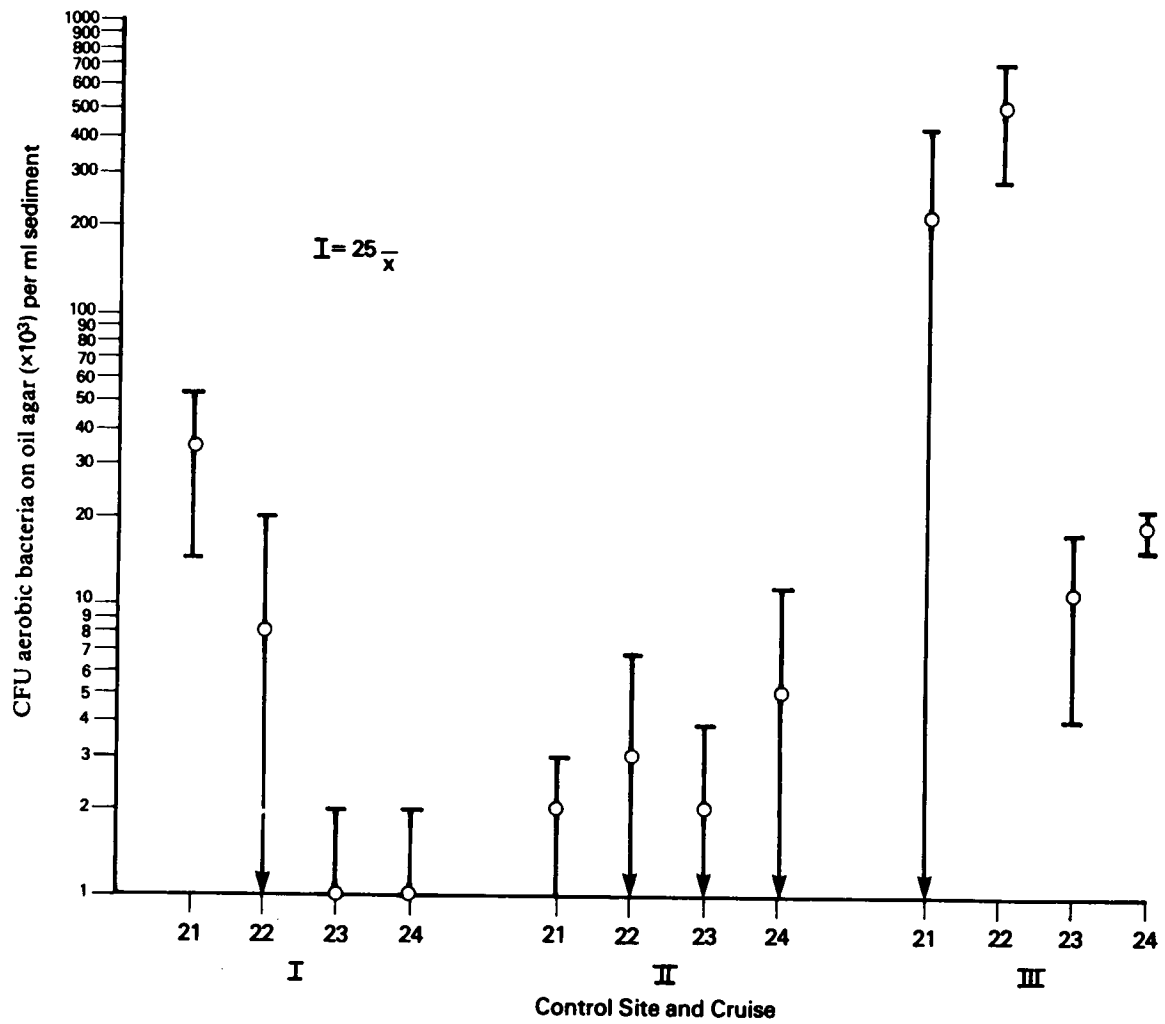


FIG. 6. Counts of bacteria on oil agar obtained at control sites during Cruises I, II, and III.

TABLE 13. Average number of aerobic bacterial CFU($\times 10^{-3}$) cultured on oil agar per ml of sediment collected at the primary platforms

Primary Site	Transect	Cruise I	Cruise II	Cruise III
P1	N	1100 \pm 480	4 \pm 3	310 \pm 93
	E	1300 \pm 800	5 \pm 2	340 \pm 250
	S	2300 \pm 500	5 \pm 2	560 \pm 86
	W	1100 \pm 670	6 \pm 2	420 \pm 85
	\bar{x}	1400 \pm 770	5 \pm 2	410 \pm 160
P2	N	2100 \pm 2300 (4100 \pm 560) ^a	19 \pm 11	860 \pm 130
	E	4500 \pm 1300	6 \pm 3	930 \pm 520
	S	1400 \pm 1600 (2700 \pm 710) ^a	13 \pm 5	1400 \pm 700
	W	1600 \pm 1100	8 \pm 4	2900 \pm 1000
	\bar{x}	(2100 \pm 460) ^a 2400 \pm 2000	12 \pm 8	1500 \pm 1000
P3	N	2900 \pm 1900	3 \pm 2	990 \pm 640
	E	2900 \pm 750	4 \pm 5	1600 \pm 250
	S	4200 \pm 970	3 \pm 1	1200 \pm 1100
	W	3200 \pm 1200	4 \pm 4	350 \pm 320
	\bar{x}	3300 \pm 1300	4 \pm 3	1000 \pm 740
P4	N	1500 \pm 260	2 \pm 1	350 \pm 230
	E	1700 \pm 310	2 \pm 1	170 \pm 170 (315 \pm 21) ^a
	S	2400 \pm 2400	3 \pm 2	580 \pm 250
	W	1400 \pm 140	3 \pm 1	310 \pm 330 (600 \pm 64) ^a
	\bar{x}	1800 \pm 1200	2 \pm 1	350 \pm 270
\bar{x}		2200 \pm 1500	6 \pm 6	820 \pm 800

^a $\bar{x} \pm S\bar{x}$ calculated after eliminating outliers

for Cruise II. Counts of bacteria on oil agar for the secondary platforms were similar to those reported for primary platforms during Cruise II, and to those reported for most control sites during all three cruises (Table 14). Counts of aerobic bacteria were distributed along an east-west transect with the highest count in the eastern portion of the study area (Fig. 7).

When the ratio of bacteria cultured on oil agar:marine agar was expressed as a percentage and used to compare primary platforms and control sites from Cruises I, II, and III, only Cruise I results provided significant differences between primary platforms and control sites (Table 15, Fig. 8). A similar trend was observed for Cruise III, but only for Control Sites C23 and C24 (Table 15). For Cruise II only Secondary Platforms S15 and S17 had significantly high ratios of bacteria on oil agar:marine agar (Fig. 9).

3. Yeasts and Fungi

Yeasts and fungi were enumerated for 304 sediment samples collected during Cruises I, II, and III on triplicate plates of Cooke Rose Bengal (RB) agar and potato dextrose agar (PDA) resulting in 1824 plates. Only counts on RB agar are reported in Tables 16-18; counts on PDA were one or two orders of magnitude lower. For the control sites, generally higher counts of yeasts and fungi were observed during Cruises II and III than during Cruise I (Table 16). The opposite results

were observed for the primary platforms; that is, the highest counts of yeasts and fungi were observed during Cruise I (Table 17). Counts of yeasts and fungi for the secondary platforms were similar to those reported for primary platforms and control sites during Cruise II (Table 18).

4. Nitrate-Reducing, Hydrocarbon-Utilizing Microorganisms

Microorganisms from 304 sediment samples collected during Cruises I, II, and III were cultured in triplicate tubes of Rosenfeld's Nitrate-Hydrocarbon Medium (RNHM), and numbers of microorganisms capable of growing in this medium were quantified by the most probable number (MPN) method. Results from this quantification revealed the numbers of microbes capable of growing in RNHM were significantly lower than aerobic bacteria cultured on marine or oil agar or than yeasts and fungi (Tables 19-21). The highest least-variable counts of microbes capable of growth in RNHM were obtained during Cruise II at Control Sites C22 and C24 (Table 19). Most other counts appeared to be within at least one order of magnitude of each other.

5. Sulfate-Reducing, Hydrocarbon-Utilizing Microorganisms

Microorganisms from 304 sediment samples collected during Cruises I, II, and III were cultured in

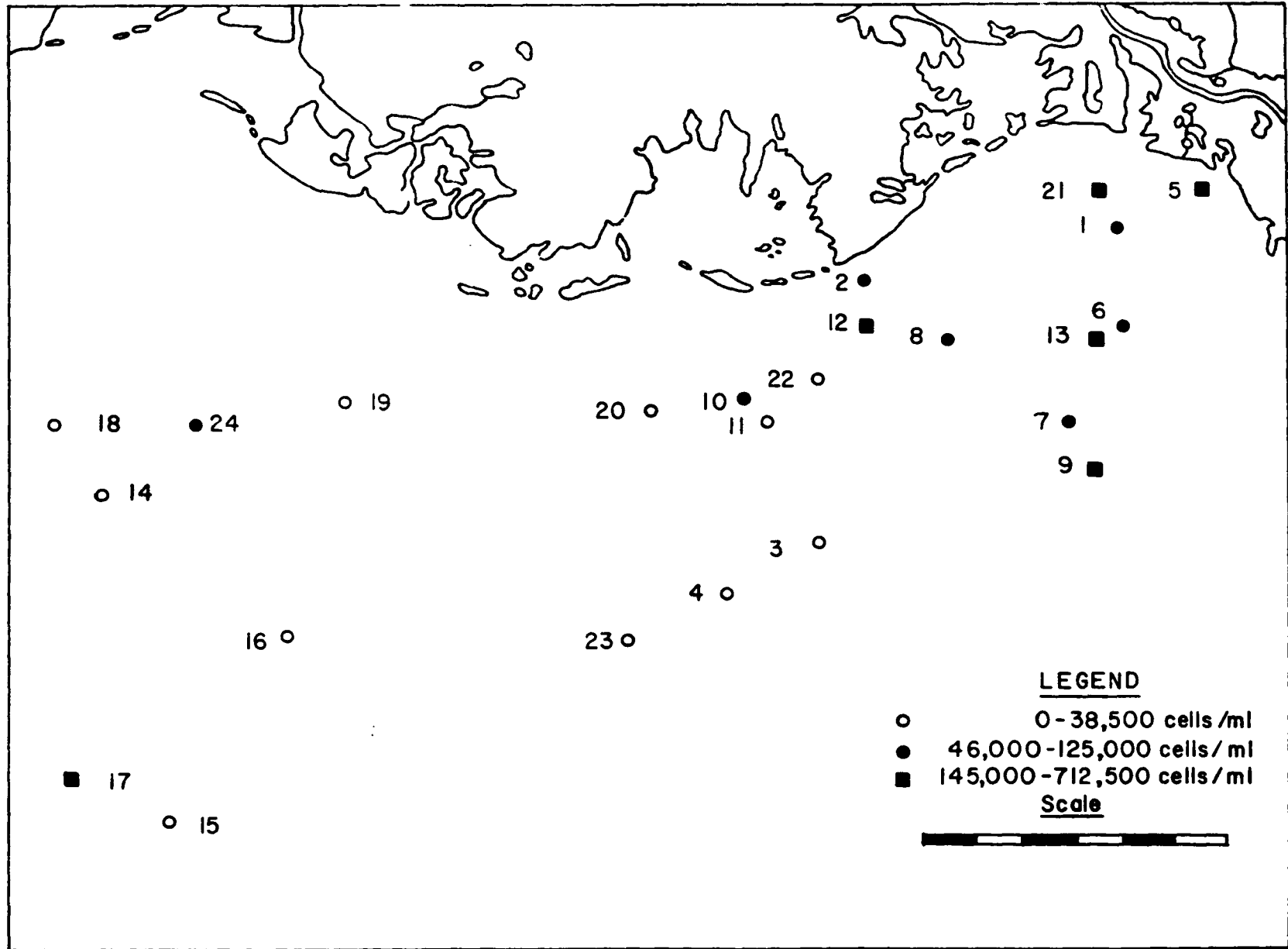


FIG. 7. Aerobic hydrocarbonoclasts based on Duncan's Multiple Range Test (SPSS).

TABLE 14. Average number of aerobic bacterial CFU ($\times 10^{-3}$) cultured on oil agar per ml sediment collected at the secondary platform during Cruise II

Secondary Site	CFU \pm S \bar{x}	Secondary Site	CFU \pm S \bar{x}
S5	71 \pm 8	S13	22 \pm 11
S6	8 \pm 1	S14	2 \pm 2
S7	12 \pm 4	S15	8 \pm 2
S8	9 \pm 3	S16	12 \pm 5
S9	15 \pm 4	S17	15 \pm 5
S10	5 \pm 4	S18	2 \pm 2
S11	4 \pm 1	S19	1 \pm 0.1
S12	14 \pm 4	S20	3 \pm 1
$\bar{x} \pm S\bar{x} = 13 \pm 17$			

TABLE 15. Distribution of bacteria cultured on oil agar expressed as a percentage of the aerobic heterotrophic bacteria cultured on marine agar

Secondary Site	Transect	Cruise I $\bar{x} \pm 2S\bar{x}$	Cruise II $\bar{x} \pm 2S\bar{x}$	Cruise III $\bar{x} \pm 2S\bar{x}$
P1	N	47 \pm 19	6 \pm 4	130 \pm 63
	E	33 \pm 23	7 \pm 3	64 \pm 15
	S	59 \pm 16	6 \pm 5	67 \pm 17
	W	26 \pm 15	8 \pm 3	55 \pm 14
P2	N	100 \pm 34	20 \pm 11	96 \pm 16
	E	96 \pm 31	7 \pm 4	114 \pm 49
	S	50 \pm 32	19 \pm 6	108 \pm 12
	W	38 \pm 26	12 \pm 8	178 \pm 41
P3	N	46 \pm 15	3 \pm 2	109 \pm 38
	E	79 \pm 30	11 \pm 13	85 \pm 20
	S	92 \pm 29	5 \pm 3	92 \pm 65
	W	75 \pm 37	7 \pm 7	84 \pm 61
P4	N	43 \pm 9	5 \pm 3	99 \pm 48
	E	42 \pm 13	5 \pm 3	99 \pm 57
	S	64 \pm 66	9 \pm 7	127 \pm 66
	W	44 \pm 8	8 \pm 4	67 \pm 12
C21	—	7 \pm 5	14 \pm 18	72 \pm 44
C22	—	1 \pm 1	5 \pm 8	56 \pm 24
C23	—	0.1 \pm 0.05	9 \pm 4	2 \pm 1
C24	—	0.1 \pm 0.05	13 \pm 12	3 \pm 0.5

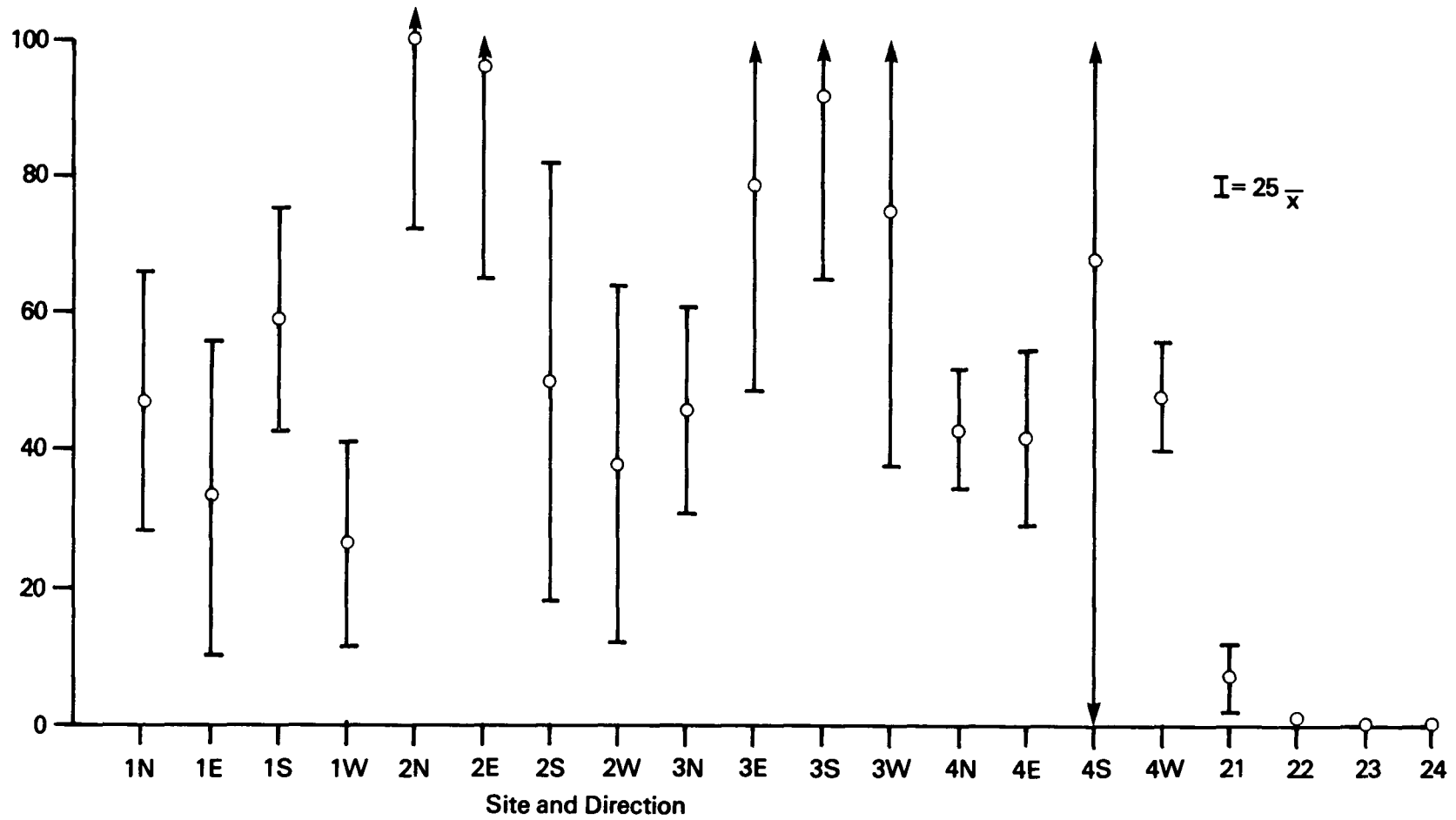


FIG. 8. Distribution of bacteria from Cruise I cultured on oil agar expressed as percentage of the bacteria cultured on marine agar.

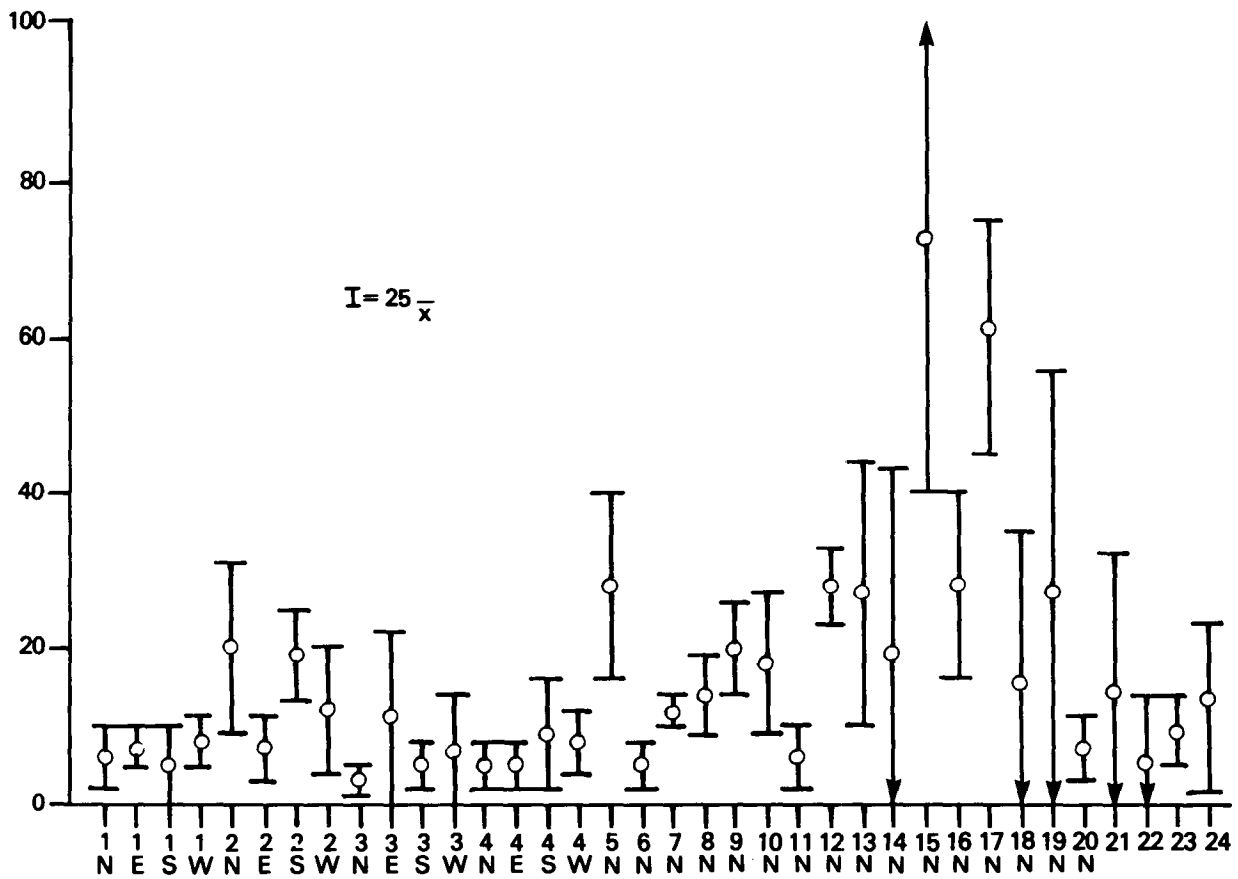


FIG. 9. Distribution of bacteria from Cruise II cultured on oil agar expressed as a percentage of the bacteria cultured on marine agar.

TABLE 16. Total number of yeast and fungal CFU cultured on Cooke's Rose Bengal Agar per ml of sediment collected at the control sites

Control Site	Cruise I	Cruise II	Cruise III
C21	50 ± 50	7800 ± 14000 (430 ± 250) ^a	450 ± 360
C22	2200 ± 1200	1300 ± 1500	680 ± 270
C23	1600 ± 2100	1200 ± 900	58 ± 56
C24	50 ± 50	1400 ± 440	250 ± 320
\bar{x}	980 ± 1450	3000 ± 7200 (1200 ± 910) ^a	360 ± 340

^a $\bar{x} \pm S\bar{x}$ calculated after eliminating outliers

TABLE 17. Total number of yeast and fungal CFU cultured on Cooke's Rose Bengal Agar per ml of sediment collected at the primary platforms

Primary Site	Transect	Cruise I	Cruise II	Cruise III
P1	N	23,000	400	350
	E	45,000	1,300	510
	S	45,000	780	330
	W	45,000	1,300	980
	\bar{x}	39,000	940	540
P2	N	190	1,800	1,100
	E	17,000	720	450
	S	2,700	150	150
	W	16,000	550	1,900
	\bar{x}	8,800	810	890
P3	N	15,000	1,800	320
	E	20,000	780	130
	S	16,000	830	320
	W	9,600	970	25
	\bar{x}	15,000	1,100	200
P4	N	17,000	620	1,200
	E	24,000	680	880
	S	8,700	800	740
	W	23,000	420	2,200
	\bar{x}	18,000	630	1,300
	$\bar{x} \pm S\bar{x}$	20,000 ± 19,000	860 ± 670	720 ± 910

TABLE 18. Total number of yeast and fungal CFU cultured on Cooke's Rose Bengal Agar per ml of sediment collected at the secondary platforms during Cruise II

Secondary Site	CFU	Secondary Site	CFU
S5	820	S13	2200
S6	900	S14	1700
S7	750	S15	920
S8	1100	S16	400
S9	1700	S17	1800
S10	1100	S18	840
S11	1800	S19	580
S12	900	S20	360
$\bar{x} \pm S\bar{x} = 1300 \pm 1400$			

TABLE 19. Total number of microorganisms cultured in Rosenfeld's nitrate-hydrocarbon medium per ml of sediment collected at the control sites

Control Site	Cruise I	Cruise II	Cruise III
C21	16 ± 8	2 ± 1	290 ± 540 (15 ± 11) ^a
C22	8 ± 10	50 ± 41	25 ± 45
C23	8 ± 10	6 ± 4	150 ± 100
C24	10 ± 9	51 ± 41	160 ± 200
\bar{x}	11 ± 9	27 ± 35	160 ± 280 (95 ± 130) ^a

^a $\bar{x} \pm S\bar{x}$ calculated after eliminating outliers

TABLE 20. Total number of microorganisms cultured in Rosenfeld's nitrate-hydrocarbon medium per ml of sediment collected at the primary platforms

Primary Site	Transect	Cruise I	Cruise II	Cruise III
P1	N	35 ± 40	10 ± 10	420 ± 510
	E	6 ± 4	5 ± 3	40 ± 39
	S	36 ± 38	3 ± 1	120 ± 220
	W	10 ± 10	6 ± 4	260 ± 240
	\bar{x}	22 ± 29	6 ± 6	200 ± 300
P2	N	150 ± 110	33 ± 51	5 ± 3
	E	19 ± 18	6 ± 4	37 ± 38
	S	140 ± 210	5 ± 3	36 ± 38
	W	80 ± 110	3 ± 1	6 ± 7
	\bar{x}	98 ± 130	12 ± 26	21 ± 29
P3	N	48 ± 52	7 ± 3	84 ± 110
	E	6 ± 4	145 ± 220	19 ± 18
	S	3 ± 4	15 ± 18	14 ± 20
	W	28 ± 43	5 ± 4	6 ± 3
	\bar{x}	22 ± 36	43 ± 110	31 ± 61
P4	N	350 ± 510	6 ± 2	8 ± 5
	E	5 ± 2	8 ± 4	14 ± 19
	S	4 ± 1	280 ± 550	8 ± 9
	W	5 ± 3	5 ± 3	14 ± 8
	\bar{x}	91 ± 280	74 ± 270	11 ± 11
	$\bar{x} \pm S\bar{x}$	57 ± 150	34 ± 150	68 ± 170

TABLE 21. Total number of microorganisms cultured in Rosenfeld's nitrate-hydrocarbon medium per ml of sediment collected at the secondary platforms during Cruise II

Secondary Site	$\bar{x} \pm S\bar{x}$	Secondary Site	$\bar{x} \pm S\bar{x}$
S5	9 ± 0	S13	58 ± 60
S6	18 ± 18	S14	8 ± 3
S7	15 ± 21	S15	2 ± 1
S8	31 ± 53	S16	2 ± 2
S9	38 ± 48	S17	1 ± 1
S10	26 ± 15	S18	6 ± 4
S11	13 ± 7	S19	2 ± 1
S12	33 ± 51	S20	6 ± 2
$\bar{x} \pm S\bar{x} = 16 \pm 29$			

triplicate tubes of Rosenfeld's Sulfate-Hydrocarbon Medium (RSHM). The number of microorganisms capable of growing in this medium were quantified by the MPN method. Results from this quantification produced numbers similar to those derived for microbes capable of growing in RNHM (Tables 22-24). Counts of these microbes could be described on an east-west transect (Fig. 10).

6. Chemical Nutrients

Sediment samples (304) collected from control sites, primary platforms and secondary platforms during Cruises I, II, and III were analyzed for phosphate phosphorus ($\text{PO}_4\text{-P}$), ammonia nitrogen ($\text{NH}_4\text{-N}$), nitrate nitrogen ($\text{NO}_3\text{-N}$), nitrite nitrogen ($\text{NO}_2\text{-N}$), total Kjeldahl nitrogen (TN), and total phosphorus (TP). Concentrations of $\text{PO}_4\text{-P}$ and $\text{NH}_4\text{-N}$ at control sites were lowest during Cruise I and highest during Cruise II

(Tables 25-27). Concentrations of TP at control sites were highest during Cruise I and lowest during Cruise III. Similar concentrations of $\text{NO}_3\text{-N}$, and TN were detected in control site sediments during all three cruises.

Concentrations of $\text{PO}_4\text{-P}$ in sediments collected at Primary Platform P1 were lowest during Cruise I and highest during Cruises II and III. The opposite results were observed for Primary Platforms P2, P3, and P4 (Tables 28-30). Concentrations of $\text{NH}_4\text{-N}$ in sediments collected at primary platforms were lower during Cruise I than during Cruises II and III. Concentrations of $\text{NO}_3\text{-N}$ and TN were similar for primary platform sediments collected during Cruises I, II, and III. Concentrations of TP at primary platforms were somewhat lower during Cruise III.

Concentrations of $\text{PO}_4\text{-P}$ in sediments collected from Secondary Platforms S5 and S6 were similar to concentrations reported for control sites and for

TABLE 22. Total number of microorganisms cultured on Rosenfeld's sulfate-hydrocarbon medium per ml of sediment collected at the control sites

Control Site	Cruise I	Cruise II	Cruise III
C21	20 ± 7	5 ± 3	35 ± 17
C22	9 ± 10	3 ± 1	9 ± 9
C23	7 ± 3	3 ± 1	5 ± 3
C24	3 ± 1	2 ± 0	6 ± 2
\bar{x}	10 ± 8	3 ± 2	13 ± 16

TABLE 23. Total number of microorganisms cultured in Rosenfeld's sulfate-hydrocarbon medium per ml of sediment collected at the primary platforms

Primary Site	Transect	Cruise I	Cruise II	Cruise III
P1	N	15 ± 9	3 ± 1	8 ± 2
	E	33 ± 12	8 ± 11	18 ± 9
	S	28 ± 10	4 ± 4	18 ± 19
	W	10 ± 9	3 ± 1	29 ± 12
	\bar{x}	21 ± 13	4 ± 6	18 ± 13
P2	N	140 ± 120	4 ± 1	20 ± 7
	E	20 ± 7	2 ± 2	18 ± 19
	S	23 ± 16	3 ± 1	10 ± 9
	W	12 ± 8	4 ± 4	4 ± 1
	\bar{x}	48 ± 76	3 ± 2	13 ± 12
P3	N	7 ± 3	3 ± 1	3 ± 1
	E	18 ± 10	3 ± 1	6 ± 6
	S	15 ± 10	3 ± 2	25 ± 14
	W	16 ± 8	3 ± 1	37 ± 41
	\bar{x}	14 ± 8	3 ± 1	18 ± 24
P4	N	20 ± 7	8 ± 11	3 ± 2
	E	9 ± 10	2 ± 0.4	3 ± 1
	S	13 ± 7	3 ± 1	5 ± 3
	W	6 ± 4	3 ± 1	4 ± 1
	\bar{x}	12 ± 9	4 ± 5	4 ± 2
$\bar{x} \pm S\bar{x}$		24 ± 41	4 ± 5	13 ± 16

TABLE 24. Total number of microorganisms cultured in Rosenfeld's sulfate-hydrocarbon medium per ml of sediment collected at the secondary platforms during Cruise II

Secondary Site	$\bar{x} \pm S\bar{x}$	Secondary Site	$\bar{x} \pm S\bar{x}$
S5	10 ± 8	S13	10 ± 9
S6	10 ± 9	S14	2 ± 0
S7	14 ± 12	S15	2 ± 0
S8	6 ± 2	S16	2 ± 0
S9	8 ± 5	S17	2 ± 0
S10	2 ± 0	S18	2 ± 0
S11	3 ± 1	S19	0 ± 0
S12	10 ± 10	S20	2 ± 0
$\bar{x} \pm S\bar{x} = 5 \pm 6$			

TABLE 25. Concentrations of inorganic nutrients ($\mu\text{g atom/kg}$ dry weight sediment) in sediments collected from control sites during Cruise I

Control Site	PO ₄ -P	NH ₄ -N	NO ₃ -N	Total N ($\times 10^{-2}$)	Total P ($\times 10^{-4}$)
C21	4.1 ± 5.3	130 ± 12	330 ± 230	230 ± 150	160 ± 57
C22	4.6 ± 6.8	77 ± 11	310 ± 140	380 ± 230	210 ± 18
C23	43.0 ± 75.0 (5.7 ± 7.7)	91 ± 14	1000 ± 170	340 ± 210	180 ± 23
C24	4.4 ± 5.6	1100 ± 1500 (380 ± 430) ^a	490 ± 82	250 ± 150	130 ± 7
\bar{x}	14 ± 38 (4.6 ± 5.6) ^a	350 ± 820 (160 ± 220) ^a	540 ± 330	300 ± 180	172 ± 41

^a $\bar{x} \pm S\bar{x}$ calculated after eliminating outlier

TABLE 26. Concentrations of inorganic nutrients ($\mu\text{g atom/kg}$ dry weight sediment) in sediments collected from control sites during Cruise II

Control Site	PO ₄ -P	NH ₄ -N	NO ₃ -N	Total N ($\times 10^{-2}$)	Total P ($\times 10^{-2}$)
C21	380 ± 260	1500 ± 140	850 ± 160	130 ± 49	96 ± 45
C22	220 ± 140	1100 ± 580	530 ± 200	110 ± 40	97 ± 7
C23	260 ± 310	1400 ± 500	960 ± 170	150 ± 83	85 ± 20
C24	140 ± 50	1300 ± 400	400 ± 250	170 ± 86	94 ± 16
\bar{x}	250 ± 210	1300 ± 420	690 ± 290	140 ± 65	93 ± 24

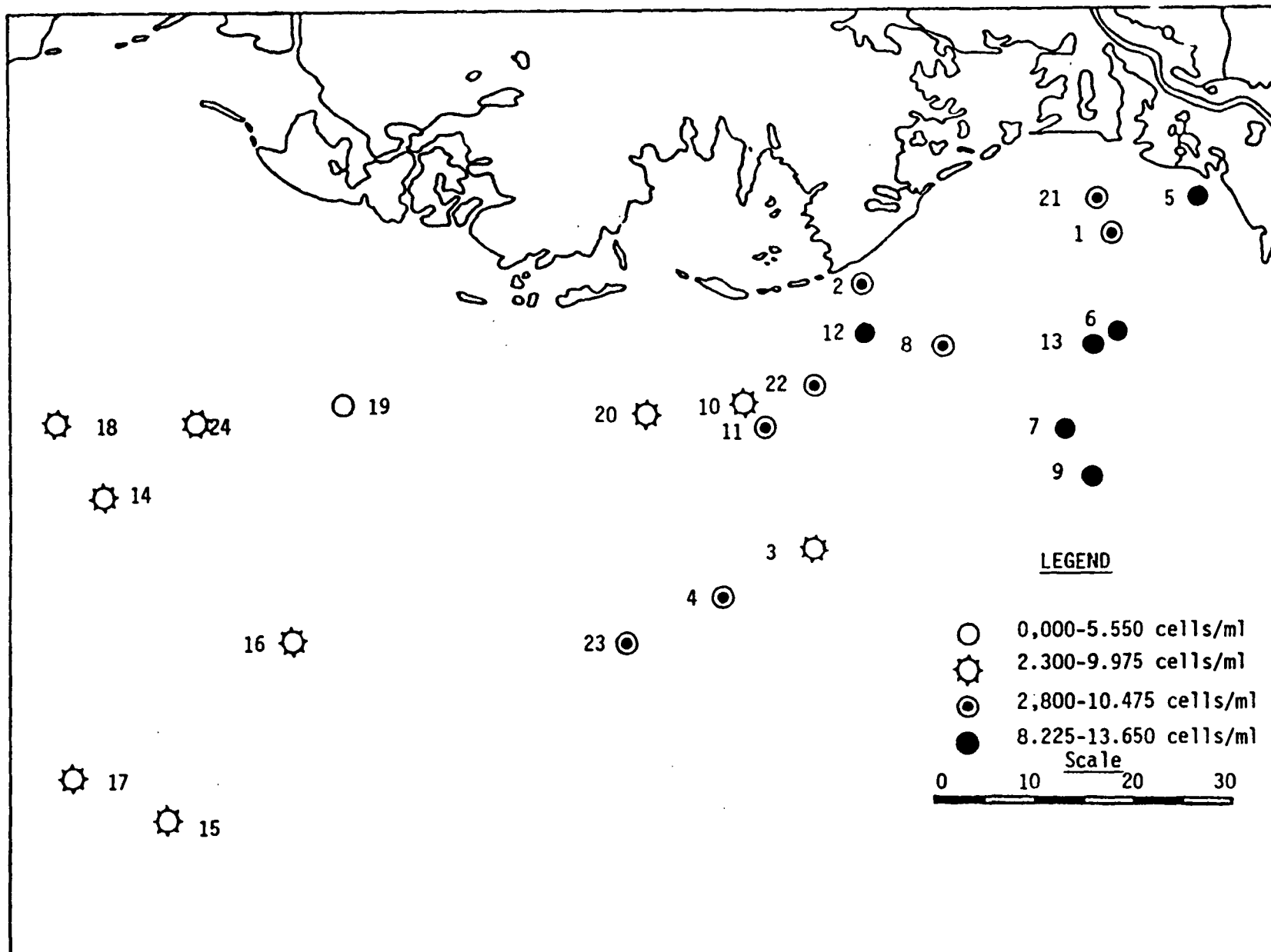


FIG. 10. Subsets of sulfate-reducing, hydrocarbon-utilizing bacteria based on Duncan's Multiple Range Test (SPSS).

**TABLE 27. Concentrations of inorganic nutrients ($\mu\text{g atom/kg dry weight sediment}$)
in sediments collected from control sites during Cruise III**

Control Site	PO ₄ -P	NH ₄ -N	NO ₃ -N	Total N ($\times 10^{-2}$)	Total P ($\times 10^{-2}$)
C21	170 \pm 59	1300 \pm 670	460 \pm 310	79 \pm 77	150 \pm 54
C22	39 \pm 46	1200 \pm 160	670 \pm 230	260 \pm 130	140 \pm 6
C23	73 \pm 34	1100 \pm 310	570 \pm 230	70 \pm 40	130 \pm 17
C24	75 \pm 19	940 \pm 290	810 \pm 40	920 \pm 1300 (270 \pm 54) ^a	120 \pm 12
\bar{x}	90 \pm 64	1100 \pm 390	630 \pm 240	330 \pm 690 (160 \pm 120) ^a	130 \pm 27

^a $\bar{x} \pm S\bar{x}$ calculated after eliminating outliers.

**TABLE 28. Concentrations of inorganic nutrients ($\mu\text{g atoms/kg dry weight sediment}$)
in sediments collected from the primary platforms during Cruise I**

Primary Site	Transect	PO ₄ -P	NH ₄ -N	NO ₃ -N	Total N ($\times 10^{-2}$)	Total P ($\times 10^{-2}$)
P1	N	8 \pm 1	180 \pm 160	270 \pm 180	100 \pm 60	100 \pm 43
	E	55 \pm 87	180 \pm 100	330 \pm 240	220 \pm 220	150 \pm 23
	S	13 \pm 1	330 \pm 140	220 \pm 240	340 \pm 230	160 \pm 150
	W	33 \pm 37	490 \pm 300	190 \pm 120	190 \pm 110	140 \pm 230
	\bar{x}	27 \pm 46	300 \pm 220	250 \pm 190	210 \pm 170	140 \pm 230
P2	N	86 \pm 11	140 \pm 88	310 \pm 89	220 \pm 110	110 \pm 16
	E	180 \pm 130	190 \pm 180	310 \pm 78	380 \pm 430	220 \pm 61
	S	110 \pm 28	130 \pm 130	420 \pm 350	150 \pm 52	100 \pm 53
	W	210 \pm 47	25 \pm 4	330 \pm 62	130 \pm 78	180 \pm 40
	\bar{x}	150 \pm 81	120 \pm 120	340 \pm 170	220 \pm 220	150 \pm 81
P3	N	120 \pm 33	28 \pm 8	370 \pm 260	140 \pm 150	260 \pm 96
	E	180 \pm 200	77 \pm 82	480 \pm 250	210 \pm 230	340 \pm 180
	S	340 \pm 95	120 \pm 130	510 \pm 150	360 \pm 81	350 \pm 120
	W	340 \pm 90	380 \pm 260	890 \pm 590	430 \pm 160	330 \pm 170
	\bar{x}	240 \pm 150	150 \pm 190	560 \pm 380	290 \pm 190	320 \pm 130
P4	N	150 \pm 58	270 \pm 340	290 \pm 120	360 \pm 170	170 \pm 160
	E	120 \pm 22	250 \pm 150	720 \pm 310	300 \pm 100	190 \pm 250
	S	95 \pm 21	98 \pm 150	250 \pm 76	250 \pm 150	150 \pm 270
	W	130 \pm 47	210 \pm 220	880 \pm 330	450 \pm 540	180 \pm 480
	\bar{x}	120 \pm 42	210 \pm 210	530 \pm 350	340 \pm 280	170 \pm 330
\bar{x}		140 \pm 120	190 \pm 200	420 \pm 310	260 \pm 220	200 \pm 110

TABLE 29. Concentrations of inorganic nutrients ($\mu\text{g atoms/kg dry weight sediment}$) in sediments collected from primary platforms during Cruise II

Primary Site	Transect	PO ₄ -P	NH ₄ -N	NO ₃ -N	Total N ($\times 10^{-2}$)	Total P ($\times 10^{-2}$)
P1	N	130 ± 20	1400 ± 470	690 ± 100	140 ± 79	81 ± 21
	E	150 ± 58	880 ± 250	570 ± 130	180 ± 180	74 ± 8
	S	240 ± 83	1400 ± 310	690 ± 70	270 ± 400	87 ± 19
	W	210 ± 67	1500 ± 420	840 ± 150	130 ± 110	110 ± 21
	\bar{x}	180 ± 69	1300 ± 410	700 ± 150	180 ± 210	88 ± 21
P2	N	12 ± 5	480 ± 190	430 ± 160	120 ± 160	74 ± 32
	E	17 ± 17	910 ± 840	440 ± 310	110 ± 160	91 ± 66
	S	10 ± 7	530 ± 280	520 ± 290	150 ± 170	99 ± 68
	W	33 ± 21	2000 ± 1900	1200 ± 1200	150 ± 140	280 ± 340
	\bar{x}	18 ± 16	970 ± 1100	650 ± 660	130 ± 140	140 ± 180
P3	N	35 ± 52	1500 ± 1700	1200 ± 1300	180 ± 230	240 ± 320
	E	40 ± 46	670 ± 550	610 ± 300	90 ± 44	150 ± 79
	S	52 ± 40	820 ± 500	650 ± 220	85 ± 69	89 ± 56
	W	9 ± 5	380 ± 150	430 ± 230	43 ± 24	67 ± 34
	\bar{x}	34 ± 40	850 ± 960	710 ± 690	100 ± 120	140 ± 160
P4	N	14 ± 2	900 ± 300	1100 ± 250	130 ± 75	95 ± 16
	E	14 ± 2	1000 ± 150	720 ± 84	100 ± 25	110 ± 33
	S	13 ± 2	940 ± 120	730 ± 82	63 ± 28	110 ± 12
	W	14 ± 1	1100 ± 96	920 ± 180	74 ± 50	110 ± 12
	\bar{x}	14 ± 2	980 ± 180	860 ± 210	93 ± 52	110 ± 19
	$\bar{x} \pm S\bar{x}$	62 ± 81	1000 ± 770	730 ± 490	130 ± 140	120 ± 120

TABLE 30. Concentrations of inorganic nutrients ($\mu\text{g atoms/kg dry weight sediment}$) in sediments collected from the primary platforms during Cruise III

Primary Site	Transect	PO ₄ -P	NH ₄ -N	NO ₃ -N	Total N ($\times 10^{-2}$)	Total P ($\times 10^{-2}$)
P1	N	130 ± 31	1400 ± 82	670 ± 70	8 ± 3	120 ± 5
	E	150 ± 22	1200 ± 130	770 ± 53	30 ± 29	140 ± 24
	S	180 ± 59	1000 ± 200	750 ± 170	15 ± 3	120 ± 33
	W	140 ± 22	1000 ± 130	620 ± 210	200 ± 120	140 ± 7
	\bar{x}	150 ± 37	1100 ± 200	700 ± 140	62 ± 97	130 ± 21
P2	N	27 ± 24	260 ± 150	430 ± 99	25 ± 7	140 ± 45
	E	9 ± 3	280 ± 43	270 ± 120	33 ± 35	81 ± 42
	S	27 ± 17	510 ± 320	410 ± 150	28 ± 24	130 ± 47
	W	39 ± 23	300 ± 120	550 ± 250	110 ± 67	120 ± 41
	\bar{x}	26 ± 20	340 ± 200	410 ± 180	48 ± 50	120 ± 45
P3	N	68 ± 36	1900 ± 590	1000 ± 190	51 ± 25	240 ± 30
	E	45 ± 23	1800 ± 970	680 ± 260	57 ± 18	190 ± 76
	S	100 ± 39	2100 ± 740	750 ± 200	140 ± 100	140 ± 26
	W	77 ± 41	1600 ± 290	760 ± 190	95 ± 130	130 ± 15
	\bar{x}	73 ± 38	1900 ± 650	800 ± 230	86 ± 85	170 ± 60
P4	N	15 ± 5	1100 ± 420	460 ± 180	150 ± 27	110 ± 21
	E	13 ± 2	1100 ± 280	360 ± 230	220 ± 50	110 ± 23
	S	12 ± 2	830 ± 150	330 ± 210	190 ± 35	110 ± 22
	W	13 ± 2	940 ± 180	260 ± 48	190 ± 55	120 ± 21
	\bar{x}	13 ± 3	1000 ± 280	350 ± 180	180 ± 46	120 ± 20
\bar{x}		66 ± 61	1100 ± 660	570 ± 260	93 ± 88	130 ± 46

Primary Platform P1 during Cruise II (Table 31). Concentrations of PO₄-P in sediments collected from Secondary Platforms S7-S9 and S12-S20 were similar to concentrations reported for Primary Platforms P2-P4 during Cruise II. Concentrations of NH₄-N, NO₃-N, TN and TP in sediments collected from secondary platforms were similar to concentrations reported for control sites and primary platforms during Cruise II. Detectable levels of NO₂-N were not found at any site on any cruise.

7. Oil Degradation

a. Sediments

For each of the three cruises, tests for oil-degrading potential of sediments were done on one composite sample from the four primary platforms and on one composite sample from the four control sites. The eight different test systems employed in these studies were designed to test the effect of temperature, oil concentration, and added nutrients (nitrogen and phosphorus) on oil degradation (Table 32). Oxygen consumption data are given in Tables 33-38. In all test systems, the slopes of the curves prepared from the oxygen consumption data were essentially the same for both the platforms and the control sites on each cruise, but differed from cruise to cruise. The sediments from Cruise II showed a greater oil-degrading potential than samples collected during Cruises I and III. This was reflected not

only in a more rapid rate of oxygen utilization but also in greater total oxygen utilization over the 60 day period of the test (Fig. 11).

The rate of oxygen utilization was essentially the same at 15 C, 20 C, and 27 C, as determined by the slope of the oxygen utilization curves (Fig. 12). It took longer to reach the stationary phase at 15 C than it did at either 27 C or 20 C.

Increasing the concentration of oil ten-fold in the sediments did not have any significant effect on the rate of oxygen utilization. Added nitrogen (ammonium chloride or potassium nitrate) and phosphorus (potassium phosphate) did not enhance oxygen utilization by the sediments.

The analysis data were erratic. In setting up the test systems, the crude oil was added to a slurry of the sediments prior to dispensing into the BOD bottles and a majority of the oil was adsorbed onto particulate matter. While every effort was made to dispense a representative amount of slurry into each bottle, the heterogeneity of the sediments themselves created problems in dispensing the same amount of sediment and oil into each bottle, even though the total volume placed in each bottle was the same. The problem was further aggravated by the presence of background hydrocarbons in the sediment samples.

When the amount of oil in the sample was determined on the basis of the amount of *n*-C₁₆ present,

TABLE 31. Concentrations of inorganic nutrients ($\mu\text{g atoms/kg dry weight sediment}$) in sediments collected from the secondary platforms during Cruise II

Secondary Site	PO ₄ -P	NH ₄ -N	NO ₃ -N	Total N ($\times 10^{-2}$)	Total P ($\times 10^{-2}$)
S5	150 \pm 36	550 \pm 170	430 \pm 88	42 \pm 33	72 \pm 19
S6	170 \pm 75	1700 \pm 290	1100 \pm 150	330 \pm 120	96 \pm 20
S7	130 \pm 230	610 \pm 49	800 \pm 340	210 \pm 140	93 \pm 44
S8	55 \pm 49	700 \pm 360	860 \pm 86	58 \pm 13	93 \pm 8
S9	95 \pm 10	680 \pm 230	1200 \pm 190	320 \pm 160	110 \pm 9
S10	a	2100 \pm 540	540 \pm 80	310 \pm 150	200 \pm 21
S11	a	1900 \pm 530	550 \pm 24	580 \pm 480	220 \pm 55
S12	83 \pm 76	490 \pm 38	680 \pm 31	44 \pm 26	73 \pm 28
S13	33 \pm 5	2400 \pm 380	1400 \pm 460	340 \pm 340	150 \pm 9
S14	89 \pm 23	850 \pm 240	480 \pm 190	150 \pm 46	120 \pm 40
S15	35 \pm 16	980 \pm 390	1000 \pm 270	120 \pm 48	83 \pm 7
S16	24 \pm 3	1400 \pm 330	1700 \pm 600	79 \pm 23	160 \pm 23
S17	86 \pm 64	1200 \pm 540	1400 \pm 450	54 \pm 24	230 \pm 35
S18	62 \pm 36	1000 \pm 270	340 \pm 130	160 \pm 38	130 \pm 71
S19	18 \pm 5	530 \pm 260	290 \pm 140	24 \pm 16	160 \pm 5
S20	54 \pm 12	670 \pm 250	350 \pm 140	100 \pm 84	130 \pm 150
$\bar{x} \pm S$	78 \pm 82	1100 \pm 660	810 \pm 480	180 \pm 210	130 \pm 65

a = samples lost

TABLE 32. Purpose of test systems shown in TABLE 6

Test System	Data Employed to Determine
1 vs 2 vs 3	Effect of temperature on oil degradation
5 vs 7 and 6 vs 8	Effect of oil concentration on oil degradation
3 vs 4 vs 5 vs 6	Effect of added phosphorus and nitrogen on oil degradation

TABLE 33. Oxygen consumption ($\bar{x} \pm S$) by microorganisms from Cruise I control site sediments in test systems 1-8 (see TABLE 6)

Incubation Time (Days)	Dissolved oxygen consumed (mg/l) in test system number							
	1	2	3	4	5	6	7	8
4	0.3 ± 0	0.3 ± 0	0.5 ± 0	0.4 ± 0	0.4 ± 0	0.4 ± 0	0.4 ± 0	0.4 ± 0
7	0.6 ± 0	0.7 ± 0	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0	0.9 ± 0.1
11	0.8 ± 0.1	0.7 ± 0	1.2 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1
14	1.3 ± 0.2	1.0 ± 0.1	1.5 ± 0.1	1.9 ± 0.2	1.8 ± 0.1	1.8 ± 0.2	2.0 ± 0.2	2.0 ± 0.2
17	1.5 ± 0.3	1.1 ± 0.1	1.9 ± 0.1	2.3 ± 0.2	2.1 ± 0.2	2.1 ± 0.2	2.6 ± 0.1	2.6 ± 0.2
20	1.5 ± 0.3	1.2 ± 0.1	2.1 ± 0.2	2.4 ± 0.2	2.4 ± 0.2	2.2 ± 0.2	3.0 ± 0.1	2.8 ± 0.2
24	1.6 ± 0.4	1.1 ± 0.1	2.3 ± 0.1	2.9 ± 0.3	3.0 ± 0.4	2.3 ± 0.1	3.5 ± 0	3.1 ± 0.3
27	1.8 ± 0.5	1.2 ± 0.1	2.6 ± 0.2	3.3 ± 0.4	3.4 ± 0.5	2.6 ± 0.1	4.2 ± 0.1	3.6 ± 0.3
31	2.4 ± 0.6	1.3 ± 0.1	2.9 ± 0.3	3.4 ± 0.4	3.8 ± 0.7	2.9 ± 0.2	4.4 ± 0.2	3.9 ± 0.3
34	2.6 ± 0.6	1.4 ± 0.1	3.0 ± 0.3	3.6 ± 0.4	3.9 ± 0.7	2.9 ± 0.2	4.6 ± 0.4	4.1 ± 0.3
40	3.1 ± 0.8	1.5 ± 0.1	3.4 ± 0.4	4.0 ± 0.4	4.1 ± 0.8	3.2 ± 0.2	4.9 ± 0.6	4.9 ± 0.3
45	3.3 ± 0.6	1.9 ± 0.1	3.8 ± 0.6	4.4 ± 0.1	4.7 ± 0.3	3.4 ± 0.2	5.5 ± 1.2	5.0 ± 0.5
49	3.5 ± 0.6	2.1 ± 0.4	3.9 ± 0.7	4.5 ± 0.1	5.5 ± 0.3	3.6 ± 0.2	5.9 ± 1.4	5.2 ± 0.5
53	3.7 ± 0.7	2.3 ± 0.6	4.0 ± 0.7	4.6 ± 0.2	5.7 ± 0.6	3.6 ± 0.2	6.2 ± 1.3	5.5 ± 0.5
60	4.1 ± 0.9	2.4 ± 0.7	4.1 ± 0.7	4.7 ± 0.2	6.0 ± 0.7	3.9 ± 0.2	7.1 ± 1.0	5.8 ± 0.7

TABLE 34. Oxygen consumption ($\bar{x} \pm S$) by microorganisms from Cruise I platform site sediments in test systems 1-8 (see TABLE 6)

Incubation Time (Days)	Dissolved oxygen consumed (mg/l) in test system number							
	1	2	3	4	5	6	7	8
4	0.3 ± 0	0.4 ± 0	0.5 ± 0	0.4 ± 0	0.4 ± 0	0.5 ± 0	0.4 ± 0	0.4 ± 0
7	0.6 ± 0	0.9 ± 0	1.0 ± 0.1	0.9 ± 0	0.8 ± 0.1	0.1 ± 0.1	1.0 ± 0.1	0.9 ± 0.1
11	1.0 ± 0.1	1.2 ± 0.1	1.5 ± 0.2	1.6 ± 0.1	1.5 ± 0.2	1.6 ± 0.2	1.6 ± 0.1	1.5 ± 0.1
14	1.6 ± 0.2	1.6 ± 0.1	1.9 ± 0.2	2.1 ± 0.2	1.9 ± 0.2	2.1 ± 0.2	2.2 ± 0.2	2.0 ± 0.1
17	1.9 ± 0.3	1.8 ± 0.1	2.3 ± 0.1	2.5 ± 0.2	2.3 ± 0.2	2.4 ± 0.2	2.8 ± 0.2	2.9 ± 0.1
20	1.9 ± 0.3	1.8 ± 0.1	2.4 ± 0.1	2.7 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	3.2 ± 0.2	2.1 ± 0.2
24	2.2 ± 0.4	1.9 ± 0.2	2.7 ± 0.2	2.9 ± 0.2	2.8 ± 0.4	2.9 ± 0.3	3.8 ± 0.1	3.6 ± 0.2
27	2.4 ± 0.5	2.0 ± 0.2	2.9 ± 0.2	3.2 ± 0.2	3.1 ± 0.6	3.1 ± 0.3	4.5 ± 0.1	4.2 ± 0.3
31	3.1 ± 0.5	2.2 ± 0.2	3.2 ± 0.2	3.3 ± 0.3	3.6 ± 0.9	3.3 ± 0.3	4.6 ± 0.1	4.4 ± 0.3
34	3.3 ± 0.5	2.2 ± 0.2	3.2 ± 0.2	3.5 ± 0.3	3.8 ± 0.8	3.4 ± 0.4	4.7 ± 0.1	4.5 ± 0.3
40	3.7 ± 0.5	2.3 ± 0.2	3.6 ± 0.2	4.0 ± 0.3	4.4 ± 0.8	3.7 ± 0.4	5.0 ± 0.1	4.8 ± 0.3
45	4.3 ± 0.4	2.6 ± 0.2	3.8 ± 0.2	4.3 ± 0.4	4.8 ± 0.4	4.0 ± 0.3	5.1 ± 0.1	5.3 ± 0.3
49	4.4 ± 0.6	2.8 ± 0.4	3.8 ± 0.3	4.7 ± 0.3	5.1 ± 0.4	4.2 ± 0.4	5.6 ± 0.3	5.7 ± 0.3
53	4.6 ± 0.6	2.9 ± 0.4	4.2 ± 0.8	3.0 ± 0.3	5.2 ± 0.3	4.3 ± 0.4	6.7 ± 0.3	6.0 ± 0.3
60	4.8 ± 1.2	3.0 ± 0.4	5.2 ± 0.7	5.3 ± 0.3	5.5 ± 0.3	5.0 ± 0.2	7.5 ± 0.1	6.6 ± 0.1

TABLE 35. Oxygen consumption ($\bar{x} \pm S$) by microorganisms from Cruise II control site sediments in test systems 1-8 (see TABLE 6)

Incubation Time (Days)	Dissolved oxygen consumed (mg/l) in test system number							
	1	2	3	4	5	6	7	8
4	3.6 ± 1.2	1.9 ± 0.1	3.2 ± 0.3	3.5 ± 0.2	3.1 ± 0.3	4.3 ± 0.1	4.6 ± 0.7	4.6 ± 0.1
7	15.2 ± 4.0	5.5 ± 0.4	15.0 ± 0.7	14.9 ± 0.8	13.0 ± 0.7	17.3 ± 1.6	15.8 ± 1.5	15.8 ± 0.6
11	28.5 ± 7.6	20.2 ± 2.1	27.8 ± 1.8	30.4 ± 1.4	27.9 ± 0.7	25.3 ± 2.0	31.9 ± 1.6	26.3 ± 0.9
14	39.1 ± 12.2	23.6 ± 1.4	34.4 ± 1.3	36.6 ± 1.9	34.2 ± 1.1	28.3 ± 2.0	39.2 ± 1.5	29.0 ± 0.9
17	45.3 ± 16.3	27.2 ± 1.5	38.7 ± 1.2	40.7 ± 1.9	37.7 ± 1.0	31.8 ± 2.0	43.4 ± 2.0	32.5 ± 0.8
20	53.1 ± 18.7	30.1 ± 1.6	41.0 ± 1.2	43.7 ± 2.2	39.9 ± 0.9	34.5 ± 1.1	46.9 ± 2.0	34.3 ± 0.7
24	61.2 ± 21.1	33.2 ± 1.6	42.5 ± 1.2	45.5 ± 2.2	41.6 ± 0.9	36.8 ± 1.4	48.7 ± 2.1	35.2 ± 0.7
27	67.0 ± 21.8	34.8 ± 1.7	44.0 ± 1.2	47.2 ± 2.2	43.2 ± 0.8	38.7 ± 1.6	51.0 ± 1.9	37.7 ± 0.6
31	70.3 ± 23.2	35.3 ± 1.9	44.0 ± 1.2	47.2 ± 2.2	43.2 ± 0.8	38.8 ± 1.6	51.0 ± 1.9	37.7 ± 0.6
34	75.3 ± 24.1	37.0 ± 2.0	45.1 ± 1.2	48.5 ± 2.2	44.7 ± 0.8	40.6 ± 1.7	52.7 ± 2.0	40.0 ± 0.9
40	86.7 ± 22.6	37.1 ± 2.2	45.5 ± 0.8	49.8 ± 1.3	45.0 ± 1.1	41.7 ± 2.7	53.2 ± 2.8	40.0 ± 1.2
45	93.7 ± 24.0	37.2 ± 2.4	46.3 ± 1.1	51.0 ± 1.5	45.9 ± 1.1	42.9 ± 2.7	53.3 ± 2.8	40.0 ± 1.0
49	96.8 ± 26.8	38.0 ± 3.1	46.4 ± 1.0	51.0 ± 1.5	45.9 ± 1.1	43.0 ± 2.9	53.9 ± 2.6	40.7 ± 0.7
53	101.2 ± 27.3	38.3 ± 3.1	47.3 ± 0.8	52.0 ± 1.7	46.4 ± 1.3	43.9 ± 2.7	55.1 ± 2.6	42.0 ± 0.8
60	103.2 ± 28.0	38.9 ± 3.1	47.5 ± 0.8	52.0 ± 1.8	47.1 ± 1.3	44.3 ± 2.4	55.7 ± 2.6	42.4 ± 0.7

TABLE 36. Oxygen consumption ($\bar{x} \pm S$) by microorganisms from Cruise II platform site sediments in test systems 1-8 (see TABLE 6)

Incubation Time (Days)	Dissolved oxygen consumed (mg/l) in test system number							
	1	2	3	4	5	6	7	8
4	2.8 ± 1.6	1.8 ± 0.1	3.1 ± 0.3	3.6 ± 0.2	3.6 ± 0.2	4.5 ± 0.1	4.5 ± 0.5	4.6 ± 0.2
7	11.3 ± 3.8	5.2 ± 0.4	15.1 ± 0.9	14.4 ± 0.7	14.8 ± 0.7	15.8 ± 0.6	14.9 ± 0.6	15.2 ± 0.6
11	21.5 ± 8.4	17.2 ± 2.3	30.3 ± 0.9	30.3 ± 0.6	29.9 ± 0.8	24.4 ± 1.6	30.5 ± 0.7	25.4 ± 0.6
14	28.8 ± 15.2	24.6 ± 2.5	38.3 ± 0.8	37.1 ± 0.8	38.4 ± 0.9	28.0 ± 2.0	38.5 ± 3.1	29.2 ± 1.1
17	33.4 ± 17.4	28.6 ± 2.9	42.5 ± 1.0	41.9 ± 0.8	43.0 ± 2.0	31.5 ± 1.9	44.2 ± 3.5	32.5 ± 1.0
20	42.9 ± 22.4	33.8 ± 2.7	46.2 ± 0.9	45.1 ± 0.8	45.5 ± 2.5	34.0 ± 1.7	47.8 ± 1.6	34.1 ± 0.5
24	50.2 ± 26.5	37.6 ± 3.3	48.3 ± 0.8	46.9 ± 0.7	47.3 ± 2.3	36.1 ± 1.8	49.3 ± 1.7	35.6 ± 0.6
27	54.6 ± 29.2	39.8 ± 3.4	50.0 ± 0.8	48.5 ± 0.8	49.0 ± 2.3	37.9 ± 1.9	51.5 ± 1.8	38.0 ± 0.6
31	56.5 ± 30.1	39.8 ± 3.4	50.0 ± 0.8	48.6 ± 0.8	49.0 ± 2.3	38.2 ± 2.1	51.5 ± 1.8	38.0 ± 0.6
34	61.0 ± 32.4	41.4 ± 3.4	51.3 ± 0.9	50.1 ± 0.8	50.5 ± 2.3	40.0 ± 2.5	57.6 ± 1.7	39.8 ± 0.6
40	42.3 ± 12.9	43.2 ± 4.1	50.9 ± 1.1	50.4 ± 1.1	51.4 ± 3.4	39.0 ± 1.2	52.5 ± 1.7	40.2 ± 0.9
45	45.2 ± 13.7	44.0 ± 3.9	51.6 ± 1.1	52.0 ± 1.1	52.7 ± 3.4	40.3 ± 1.2	52.8 ± 1.8	40.7 ± 1.2
49	46.1 ± 13.7	45.8 ± 4.2	52.2 ± 1.1	52.0 ± 1.1	52.7 ± 3.4	40.3 ± 1.2	53.7 ± 2.0	41.4 ± 1.4
53	48.1 ± 14.2	46.6 ± 4.3	53.1 ± 1.1	53.0 ± 1.2	53.4 ± 3.5	40.8 ± 1.9	54.7 ± 2.0	42.5 ± 1.4
60	49.3 ± 14.6	47.3 ± 4.4	53.5 ± 1.0	53.7 ± 1.2	54.1 ± 3.4	41.9 ± 1.9	55.3 ± 1.9	42.9 ± 1.5

TABLE 37. Oxygen consumption ($\bar{x} \pm S$) by microorganisms from Cruise III control site sediments in test systems 1-8 (see TABLE 6)

Incubation Time (Days)	Dissolved oxygen consumed (mg/l) in test system number							
	1	2	3	4	5	6	7	8
4	0.2 ± 0	0.3 ± 0	0.4 ± 0	0.3 ± 0	0.4 ± 0	0.4 ± 0	10.4 ± 0	0.4 ± 0
7	0.4 ± 0	0.7 ± 0.1	0.9 ± 0	0.7 ± 0	0.7 ± 0.1	0.7 ± 0	0.9 ± 0.1	0.8 ± 0
11	0.7 ± 0	0.9 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0	1.0 ± 0.1	0.9 ± 0
14	0.8 ± 0	1.1 ± 0.1	1.3 ± 0.6	1.1 ± 0.1	1.1 ± 0.2	0.9 ± 0	1.2 ± 0.1	1.0 ± 0
17	1.1 ± 0.1	1.2 ± 0.1	1.6 ± 0.8	1.3 ± 0.1	1.3 ± 0.2	1.0 ± 0.1	1.3 ± 0.1	1.1 ± 0
20	1.3 ± 0	1.2 ± 0.2	1.7 ± 0.7	1.4 ± 0.1	1.4 ± 0.3	1.2 ± 0.1	1.4 ± 0.1	1.2 ± 0
24	1.5 ± 0.1	1. ± 0.2	1.8 ± 0.7	1.5 ± 0.1	1.5 ± 0.3	1.3 ± 0.1	1.5 ± 0.1	1.2 ± 0
27	1.5 ± 0.1	1.4 ± 0.2	1.8 ± 0.7	1.5 ± 0.1	1.7 ± 0.3	1.3 ± 0.1	1.5 ± 0.1	1.3 ± 0
31	1.5 ± 0.1	1.4 ± 0.2	1.8 ± 0.7	1.5 ± 0.1	1.7 ± 0.3	1.3 ± 0.1	1.7 ± 0.1	1.4 ± 0
34	1.6 ± 0.1	1.4 ± 0.2	2.0 ± 0.1	1.7 ± 0.1	1.8 ± 0.4	1.5 ± 0	1.9 ± 0.1	1.6 ± 0
40	1.8 ± 0.1	1.4 ± 0.2	2.0 ± 0.1	1.7 ± 0.1	1.8 ± 0.2	1.5 ± 0.1	2.0 ± 0.1	1.6 ± 0
45	2.0 ± 0.1	1.5 ± 0.2	2.0 ± 0.1	1.7 ± 0.1	1.9 ± 0.2	1.6 ± 0.1	2.2 ± 0.2	1.7 ± 0.1
49	2.3 ± 0.1	1.5 ± 0.2	2.2 ± 0.1	1.8 ± 0.1	2.0 ± 0.2	2.0 ± 0.2	2.3 ± 0.1	1.8 ± 0.1
53	2.5 ± 0	1.6 ± 0.2	2.4 ± 0	1.9 ± 0.1	2.3 ± 0.4	2.5 ± 0.3	2.4 ± 0.2	2.0 ± 0.1
60	2.6 ± 0.1	1.6 ± 0.2	2.7 ± 0.3	1.9 ± 0.1	2.7 ± 0.9	2.6 ± 0.3	2.7 ± 0.3	2.0 ± 0.1

TABLE 38. Oxygen consumption ($\bar{x} \pm S$) by microorganisms from Cruise III platform site sediments in test systems 1-8 (see TABLE 6)

Incubation Time (Days)	Dissolved oxygen consumed (mg/l) in test system number							
	1	2	3	4	5	6	7	8
4	0.2 ± 0	0.1 ± 0	0.4 ± 0	0.4 ± 0	0.4 ± 0	0.4 ± 0	0.4 ± 0	0.4 ± 0
7	0.4 ± 0.1	0.6 ± 0	1.0 ± 0.1	0.9 ± 0	0.9 ± 0.1	0.8 ± 0	0.9 ± 0.1	0.8 ± 0.1
11	0.7 ± 0.2	0.9 ± 0	1.1 ± 0.1	1.1 ± 0	1.1 ± 0.1	1.0 ± 0	1.1 ± 0.1	1.0 ± 0.1
14	0.9 ± 0.4	1.0 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	1.3 ± 0.2	1.1 ± 0.1
17	1.2 ± 0.5	1.2 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.3 ± 0.1	1.5 ± 0.3	1.2 ± 0.1
20	1.3 ± 0.7	1.3 ± 0.1	1.7 ± 0.2	1.7 ± 0.2	1.8 ± 0.2	1.4 ± 0.1	1.8 ± 0.3	1.3 ± 0.1
24	1.6 ± 0.6	1.5 ± 0.1	1.8 ± 0.2	1.8 ± 0.2	2.0 ± 0.4	1.5 ± 0.1	1.8 ± 0.3	1.4 ± 0.1
27	1.6 ± 0.6	1.5 ± 0.1	1.8 ± 0.2	1.9 ± 0.2	2.2 ± 0.3	1.5 ± 0.1	1.8 ± 0.3	1.4 ± 0.1
31	1.6 ± 0.6	1.5 ± 0.1	2.0 ± 0.2	2.0 ± 0.3	2.3 ± 0.4	1.6 ± 0.1	1.9 ± 0.3	1.5 ± 0.1
34	1.7 ± 0.7	1.5 ± 0.1	2.1 ± 0.3	2.2 ± 0.3	2.8 ± 0.3	1.8 ± 0.1	2.1 ± 0.2	1.8 ± 0.1
40	2.1 ± 1.1	1.4 ± 0	2.1 ± 0.1	2.1 ± 0.1	3.0 ± 0.1	2.0 ± 0.3	2.2 ± 0.4	1.8 ± 0.1
45	2.4 ± 1.2	1.5 ± 0	2.2 ± 0.3	2.3 ± 0.3	3.5 ± 0.3	2.6 ± 0.5	2.4 ± 0.5	1.9 ± 0.1
49	2.7 ± 1.3	1.6 ± 0	2.6 ± 0.5	2.6 ± 0.5	4.0 ± 0.5	3.0 ± 0.3	2.7 ± 0.8	1.9 ± 0.1
53	2.9 ± 1.4	1.6 ± 0	3.2 ± 0.3	2.9 ± 0.6	4.3 ± 0.7	3.3 ± 0.2	3.0 ± 1.3	2.3 ± 0.5
60	2.9 ± 1.4	1.6 ± 0	3.4 ± 0.4	3.3 ± 0.3	4.4 ± 0.7	3.4 ± 0.2	3.5 ± 1.6	2.7 ± 0.4

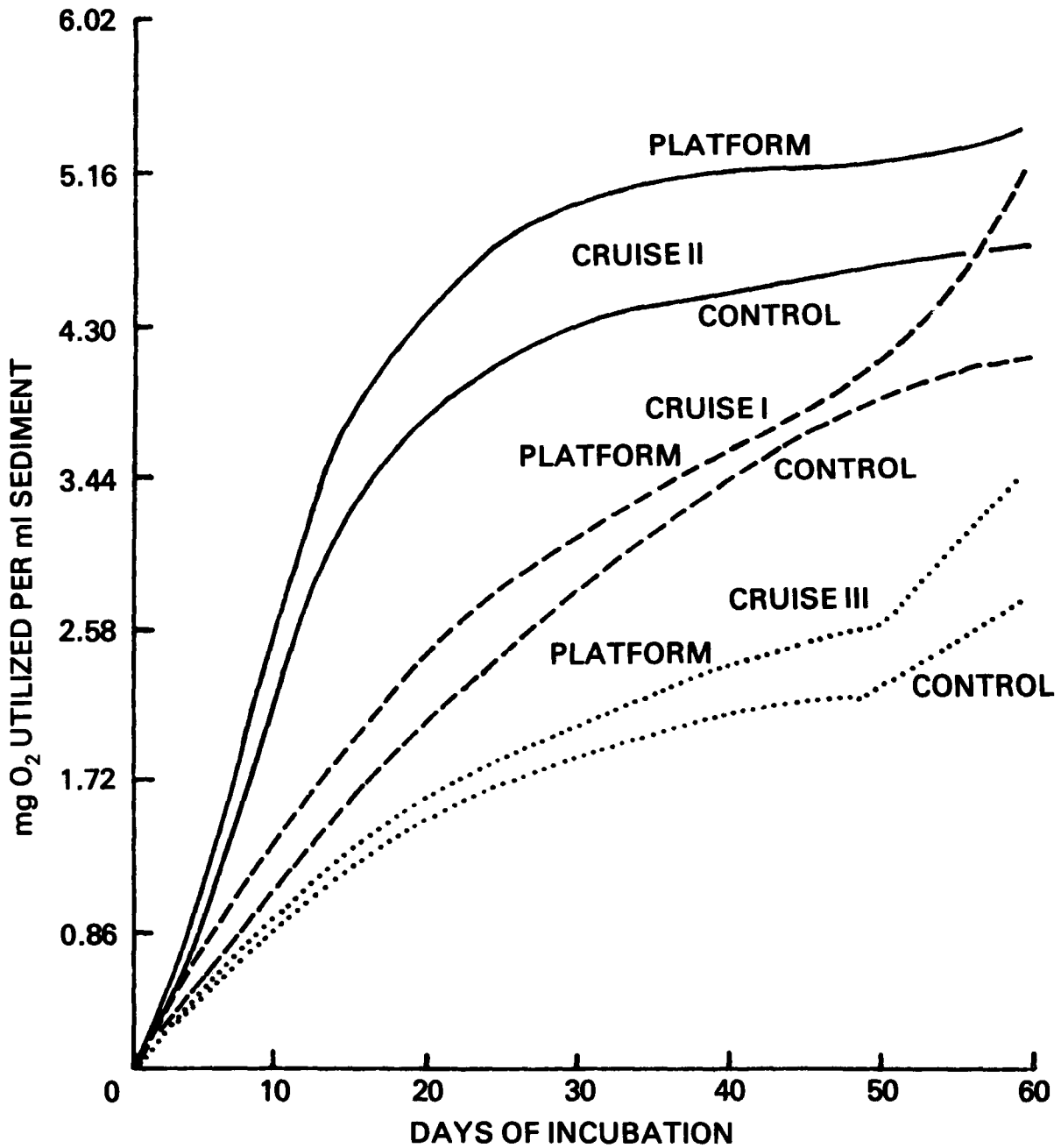


FIG. 11. Oxygen utilization by sediments during incubation with oil at 27 C in BOD bottles.

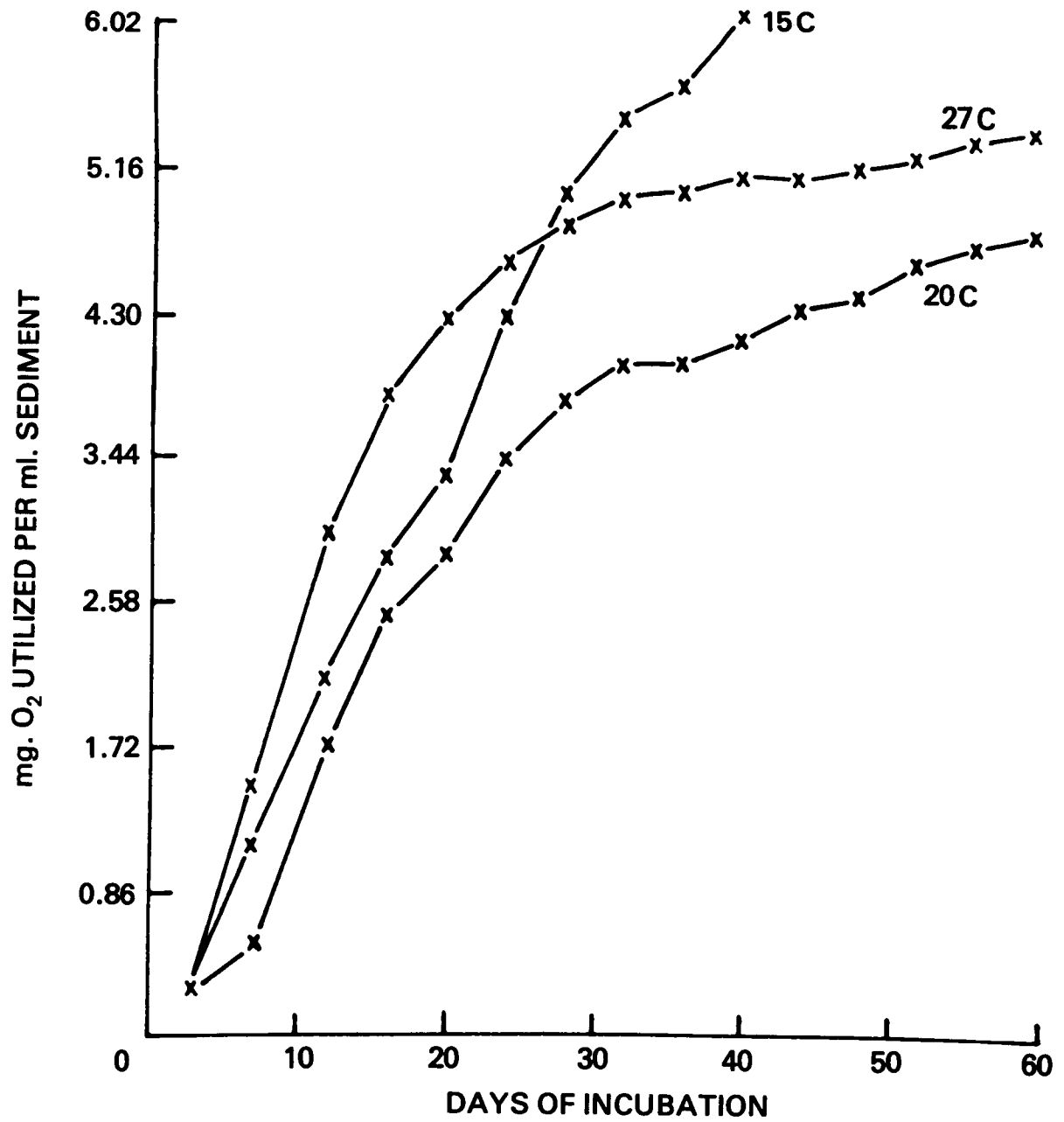


FIG. 12. Effect of temperature on oxygen utilization by sediments taken from platform areas (Cruise II) during incubation with oil in BOD bottles.

the data indicated that all of the oil was consumed within the first 20 days of incubation.

The data obtained by measuring UV absorption at 277 nm were highly variable and sometimes the values increased with increasing time of incubation. Part of this observation may be explained on the basis of the production by the microflora of compounds that absorb 277 nm UV light.

Samples were also analyzed by fluorescence using a 274 nm excitation wavelength and measuring emission at 370 nm. The values obtained by this method of analysis were low and decreased with increasing time of incubation.

Analyses were also conducted by measuring fluorescence at 418 nm after excitation at 403 nm. This method of analysis detects petrogenic hydrocarbons but not biogenic hydrocarbons. On the basis of these analyses the hydrocarbon content decreased with increasing time of incubation and was usually absent after 20 days of incubation.

The colonial types developing on oil agar streaked from the BOD bottles after incubation were similar for both platform and control samples. Only several distinct colonial types were present and the majority of these resembled pseudomonads. Four of the predominant type colonies were subjected to purification procedures and were employed in the next section of the study. Two of the organisms were identified as *Pseudomonas* species and two were identified as *Flavobacterium* species.

b. Mixed and Pure Cultures

Studies on the degradation of crude oil by mixed and pure cultures were carried out in 125-ml Warburg flasks.

In the first experiment, 50 μ l of Empire Mix crude oil in the test flasks was inoculated with a slurry prepared from a mixture of sediments from control and platform sites from Cruise I. Flasks without oil served as the endogenous controls. Oxygen consumption and carbon dioxide production are shown in Fig. 13. As may be observed, the rate of oxygen utilization and carbon dioxide production rose rapidly after 18 hours, remained high for nearly 70 hours, and then diminished. The respiratory quotient (RQ) increased during the first 100 hours and then remained essentially constant at 0.7. Since the RQ for a medium sized alkane (*n*-C₁₆) is 0.65 and for an aromatic (naphthalene) is 0.83, the RQ of 0.7 indicates that the crude oil was almost completely oxidized to carbon dioxide.

Data from the chemical analyses of the flask contents showed that with time the percent of alkanes

decreased, then increased, suggesting the production of these compounds by the microflora (Fig. 14). The ratios of heptadecane to pristane, and octadecane to phytane decreased with time, indicating a preference for the straight-chain compounds over the branched-chain compounds (Fig. 15).

The next experiment was done using one enrichment culture derived from platform sediments and one enrichment culture derived from control sediments. For Cruise I, the data in Fig. 16 show that oxygen utilization and carbon dioxide production were greater for the platform sediments than for the control sediments. Furthermore, the rate of oxygen utilization and carbon dioxide production was greater for the platform sediments. Respiratory rates for the enrichment cultures are shown in Table 39. Analyses of the flask contents after incubation indicated that the aliphatic compounds were reduced in concentration in the control enrichment flasks and were essentially depleted in the platform enrichment flasks (Fig. 17). There appeared to be very little utilization of the aromatic compounds by the control enrichment culture while the platform enrichment culture substantially reduced the aromatics (Fig. 18). The GC-MS analyses performed on these samples confirmed the results of the GC and LC data given in Figs. 17 and 18, respectively. The terminal RQ for the platform sediments was 0.59 while that of the control sediment was 0.31.

This experiment was repeated with another set of enrichment cultures prepared from samples collected on the second cruise. As in the previous experiment, both the total amount of oxygen consumed and the total amount of carbon dioxide produced were greater with the platform sediments but the difference was not as pronounced (Fig. 19). Similarly, the rate of oxygen utilization and the rate of carbon dioxide production were somewhat greater for the platform sediments (Table 39). The RQ values for the platform sediments and the control sediments were 0.45 and 0.53, respectively. Evaluation of the data from chemical analyses confirmed that more oil was degraded by the platform sediments than was degraded by the control sediments. In both cases the straight-chain aliphatics were degraded to a greater extent than were the branched-chain aliphatics and the aromatics.

Experiments were also conducted using pure cultures of oil-degrading organisms (Table 40). The O₂ utilization and CO₂ production by *Pseudomonas* sp. I during growth on Empire Mix crude oil is shown in Fig. 20. The final RQ was 0.39. Chemical analyses of the residual oil showed that the straight-chain aliphatics were

TABLE 39. Respiration rates for enrichment cultures of primary platform and control site sediment microorganisms during oxidation of Empire Mix crude oil

Cruise	Site	Respiration Rate (microliters per hour)	
		O ₂ Consumption	CO ₂ Production
I	Platform	120	40
I	Control	30	13
II	Platform	140	100
II	Control	100	32

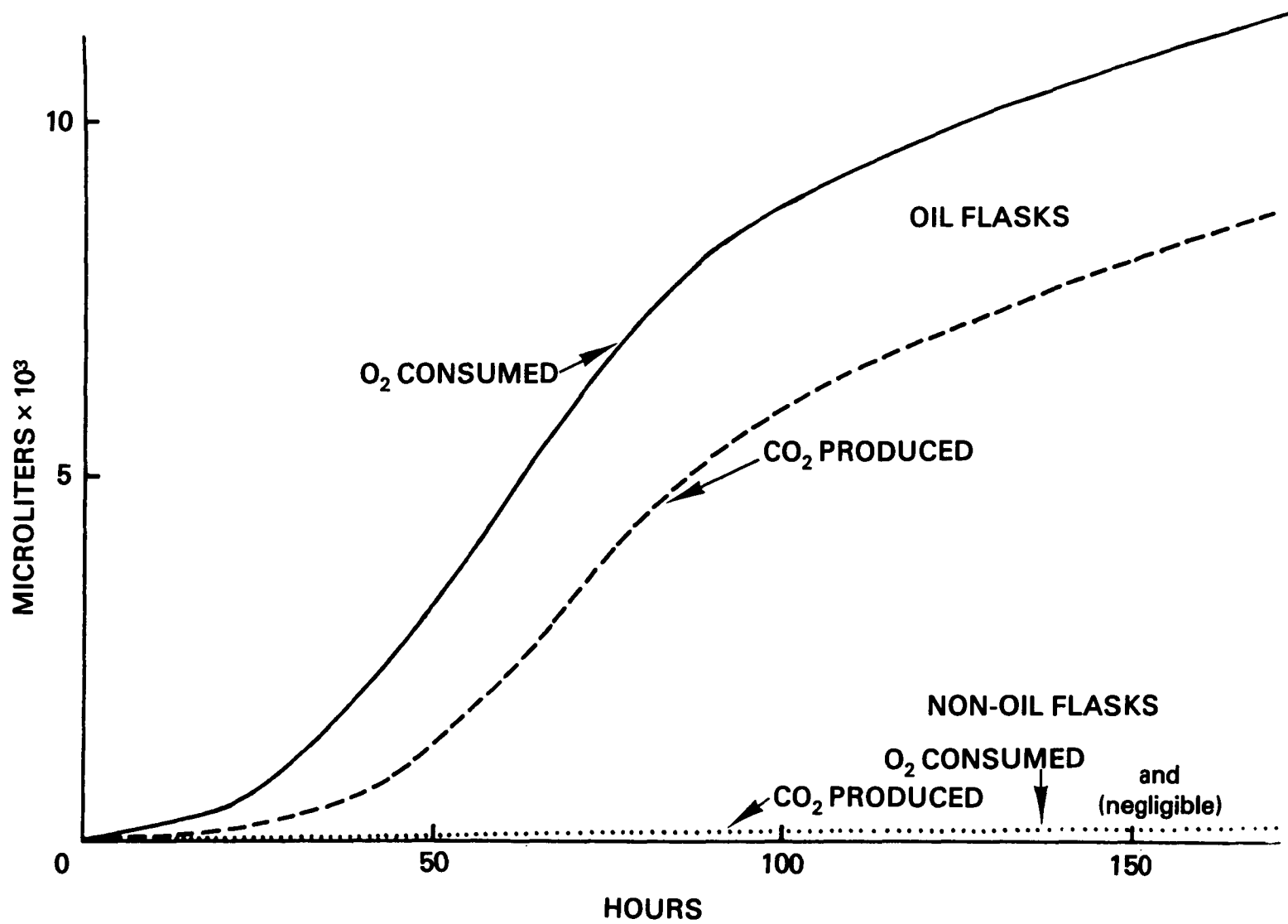


FIG. 13. Oxygen consumption and carbon dioxide production by a sediment sample from the Gulf of Mexico.

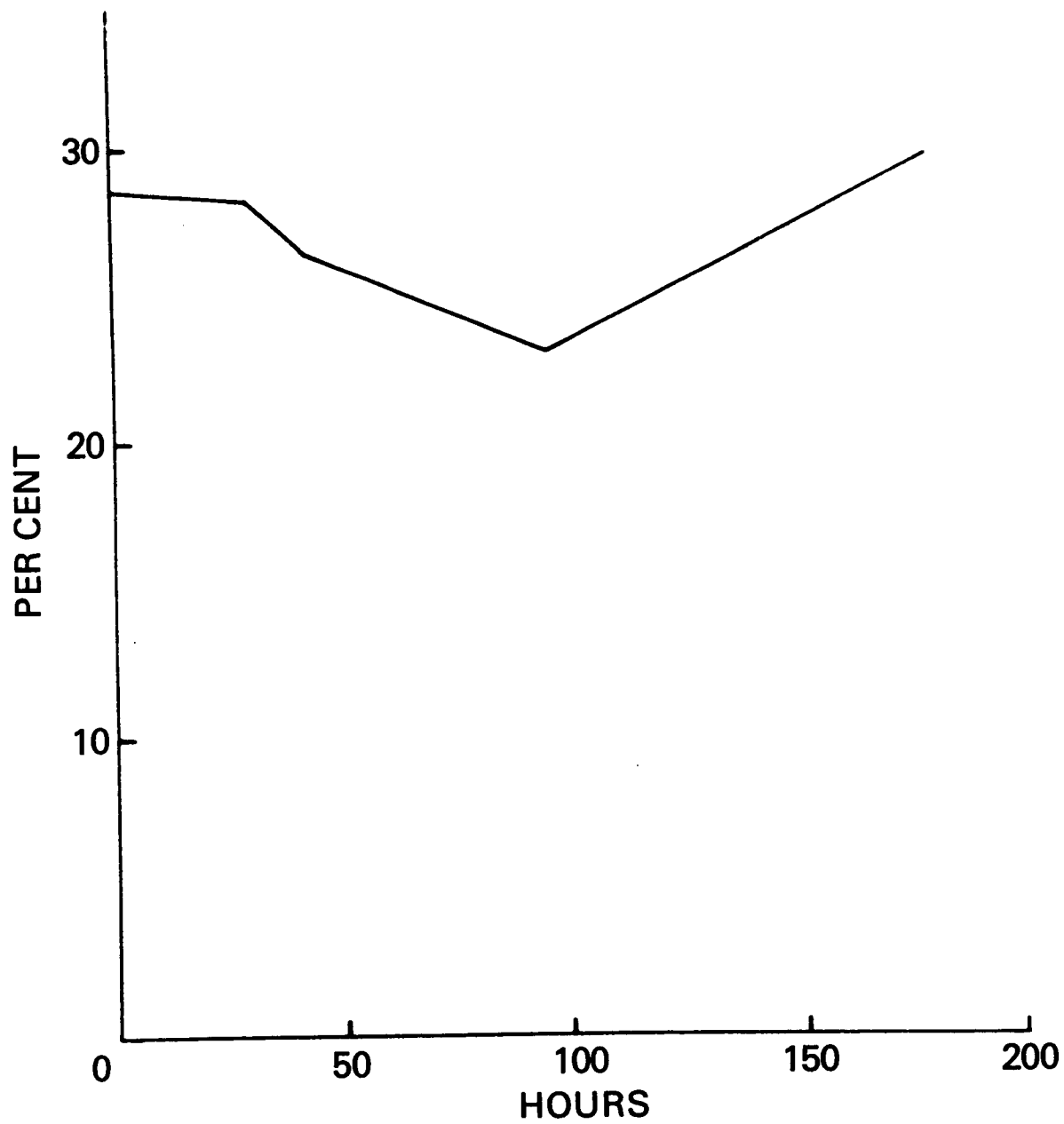


FIG. 14. Percent alkanes (heptadecane through eicosane, and pristane and phytane) as a percent of the total oil during the microbial degradation of Empire Mix crude oil.

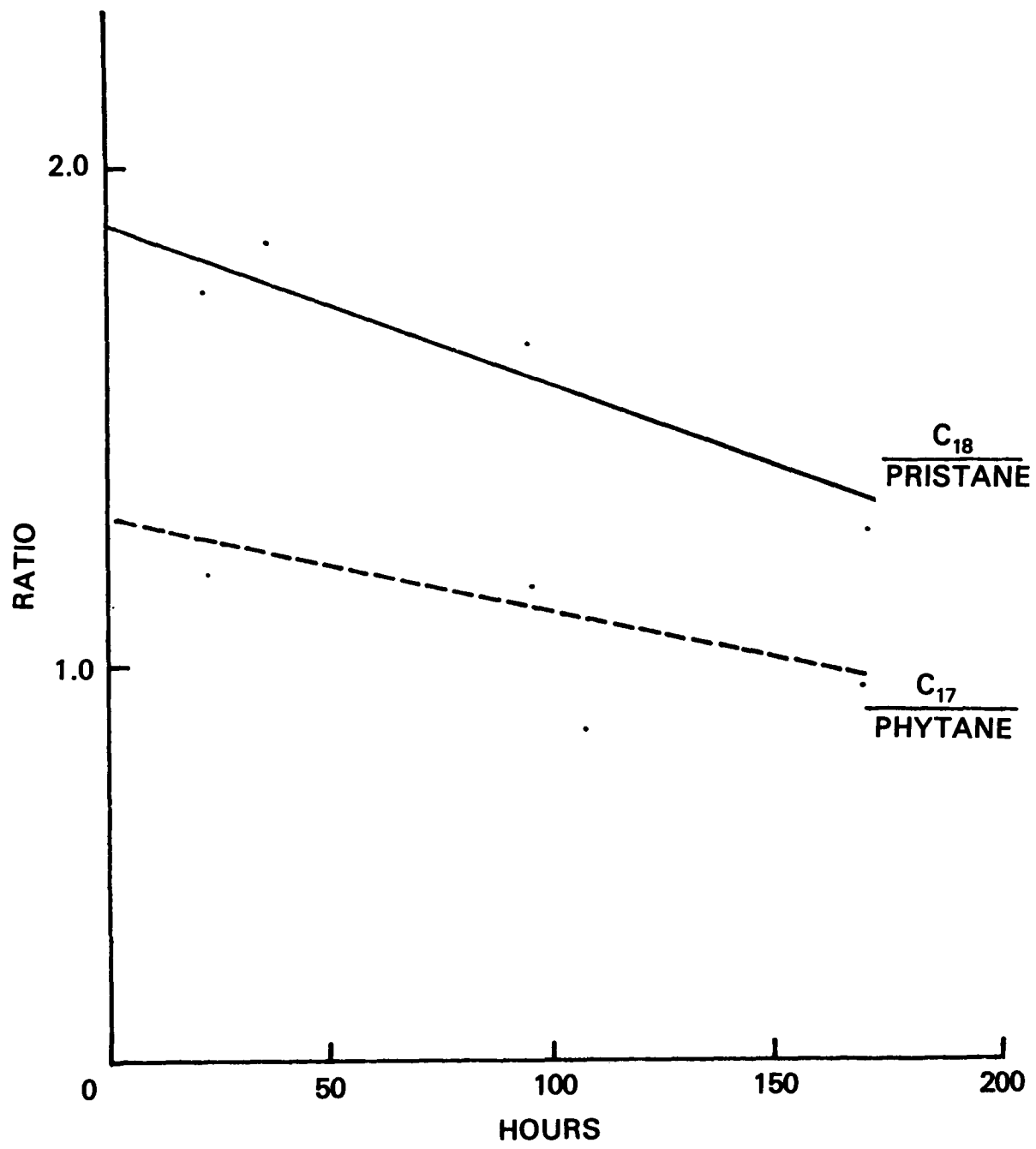


FIG. 15. Changes in the ratios of heptadecane ($n\text{-}C_{17}$) to pristane and octadecane ($n\text{-}C_{18}$) to phytane (straight-to branched-chains) during the microbial degradation of Empire Mix crude oil.

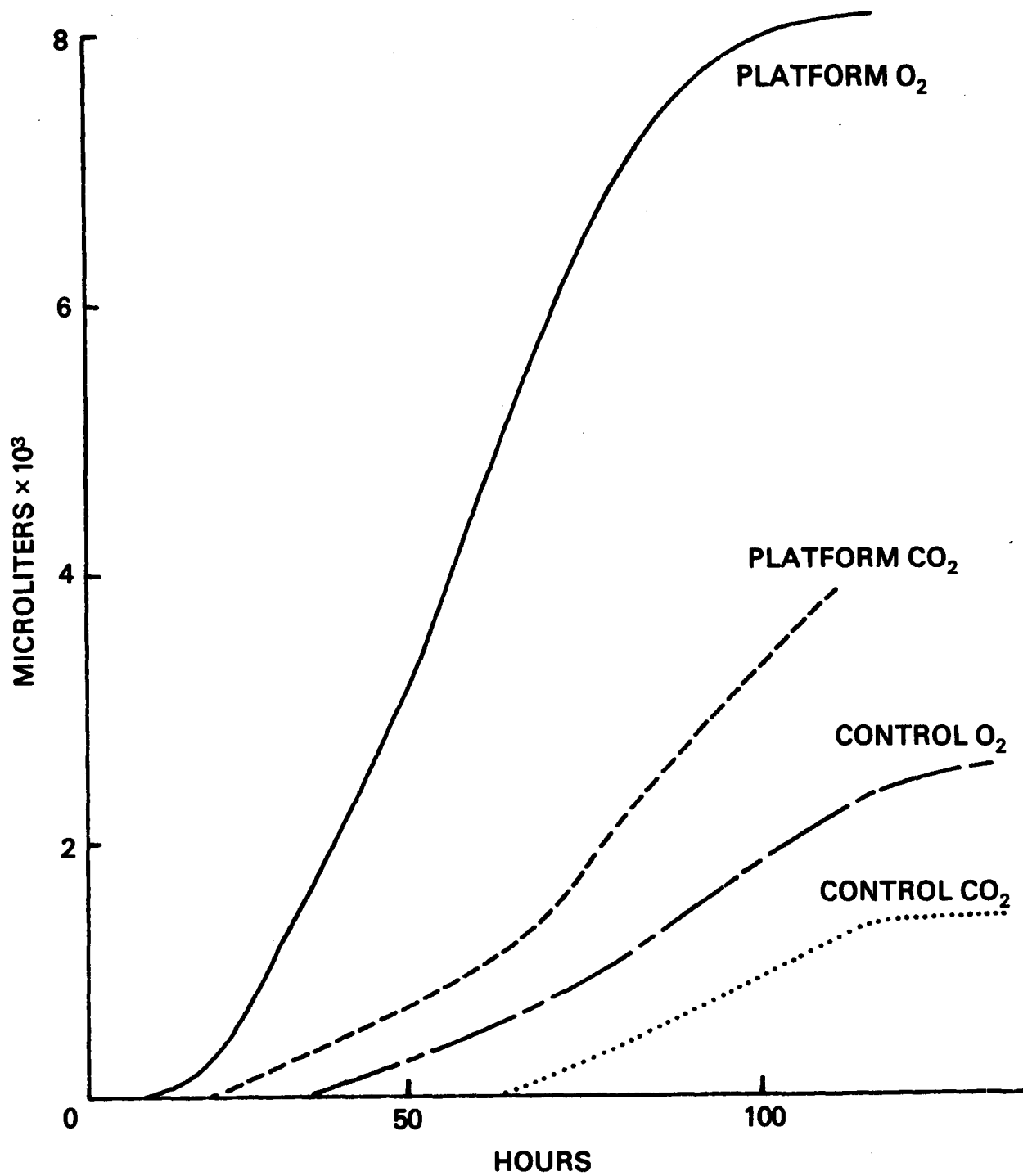


FIG. 16. Oxygen consumption and carbon dioxide production during the microbial metabolism of Empire Mix crude oil by enrichments prepared from samples taken on Cruise I.

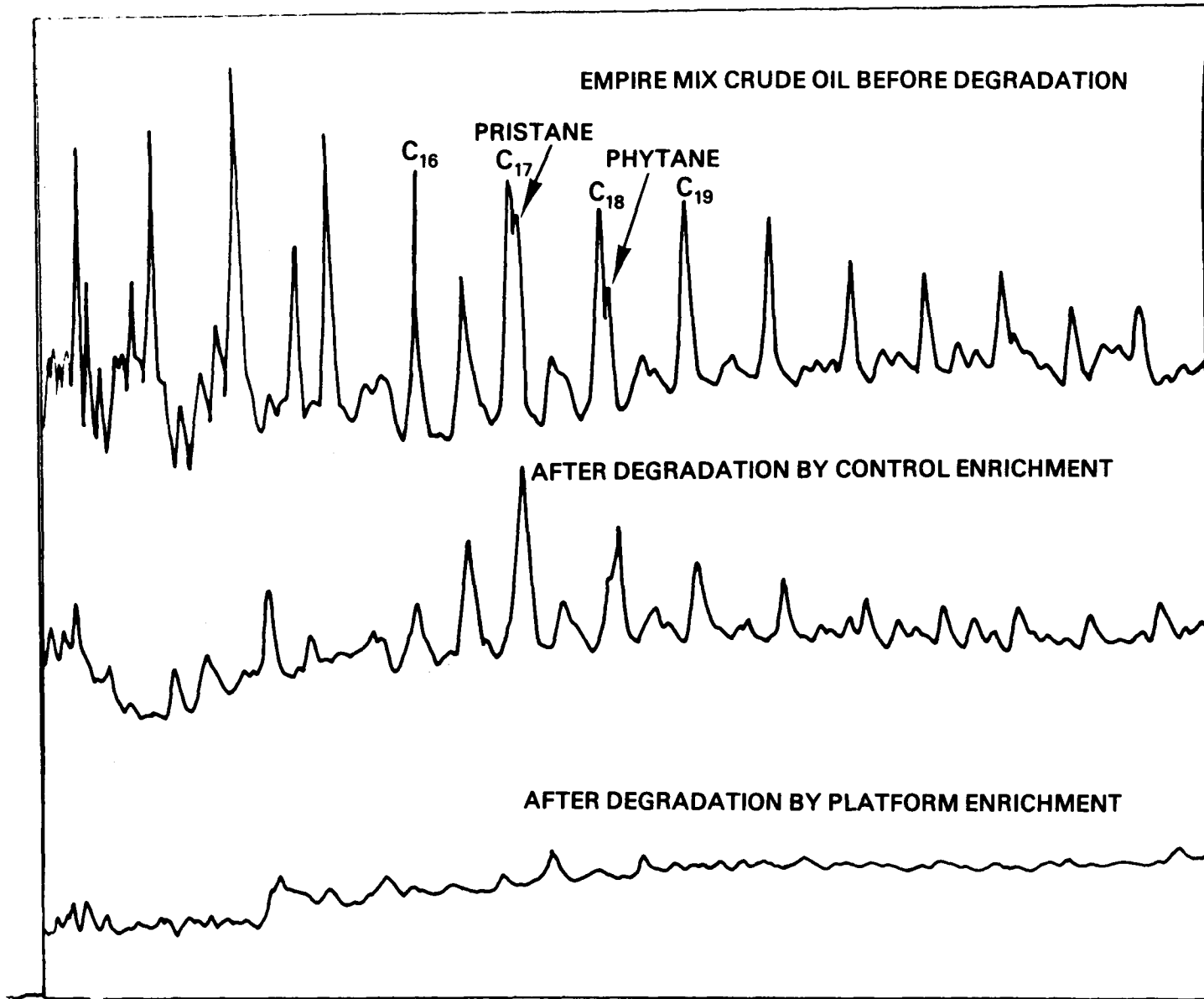


FIG. 17. Gas chromatography tracings of the aliphatics before and after microbial degradation by a control enrichment culture and a platform enrichment culture.

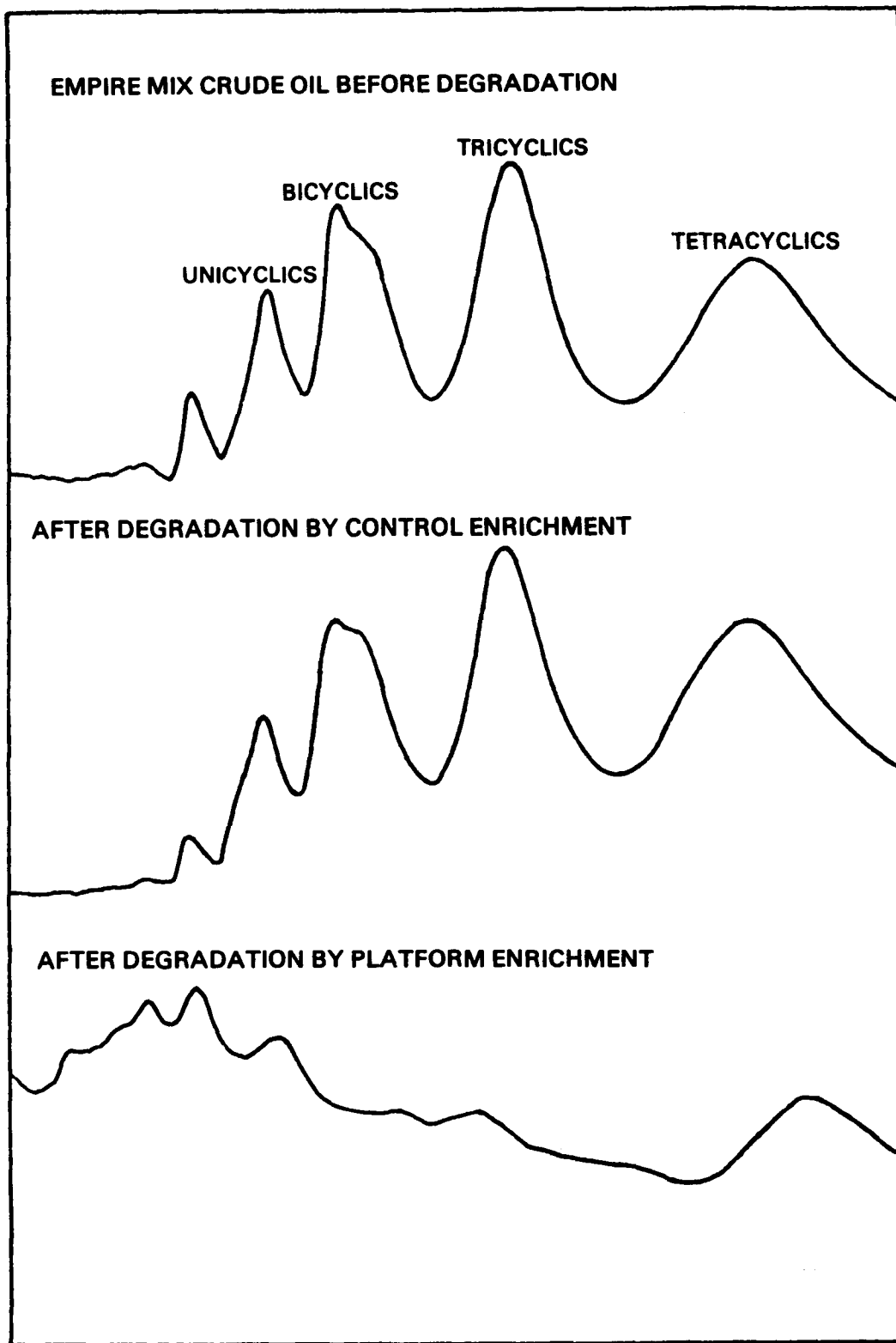


FIG. 18. Liquid chromatography tracings of the aromatics in Empire Mix crude oil before and after microbial degradation by a control enrichment culture and a platform enrichment culture.

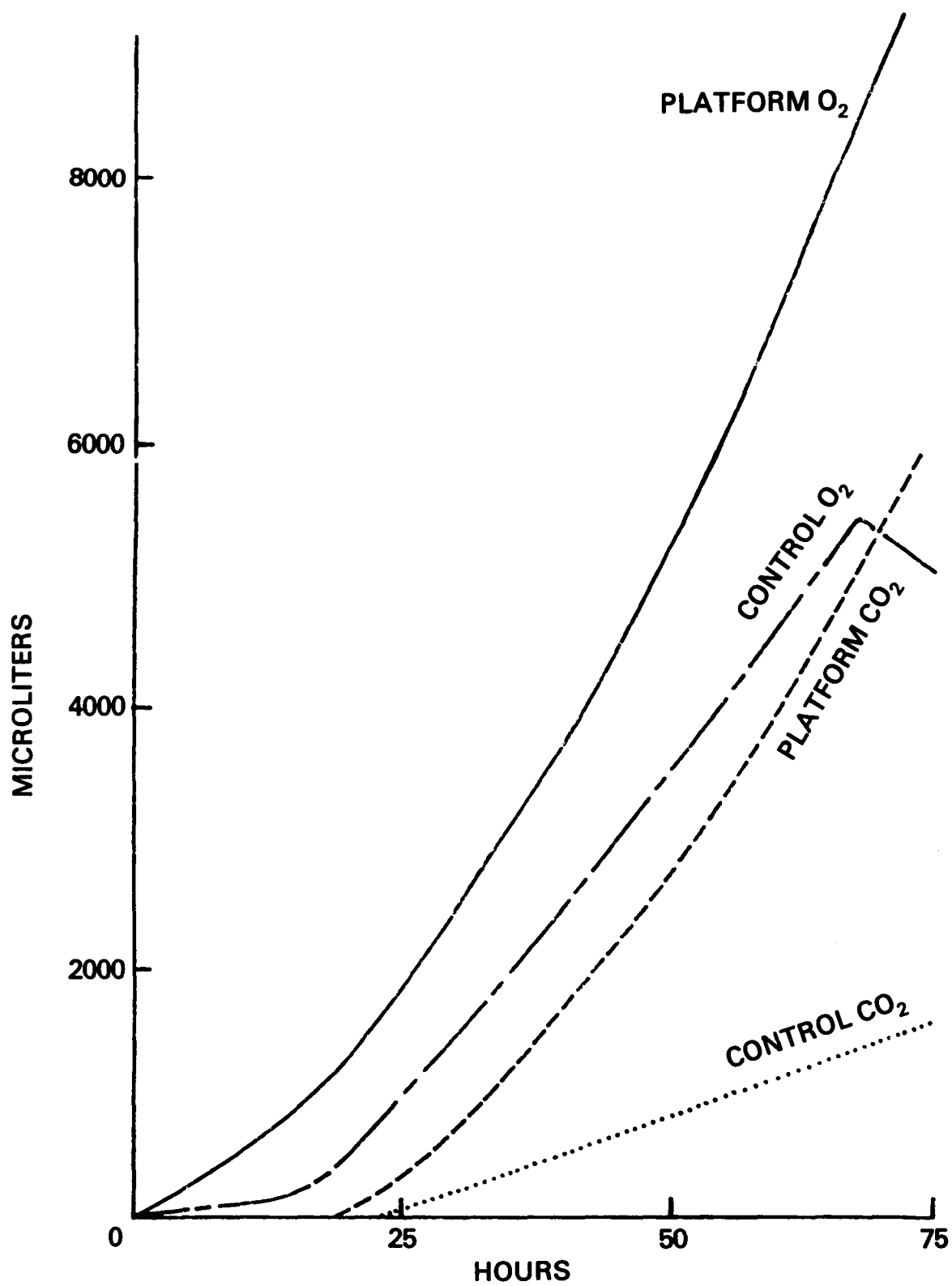


FIG. 19. Oxygen consumption and carbon dioxide production during the microbial metabolism of Empire Mix crude oil by enrichments prepared from samples taken on Cruise II.

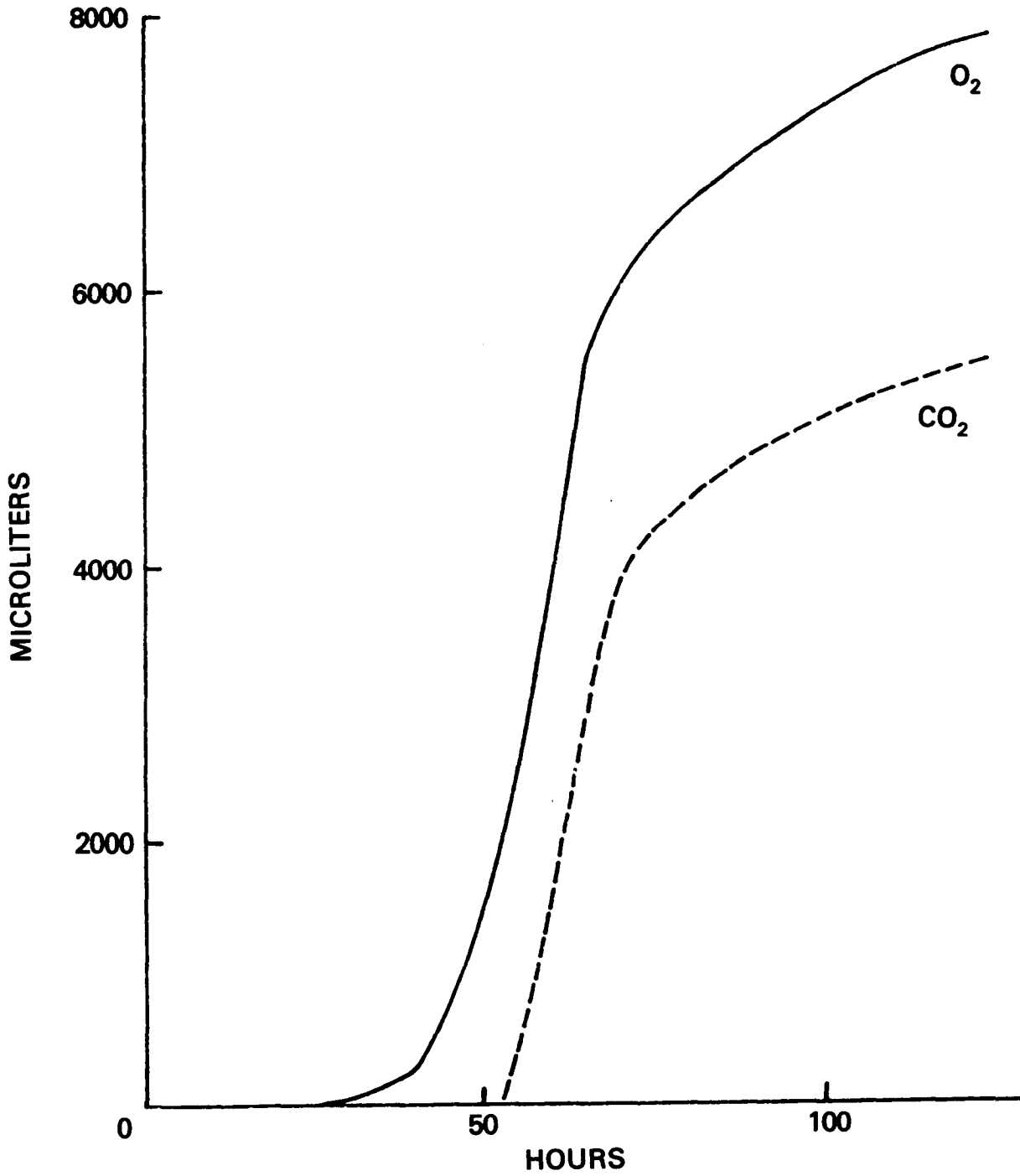


FIG. 20. Oxygen consumption and carbon dioxide production by *Pseudomonas* sp 1 during the metabolism of Empire Mix crude oil.

TABLE 40. Respiration rate for pure cultures during oxidation of Empire Mix crude oil

Isolate	Respiration Rate (microliters per hour)	
	O ₂ Consumption	CO ₂ Production
<i>Pseudomonas</i> sp. 1	200	200
<i>Flavobacterium</i> sp. 1	200	180
<i>Pseudomonas</i> sp. 2	30	20
<i>Flavobacterium</i> sp. 2	30	20

absent, and the branched-chain aliphatics had been reduced. Some degradation of aromatics was indicated.

The O₂ utilization and CO₂ production by *Flavobacterium* sp. 1 was similar to that observed for *Pseudomonas* sp. 1 (Fig. 21). The final RQ (0.31) and the fingerprint of the residual oil was essentially the same as that displayed by *Pseudomonas* sp. 1.

A similar experiment was conducted on two additional oil-degrading isolates (Fig. 22 and 23). The RQ values were 0.70 and 0.58 for *Pseudomonas* sp. 2 and *Flavobacterium* sp. 2, respectively. Essentially all of the straight-chain aliphatics were degraded by the *Pseudomonas*, along with about 50% of the branched-chain aliphatics. Nearly all of the two-ring aromatics and approximately two-thirds of the three-ring aromatics were degraded, and there appeared to be some slight degradation of the larger aromatics. There were some higher molecular weight straight-chain aliphatics (about C₃₀) and a large amount of branched-chain (C₂₄ or C₂₅ aliphatics produced. There also appeared to be some aromatics produced by the cells.

The results of the chemical analyses on the residual material from *Flavobacterium* sp. 2 were similar to those for *Pseudomonas* sp. 2, but there appeared to be less degradation of the three-ring aromatics, and the production of aromatics by the isolate was not evident. This isolate produced the same aliphatics as did *Pseudomonas* sp. 2 but in a lesser quantity.

8. Nitrification

Forty composite sediment samples prepared from primary platform and control site sediments were examined for nitrification. None of the samples demonstrated detectable nitrification activity, as exemplified by the data for sediment microorganisms from Primary Platform P1 collected during Cruise I (Table 41). Comparisons using fresh and frozen samples produced similar (insignificant) rates of nitrification.

9. Nitrogen Fixation

Forty composite samples prepared from primary platform and control site sediments were examined for nitrogen fixation. None of the samples demonstrated detectable nitrogen fixation activity, as exemplified by the data for sediment microorganisms from Primary Platform P1 collected during Cruise I (Table 42). Comparisons using fresh and frozen samples produced similar (insignificant) rates of nitrogen fixation.

10. Denitrification

Forty composite samples prepared from primary platform and control site sediments were

examined for denitrification. None of the samples demonstrated detectable denitrification activity, as exemplified by the data for sediment microorganisms from Primary Platform P1 collected during Cruise I (Table 43). Comparisons using fresh and frozen samples produced similar (insignificant) rates of denitrification.

11. Sulfate Reduction

Forty composite samples prepared from primary platform and control site sediments were examined for sulfate reduction. Most of the samples did not demonstrate detectable sulfate reduction activity, with the possible exception of the 10X OIL sample from replicate 2 Control Site C22 collected during Cruise I, and the 10X OIL and 1X OIL samples from replicates 1 and 2 Primary Platform P2 collected during Cruise II (Table 44). Comparisons using fresh and frozen samples produced similar (insignificant) rates of sulfate reduction.

12. Sulfur Oxidation

Forty composite samples prepared from primary platform and control site sediments were examined for the occurrence and magnitude of sulfur oxidation. Valid results were obtained only from samples taken during Cruises II and III. Samples demonstrated significant sulfur oxidation activity as exemplified by the data from sediment microorganisms from Primary Platform P1 (Table 45). Analysis of this data revealed that at least three patterns in production of water-soluble ³⁵S could be observed (Fig. 24): (1) rapid production of water-soluble ³⁵S in samples ± oil (Cruise II, Platform P4), (2) delayed production of water-soluble ³⁵S in samples + 10X OIL (Cruise II, Control Site C22), (3) delayed production of water-soluble ³⁵S in samples + 1X or 10X OIL (Cruise III, Platform P4). Comparisons using fresh and frozen samples produced similar rates of sulfur oxidation.

13. Photosynthesis

Forty-eight composite samples prepared from primary platform and control site sediments were examined for photosynthesis. None of the samples demonstrated detectable photosynthetic activity, as shown by the data in Table 46.

14. Heterotrophic Activity

Forty-eight composite samples prepared from primary platform and control site sediments were examined for heterotrophic activity. Most of the samples demonstrated rapid glucose degradation, as shown by the data for sediment microorganisms from Cruises I, II, and III (Tables 47-49, Fig. 25).

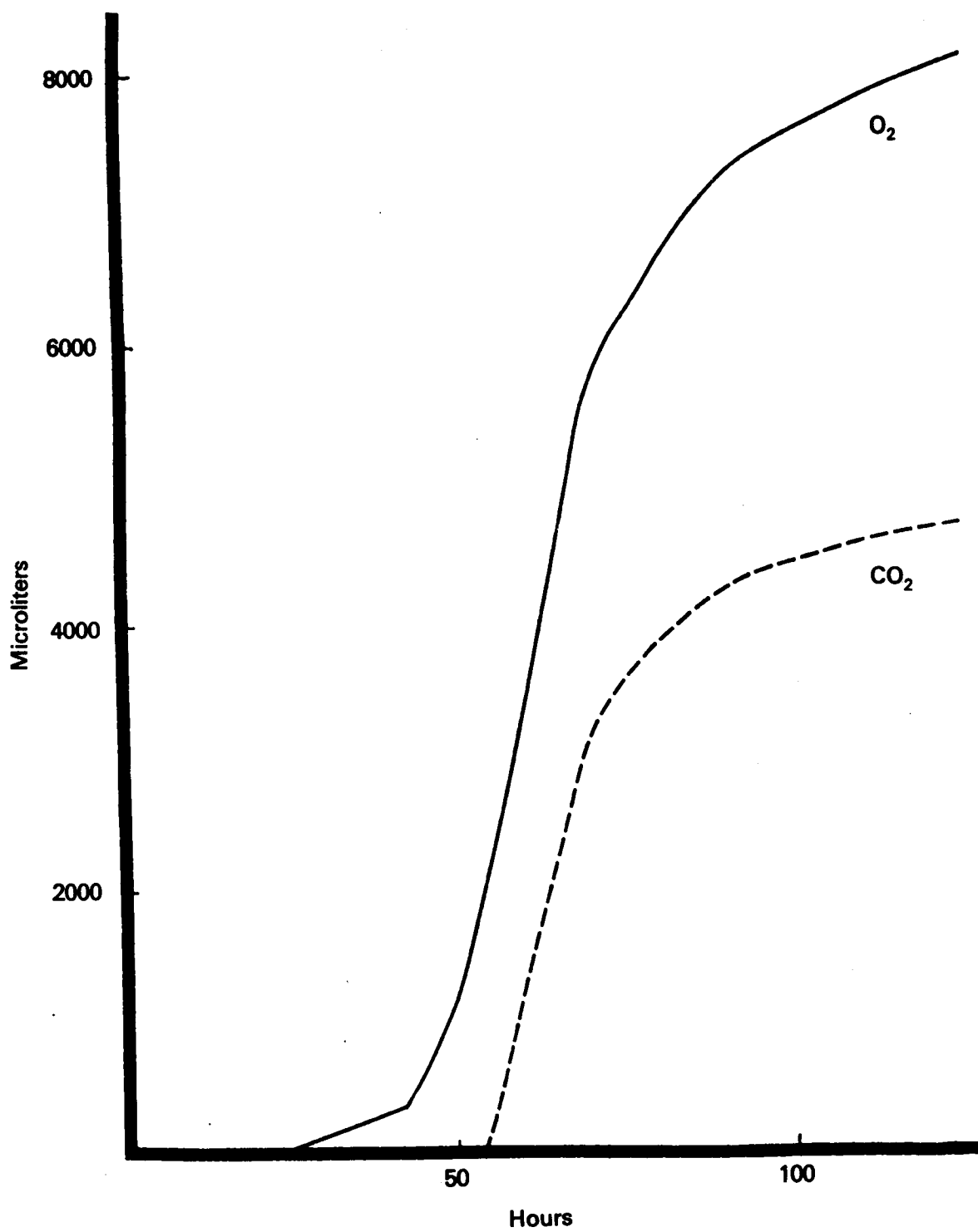


FIG. 21. Oxygen consumption and carbon dioxide production by *Flavobacterium* sp 1 during the metabolism of Empire Mix crude oil.

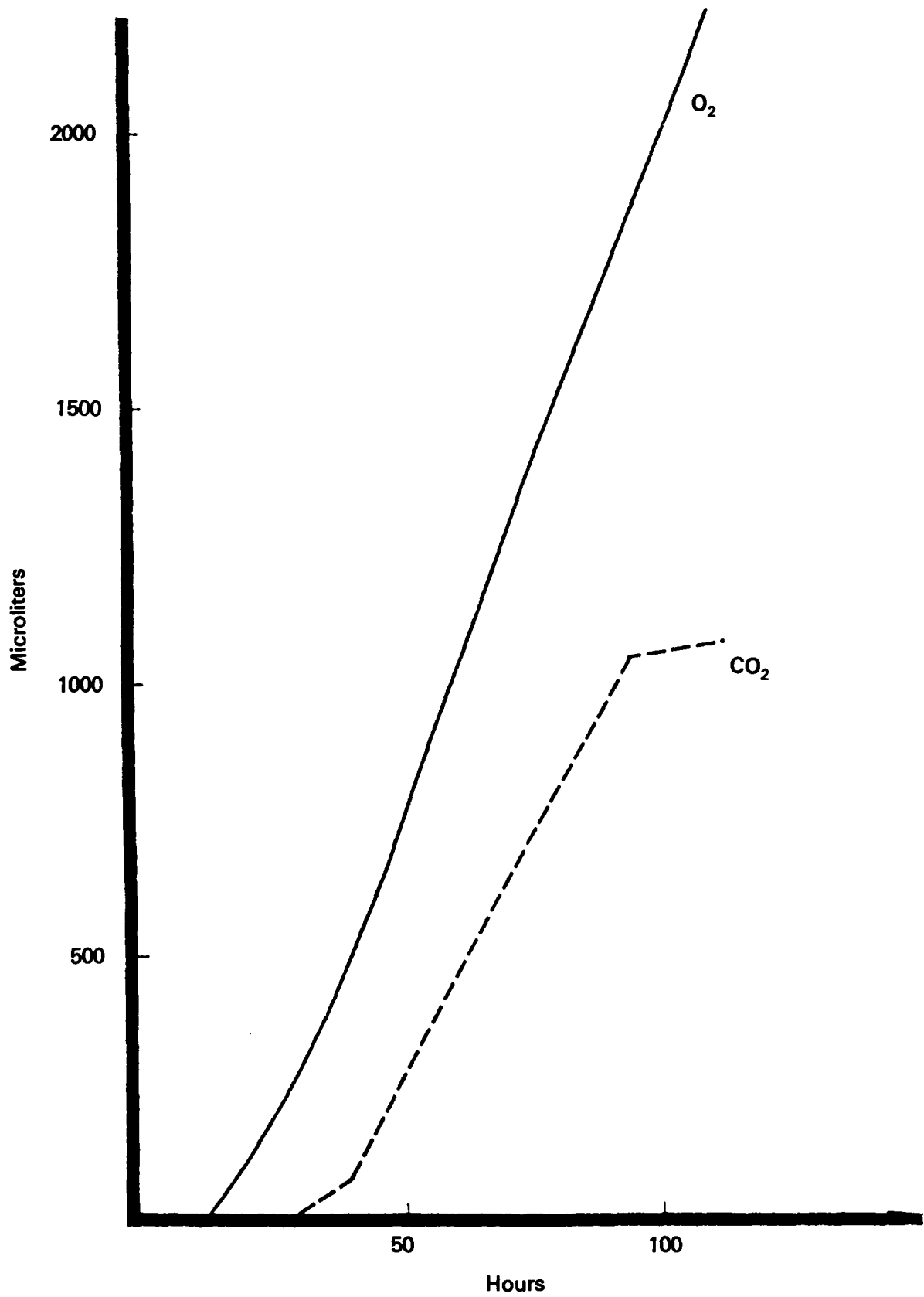


FIG. 22. Oxygen consumption and carbon dioxide production by *Pseudomonas* sp 2 during the metabolism of Empire Mix crude oil.

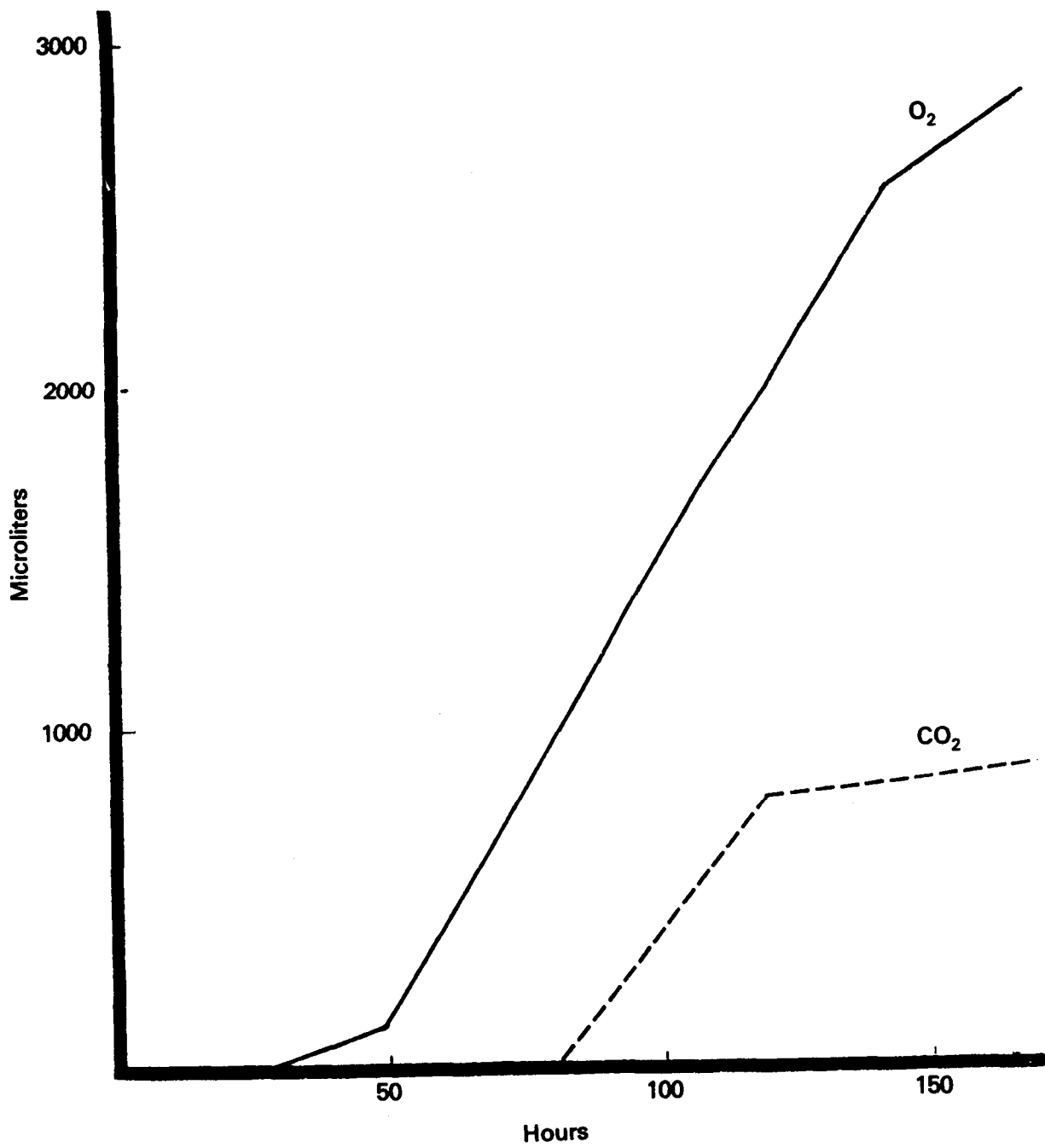


FIG. 23. Oxygen consumption and carbon dioxide production by *Flavobacterium* sp 2 during the metabolism of Empire Mix crude oil.

TABLE 41. Concentrations of NH₄-N, NO₃-N, and NO₂-N in systems inoculated with sediment from Primary Platform 1 collected during Cruise I and tested for nitrification

Incubation Time (weeks)	Form of Nitrogen	Concentration (µg N/ml sediment)			
		Test ¹	Control ¹	1X oil	10X oil
0	NH ₄ -N	12000	12000	12000	13000
	NO ₃ -N	17	17	16	16
	NO ₂ -N	890	890	870	750
1	NH ₄ -N	14000	12000	14000	13000
	NO ₃ -N	17	16	16	17
	NO ₂ -N	320	320	320	320
2	NH ₄ -N	14000	12000	12000	12000
	NO ₃ -N	17	21	17	19
	NO ₂ -N	20	400	200	99
3	NH ₄ -N	11000	12000	13000	12000
	NO ₃ -N	22	20	22	22
	NO ₂ -N	20	750	20	1600

¹Zero time "Test" and "Control" values obtained from the same reaction vessel

TABLE 42. Concentration of nitrogen (N₂) in test, control, and oil-supplemented systems inoculated with sediment from Primary Platform 1 collected during Cruise I and tested for nitrogen fixation

Incubation Time (weeks)	Concentration of N ₂ (µg/ml sediment)			
	Test	Control	1X oil	10X oil
0	22	22	22	22
1	19	18	18	18
2	22	20	20	20
3	22	20	20	21

TABLE 43. Concentration of nitrogen (N₂) in systems inoculated with sediment from Primary Platform 1 collected during Cruise I and tested for denitrification

Incubation Time (weeks)	Concentration of N ₂ (µg/ml sediment)			
	Test	Control	1X oil	10X oil
0	18	18	18	17
1	19	19	17	19
2	21	21	20	21
3	23	21	20	21

TABLE 44. Concentrations of insoluble (I) and soluble (S) ³⁵S in test, control, and oil-supplemented systems inoculated with sediment and tested for sulfate reduction

Site	Inoculum Cruise	Replicate	Incubation Time (weeks)	Form of ³⁵ S	Concentration of ³⁵ S (μg/ml sediment)			
					Test ¹	Control ¹	1X oil	10X oil
C22	I	2	0	I	11	11	7	14
				S	4496	4496	4500	4493
			1	I	25	3	4	13
				S	4482	4504	4503	4994
			2	I	5	0	7	23
				S	4501	4507	4500	4484
			3	I	2	3	21	39
				S	4505	4504	4486	4468
P2	II	1	0	I	312	312	377	223
				S	5322	5322	5257	5411
			1	I	237	314	337	391
				S	5397	5320	5297	5243
			2	I	177	310	101	419
				S	5457	5324	5533	5214
			3	I	378	471	353	714
				S	5256	5163	5281	4920
P2	II	2	0	I	4415	4415	249	2891
				S	1219	1219	5385	2743
			1	I	3116	673	436	382
				S	2518	4961	5198	5252
			2	I	283	378	424	382
				S	5351	5296	5210	5252
			3	I	480	1286	922	423
				S	5154	4348	4718	5211

¹Zero Time "Test" and "Control" values obtained from one reaction vessel per replicate

TABLE 45. Concentrations of insoluble (I) and soluble (S) ³⁵S in test, control, and oil-supplemented systems inoculated with sediment from replicate 1, Primary Platform 1, and tested for sulfur oxidation

Cruise	Incubation Time (weeks)	Form of ³⁵ S	Concentration of ³⁵ S (μg/ml sediment)			
			Test ¹	Control ¹	1X oil	10X oil
II	0	I	18159	18159	21455	20799
		S	6841	6841	3545	4201
	1	I	1214	22799	11033	3727
		S	23786	2201	13967	21273
	2	I	614	17408	1677	3691
		S	24386	7592	23323	21309
3	I	9555	17897	325	277	
	S	15445	1703	24675	24723	
III	0	I	24319	24319	24525	24530
		S	681	681	475	470
	1	I	23935	24826	21317	24182
		S	1065	174	3683	818
	2	I	9905	24610	12558	11352
		S	15095	390	12442	13648
3	I	5751	24410	11880	14105	
	S	19249	590	13120	10895	

¹Zero time "Test" and "Control" values obtained from one reaction vessel per replicate

TABLE 46. Change in dissolved oxygen concentration (DO) in systems inoculated with sediment collected during Cruise I and tested for photosynthesis (PP) and metabolic activity (MA)

Site	Replicate	Change in DO (μg/ml sediment)							
		Test		Control		1X oil		10X oil	
		PP	MA	PP	MA	PP	MA	PP	MA
P1	1	+6	-42	0	-30	+6	-30	-6	-18
	2	-36	-54	0	-54	0	-48	0	-48
P2	1	-45	-90	0	-48	-48	-84	-72	-48
	2	-36	-72	+60	-42	-33	-69	-45	-66
P3	1	0	-24	0	-18	0	-24	0	-24
	2	+6	-24	0	-18	+6	-24	+6	-24
P4	1	+12	-57	-6	-39	3	-48	-6	-39
	2	+12	-60	-48	0	+12	-66	-6	-60
C21	1	-6	-54	0	0	-27	-48	-12	-54
	2	0	-36	0	0	-6	-36	0	-30
C22	1	-6	-42	0	-24	-27	-12	-12	-42
	2	-9	-54	+18	-54	-6	-42	0	-30
C23	1	+6	-30	-6	-24	-6	-24	0	-24
	2	0	-36	0	-36	0	-36	0	-36
C24	1	+6	-30	0	-30	+3	-30	-6	-18
	2	0	-33	+3	-39	0	-36	+3	-36
\bar{x}	Control Sites	-1	-39	+2	-38	+5	-33	-33	-36
\bar{x}	Platform Sites	-10	-53	-6	-31	+8	-49	-16	-41

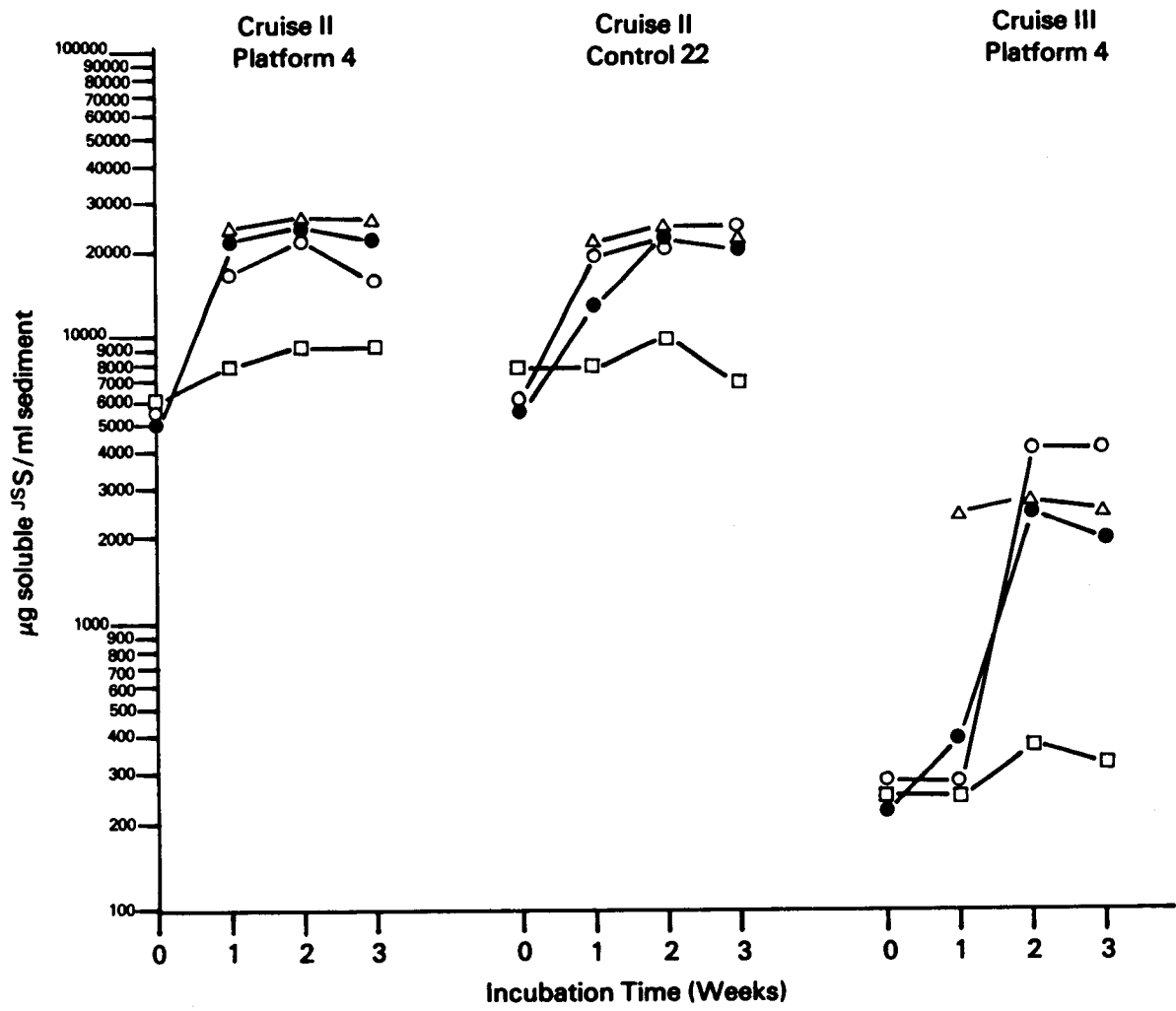


FIG. 24. Concentrations of water-soluble ^{35}S at weeks 0, 1, 2, and 3 in Test (Δ), Control (\square), 1×Oil (\circ), and 10×Oil (\bullet) inoculated with sediment microorganisms from platforms and controls

TABLE 47. CO₂ produced from glucose (expressed as percent CO₂-C/Total C) in systems inoculated with sediment collected during Cruise I and tested for heterotrophic activity

Site	Incubation Time (days)	Percent CO ₂ Produced							
		Test ¹		Control ¹		1X oil		10X oil	
		1 ²	2	1	2	1	2	1	2
P1	0	0	0	0	0	0	0	0	0
	2	6	3	0	0	6	5	4	3
	4	14	11	0	1	17	11	17	13
	6	18	16	1	2	16	13	17	15
C21	0	0	0	0	0	0	0	0	0
	2	1	1	0	1	1	1	1	1
	4	10	8	0	0	13	9	6	6
	6	12	8	0	0	12	11	6	11
P2	0	0	2	0	2	0	0	0	0
	2	17	20	0	0	14	16	11	16
	4	12	7	0	0	14	10	14	10
	6	15	16	0	0	13	12	10	13
C22	0	0	0	0	0	0	0	0	0
	2	1	6	0	5	2	5	2	3
	4	9	10	0	0	13	9	12	7
	6	10	14	0	0	12	11	11	9
P3	0	0	2	0	2	2	0	0	1
	2	17	0	0	0	21	6	19	10
	4	10	9	0	0	15	14	15	12
	6	13	9	0	0	10	9	17	17
C23	0	0	0	0	0	0	0	0	0
	2	3	4	0	0	3	3	2	3
	4	12	0	0	0	12	13	12	12
	6	13	8	0	0	11	10	11	9
P4	0	0	0	0	0	1	1	0	0
	2	21	4	0	0	17	12	14	8
	4	12	10	0	0	12	8	13	10
	6	16	11	0	0	13	14	15	12
C24	0	0	0	0	0	1	0	0	0
	2	4	3	0	0	3	4	3	3
	4	12	13	0	1	13	12	10	14
	6	12	14	0	0	14	8	14	13

¹Zero time "Test" and "Control" values obtained from one reaction vessel per replicate

²Replicate

TABLE 48. CO₂ produced from glucose (expressed as percent CO₂-C/Total C) in systems inoculated with sediment collected during Cruise II and tested for heterotrophic activity

Site	Incubation Time (days)	Percent CO ₂ Produced							
		Test ¹		Control ¹		1X oil		10X oil	
		1 ²	2	1	2	1	2	1	2
P1	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0	6
	3	1	2	0	0	8	2	4	2
	5	13	7	0	0	11	9	15	10
C21	0	1	0	1	0	0	0	0	0
	1	1	0	0	0	0	0	0	0
	3	5	2	0	0	1	2	2	2
	5	12	13	0	0	6	12	8	12
P2	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0	0
	3	1	0	0	0	1	0	0	0
	5	2	1	0	0	4	2	5	3
C22	0	2	0	2	0	1	0	0	0
	1	3	3	1	0	2	3	3	3
	3	2	3	2	0	3	4	4	3
	5	9	17	3	0	7	11	10	12
P3	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0	0
	3	0	1	0	0	0	1	0	1
	5	3	4	0	0	2	6	3	5
C23	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0	0
	3	2	2	0	0	3	2	2	2
	5	5	10	0	0	8	9	11	11
P4	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0	0
	3	0	2	0	0	1	1	1	1
	5	9	10	0	0	11	9	12	10
C24	0	0	1	0	1	0	0	0	0
	1	0	1	0	0	0	0	0	0
	3	0	7	6	0	0	6	0	2
	5	3	5	0	0	5	5	3	16

¹Zero time "Test" and "Control" values obtained from one reaction vessel per replicate

²Replicate

TABLE 49. CO₂ produced from glucose (expressed as percent CO₂-C/Total C) in systems inoculated with sediment collected during Cruise III and tested for heterotrophic activity

Site	Incubation Time (days)	Percent CO ₂ Produced							
		Test ¹		Control ¹		1X oil		10X oil	
		1 ²	2	1	2	1	2	1	2
P1	0	0	0	0	0	0	0	0	0
	1	6	5	0	0	7	6	7	6
	3	10	11	0	0	8	8	11	8
	5	13	9	0	0	16	14	17	10
C21	0	0	0	0	0	0	0	0	0
	1	5	5	0	0	4	6	4	5
	3	11	8	0	0	8	9	8	8
	5	9	9	0	0	14	16	10	11
P2	0	0	0	0	0	0	0	0	0
	1	9	10	0	0	10	7	9	10
	3	12	14	0	0	12	16	16	15
	5	32	30	0	0	30	28	30	26
C22	0	0	0	0	0	0	0	0	0
	1	5	7	0	0	5	6	5	6
	3	8	10	0	0	7	8	8	7
	5	14	14	0	0	12	11	10	12
P3	0	0	0	0	0	0	0	0	0
	1	1	1	0	0	1	1	1	2
	3	1	2	0	0	1	1	1	1
	5	2	4	0	0	2	5	2	4
C23	0	0	0	0	0	0	0	0	0
	1	0	1	0	0	2	1	3	1
	3	4	4	0	0	4	5	4	6
	5	13	31	0	0	12	30	8	17
P4	0	0	0	0	0	0	0	0	0
	1	5	3	0	0	6	2	6	2
	3	11	4	0	0	10	5	12	7
	5	32	30	0	0	28	14	25	16
C24	0	0	0	0	0	0	0	0	0
	1	5	3	0	0	4	2	5	2
	3	7	8	0	0	6	7	8	4
	5	31	29	0	0	32	30	28	25

¹Zero time "Test" and "Control" values obtained from one reaction vessel per replicate

²Replicate

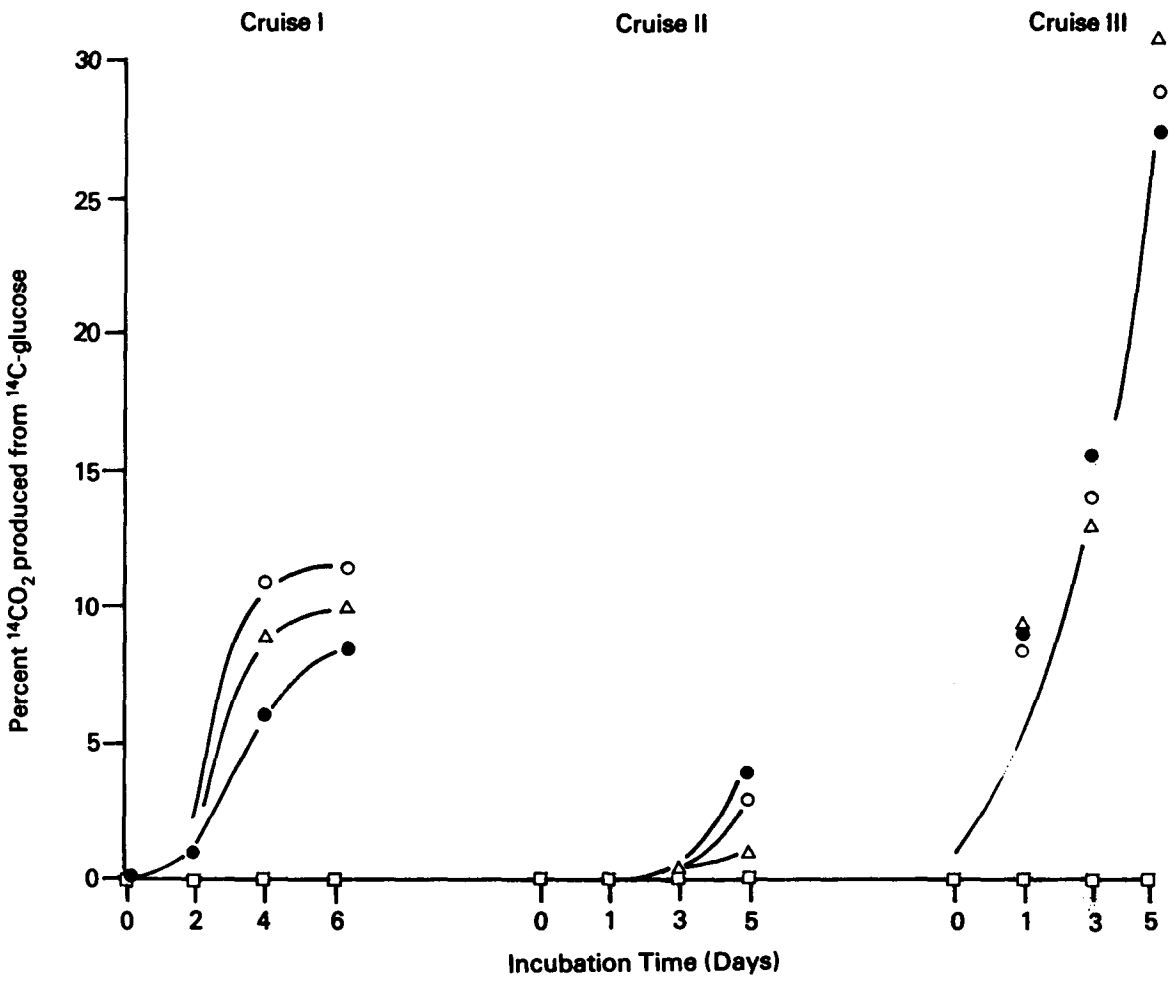


FIG. 25. Percent ¹⁴CO₂ produced from ¹⁴C-glucose by Platform 2 sediment microorganisms collected during Cruises I, II, and III.

15. Phosphorus Uptake

Forty composite samples prepared from primary platform and control site sediments were examined for phosphorus uptake. None of the samples demonstrated detectable phosphorus uptake activity, as exemplified by the data for sediment microorganisms from Primary Platform P1 collected during Cruise I (Table 50). Comparisons using fresh and frozen samples produced similar (insignificant) rates of phosphorus uptake).

16. Lipolysis

Forty-eight composite samples prepared from primary platform and control site sediments were examined for lipolytic activity. Most of the samples demonstrated detectable lipolytic activity, as shown by the data for sediment microorganisms from Cruises I, II, and III (Tables 51-53).

17. Chitinolysis

Forty-eight composite samples prepared from primary platform and control site sediments were examined for chitinolytic activity. Most of the samples demonstrated detectable chitinolysis, as exemplified by the data in Tables 54-56. Rate and/or yield of CO₂ production was often higher in test systems of sediments (from both platforms and control sites) than in oil-supplemented systems (Fig. 26).

18. Cellulolysis

Forty-eight composite samples prepared from primary platform and control site sediments were examined for cellulolytic activity. Most of the samples demonstrated detectable cellulolysis, as exemplified by the data for sediment microorganisms from Cruises I, II, and III (Tables 57-59).

19. Proteolysis

Forty-eight composite samples prepared from primary platform and control site sediments were examined for proteolytic activity. Most of the samples demonstrated significant proteolytic activity as exemplified by the data for sediment microorganisms from Cruises I, II, and III (Tables 60-62).

B. Pure Culture Studies

Neither a pure culture of *Nitrosomonas europa* nor a nitrifying enrichment culture used to measure the effects of oil on nitrification exhibited nitrification in the presence of Empire Mix crude oil.

A pure culture of *Nostoc muscorum* used to measure the effects of oil on photosynthesis exhibited similar rates of photosynthesis in the presence and absence of Empire Mix crude oil (Table 63).

Two pure cultures of *Pseudomonas* used to measure the effects of oil on proteolysis exhibited similar rates and yields of CO₂ production in the presence and absence of Empire Mix crude oil (Fig. 27).

A pure culture of *Enterobacter* used to measure the effects of oil on glucose oxidation exhibited similar rates of CO₂ production in the presence and absence of Empire Mix crude oil (Fig. 28).

Two pure cultures of *Cellulomonas* used to measure effects of oil on cellulose oxidation exhibited similar rates of CO₂ production after 5 to 10 days of incubation in the presence and absence of Empire Mix crude oil (Fig. 29).

A pure culture of *Pseudomonas* used to measure effects of oil on lipolysis exhibited similar rates of CO₂ production after 10 days of incubation in the presence and absence of Empire Mix crude oil (Fig. 30).

A pure culture of *Pseudomonas* used to measure effects of oil on chitin hydrolysis exhibited similar rates of CO₂ production in the presence and absence of Empire Mix crude oil (Fig. 31).

TABLE 50. Concentrations of insoluble (I) and soluble (S) ³²P in systems inoculated with sediment collected from Primary Platform 1 during Cruise I and tested for phosphate uptake

Incubation Time (days)	Form of ³² P	Concentration of ³² P (μg/ml sediment)			
		Test ¹	Control ¹	1X oil	10X oil
0	I	42	42	39	35
	S	6300	6300	6300	6300
5	I	53	66	46	45
	S	6300	6300	6300	6300
10	I	66	66	53	36
	S	6300	6300	6300	6300
15	I	24	23	36	29
	S	6300	6300	6300	6300

¹Zero time "Test" and "Control" values obtained from one reaction vessel per replicate

TABLE 51. CO₂ produced from tripalmitin (expressed as percent CO₂-C/Total C) in systems inoculated with sediment collected during Cruise I and tested for lipolysis

Site	Incubation Time (weeks)	Percent CO ₂ Produced							
		Test ¹		Control ¹		1X oil		10X oil	
		1 ²	2	1	2	1	2	1	2
P1	0	0	0	0	0	0	0	0	2
	1	14	5	0	0	10	36	21	8
	2	0	0	0	0	2	7	7	1
	3	2	1	0	0	10	1	13	24
P2	0	0	0	0	0	0	1	0	1
	1	1	0	2	0	0	0	0	0
	2	1	4	1	5	2	0	1	5
	3	1	0	0	0	1	8	3	1
P3	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	1	0	0
	2	1	2	2	1	3	3	0	2
	3	0	1	1	0	0	0	2	1
P4	0	1	1	1	1	0	0	1	1
	1	4	0	2	1	0	0	1	0
	2	0	1	0	1	4	0	5	3
	3	0	1	0	3	1	5	1	3
C21	0	0	2	0	2	0	2	0	2
	1	16	2	0	0	3	4	1	3
	2	0	8	1	8	0	2	0	0
	3	7	1	0	4	7	8	3	15
C22	0	0	0	0	0	1	0	0	6
	1	9	7	0	0	0	2	6	5
	2	0	0	0	1	0	1	2	3
	3	0	0	3	1	5	4	5	5
C23	0	0	0	0	0	4	0	0	0
	1	11	1	0	0	4	15	11	4
	2	0	3	0	0	1	2	0	6
	3	1	3	0	0	3	33	7	9
C24	0	1	0	1	0	1	1	0	0
	1	3	5	0	0	12	9	6	9
	2	2	0	0	1	1	3	6	4
	3	2	0	0	1	7	14	12	14

¹Zero time "Test" and "Control" values obtained from one reaction vessel per replicate

²Replicate

TABLE 52. CO₂ produced from tripalmitin (expressed as percent CO₂-C/Total C) in systems inoculated with sediment collected during Cruise II and tested for lipolysis

Site	Incubation Time (weeks)	Percent CO ₂ Produced							
		Test ¹		Control ¹		1X Oil		10X Oil	
		1 ²	2	1	2	1	2	1	2
P1	0	0	0	0	0	0	0	0	0
	1	6	3	0	0	2	2	0	9
	2	10	21	0	0	60	0	0	0
	3	18	21	1	1	11	15	21	28
P2	0	0	0	0	0	0	0	0	0
	1	2	0	0	1	2	1	0	0
	2	1	2	0	0	0	3	1	0
	3	5	6	3	4	16	2	21	4
P3	0	0	0	0	0	0	0	0	0
	1	0	1	0	0	1	2	1	2
	2	1	0	0	1	1	2	2	0
	3	9	4	0	0	3	3	17	1
P4	0	0	0	0	0	0	0	0	0
	1	2	3	0	0	1	4	3	4
	2	1	9	0	0	0	2	1	2
	3	7	4	1	0	14	19	10	10
C21	0	0	0	0	0	0	0	0	0
	1	14	1	0	0	3	0	2	8
	2	9	14	0	0	2	5	8	11
	3	32	28	3	2	5	2	14	17
C22	0	0	0	0	0	1	0	0	0
	1	3	9	7	0	3	3	5	7
	2	9	9	4	2	9	5	25	13
	3	16	3	10	0	24	15	23	25
C23	0	0	0	0	0	0	0	0	0
	1	13	0	0	0	0	1	0	0
	2	1	1	0	0	2	0	1	10
	3	15	22	1	1	13	32	31	19
C24	0	0	0	0	0	0	0	0	0
	1	3	2	0	0	5	2	2	3
	2	3	9	0	0	3	1	10	4
	3	8	11	0	0	9	15	22	22

¹Zero time "Test" and "Control" values obtained from one reaction vessel per replicate

²Replicate

TABLE 53. CO₂ produced from tripalmitin (expressed as percent CO₂-C/Total C) in systems inoculated with sediment collected during Cruise III and tested for lipolysis

Site	Incubation Time (weeks)	Percent CO ₂ Produced							
		Test ¹		Control ¹		1X oil		10X oil	
		1 ²	2	1	2	1	2	1	2
P1	0	0	0	0	0	0	0	0	0
	1	5	3	0	0	5	3	3	6
	2	2	3	0	0	1	5	0	4
	3	9	9	0	0	8	9	11	15
P2	0	0	0	0	0	0	0	0	0
	1	3	3	0	0	2	2	3	2
	2	3	6	0	0	2	3	1	2
	3	5	7	0	0	4	5	5	4
P3	0	0	0	0	0	0	0	0	0
	1	6	6	0	0	3	1	1	5
	2	8	5	0	0	3	4	1	0
	3	5	7	0	0	8	7	2	5
	0	0	0	0	0	0	0	0	0
P4	1	5	5	1	0	1	2	4	3
	2	5	6	0	0	4	4	2	3
	3	5	9	1	0	5	8	10	19
C21	0	0	0	0	0	0	0	0	0
	1	3	3	1	1	4	2	4	4
	2	5	7	0	0	6	8	3	3
	3	11	12	0	0	6	7	7	10
C22	0	0	0	0	0	0	0	0	0
	1	5	3	0	1	5	2	4	1
	2	6	4	0	0	9	5	3	3
	3	15	6	0	0	8	9	16	10
C23	0	0	0	0	0	0	0	0	0
	1	6	4	0	0	6	4	12	6
	2	11	7	0	0	7	7	8	9
	3	17	7	0	1	16	13	14	23
C24	0	0	0	0	0	0	0	0	0
	1	2	5	0	0	3	3	3	4
	2	6	6	0	0	9	5	4	7
	3	9	11	0	0	10	25	23	18

¹Zero time "Test" and "Control" values obtained from one reaction vessel per replicate

²Replicate

TABLE 54. Quantity of CO₂ produced from chitin in systems inoculated with sediment from Cruise I

Site	Incubation Time (weeks)	CO ₂ Produced (µg CO ₂ /ml sediment)							
		Test		Control		1X oil		10X oil	
		1 ¹	2	1	2	1	2	1	2
P1	0	31	31	31	31	31	31	31	31
	1	3300	2800	120	120	3000	3100	2700	2800
	2	4400	3300	150	120	2900	3100	3400	3900
	3	4900	5600	220	150	1700	2800	2800	2800
P2	0	31	31	31	31	31	31	31	31
	1	930	1000	120	120	1400	770	990	590
	2	1000	990	150	120	1100	1500	1100	650
	3	4200	2800	220	220	1800	1700	1700	1300
P3	0	31	31	31	31	31	31	31	31
	1	1300	1400	62	62	770	1200	930	1000
	2	1800	1800	62	62	1600	1800	1200	990
	3	2800	2800	120	120	1300	1500	1800	1400
P4	0	31	31	31	31	31	31	31	31
	1	1300	930	120	62	1300	710	890	650
	2	1800	1500	120	62	930	1200	930	930
	3	4000	2800	150	120	1300	2200	2000	1200
C21	0	31	31	31	31	31	31	31	31
	1	560	710	120	62	1800	2300	2300	1200
	2	5200	3100	150	120	1300	1900	5100	1800
	3	4800	4800	150	120	1800	2200	2400	1600
C22	0	31	31	31	31	31	31	31	31
	1	2200	2300	62	150	2900	2000	2300	2300
	2	3600	3500	150	150	2700	2000	2800	2100
	3	4900	4300	150	220	280	1500	1900	2800
C23	0	31	31	31	31	31	31	31	31
	1	1700	1700	62	62	1300	2200	1800	1300
	2	3300	3500	62	62	1500	1500	2300	1500
	3	4100	4200	120	62	1800	2000	2100	2300
C24	0	31	31	31	31	31	31	31	31
	1	1700	3300	120	120	1600	2400	1700	2000
	2	3500	3100	120	150	1500	2600	1800	2600
	3	4700	5400	120	120	1000	2100	2200	2800

¹Replicates

TABLE 55. Quantity of CO₂ produced from chitin in systems inoculated with sediment from Cruise II

Site	Incubation Time (weeks)	CO ₂ Produced (µg CO ₂ /ml sediment)							
		Test		Control		1X oil		10X oil	
		1 ¹	2	1	2	1	2	1	2
P1	0	69	69	69	69	69	69	69	69
	1	620	550	140	140	760	690	620	690
	2	7300	1600	210	210	1800	1100	1400	1800
	3	2600	2200	280	210	2800	1700	2100	1700
P2	0	69	69	140	69	69	69	140	140
	1	410	340	210	140	690	410	550	410
	2	1900	1100	280	140	1900	830	830	5900
	3	5700	2100	280	210	830	2100	2000	2200
P3	0	69	69	69	69	69	69	69	69
	1	480	3700	69	140	280	1400	480	2500
	2	1500	8900	140	140	280	8000	690	2800
	3	3600	11000	69	210	2500	760	1700	11000
P4	0	69	69	69	69	69	69	69	69
	1	690	550	69	69	550	1000	480	1300
	2	7000	3000	140	140	4900	5700	1500	3400
	3	3600	7000	140	140	1700	8500	2200	3700
C21	0	69	69	69	69	69	69	140	140
	1	690	1000	140	140	620	690	620	1000
	2	1600	210	140	210	1700	1900	1700	1900
	3	2700	2100	140	140	2100	2000	3000	3400
C22	0	900	620	210	210	210	210	210	210
	1	830	830	210	280	1200	1000	1200	1200
	2	2800	1100	210	280	3400	5100	5000	1900
	3	2300	1100	280	210	1200	8300	2800	4000
C23	0	69	69	69	69	69	69	69	69
	1	1400	2200	69	0	340	620	2100	830
	2	5600	5500	140	140	3900	5500	2300	5900
	3	9000	7800	69	140	8300	5600	4900	8600
C24	0	3500	1800	140	140	140	140	69	140
	1	1600	760	140	140	830	1000	830	1200
	2	3300	3700	140	69	5200	2600	7700	3400
	3	8000	5400	140	140	8600	3000	4800	3100

¹Replicates

TABLE 56. Quantity of CO₂ produced from chitin in systems inoculated with sediment from Cruise III

Site	Incubation Time (weeks)	CO ₂ Produced (µg/CO ₂ /ml sediment)							
		Test		Control		1X oil		10X oil	
		1 ¹	2	1	2	1	2	1	2
P1	0	69	410	69	69	69	69	69	69
	1	1600	620	69	69	2400	830	1800	620
	2	2400	140	69	69	2200	1900	1700	1900
	3	2300	210	69	69	3900	3200	2900	1700
P2	0	340	550	140	69	140	210	140	280
	1	1700	340	210	140	620	1200	1200	480
	2	900	690	140	69	620	3900	1400	1300
	3	1600	1700	140	69	1700	550	4300	1200
P3	0	210	410	69	69	69	69	69	69
	1	410	69	69	69	620	900	340	480
	2	970	1000	69	69	620	1400	1400	620
	3	1000	1800	69	69	1400	4000	2700	4100
P4	0	280	550	69	69	69	69	69	69
	1	140	410	69	140	140	69	340	140
	2	620	620	69	69	690	550	900	830
	3	1200	3900	69	140	1100	1400	1900	1200
C21	0	1000	760	140	69	69	69	69	69
	1	1700	1600	140	69	1400	1700	1100	1600
	2	3200	1700	140	69	3100	2300	5500	5500
	3	4500	550	210	69	3200	1800	7400	4600
C22	0	140	690	69	69	140	69	69	140
	1	1200	280	140	140	1200	1700	2500	620
	2	1400	620	69	69	690	1200	2700	1200
	3	1500	2100	69	140	1000	1500	2900	2600
C23	0	140	69	69	69	69	69	69	69
	1	2800	280	69	69	69	140	140	1200
	2	410	760	69	69	280	280	340	340
	3	690	690	3400	69	69	1700	1000	620
C24	0	69	2200	2100	69	1900	69	69	2600
	1	2700	3200	210	69	69	2100	2100	4800
	2	3300	2000	69	69	2200	4500	2300	480
	3	3000	1000	69	69	1900	4200	1800	1700

¹Replicate

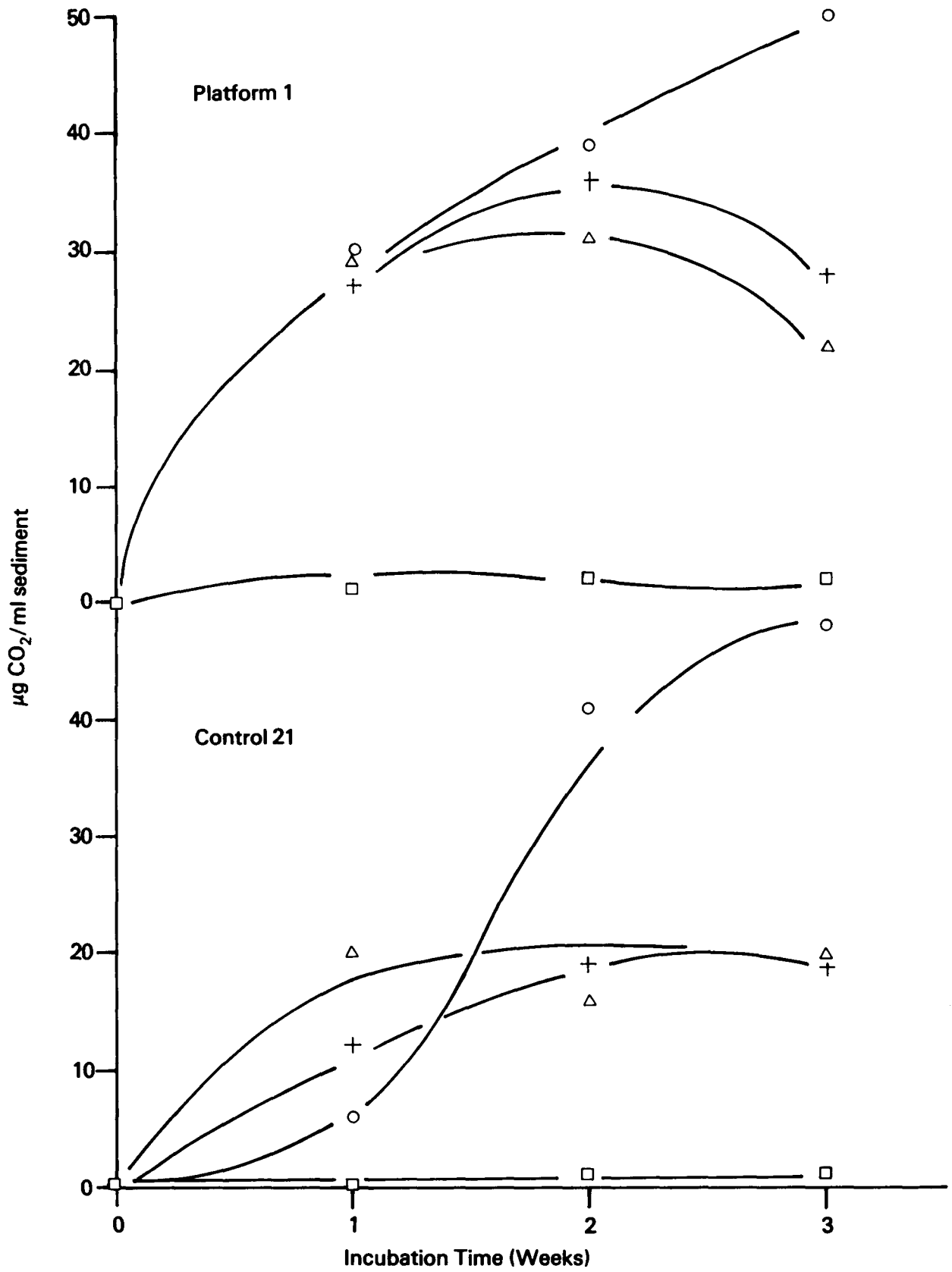


FIG. 26. Production of CO₂ from chitin in Test (○), Control (□), 1xOil (Δ), and 10xOil (+) systems.

TABLE 57. CO₂ produced from cellulose (expressed as percent CO₂-C/Total C) in systems inoculated with sediment collected during Cruise I

Site	Incubation Time (weeks)	Percent CO ₂ Produced							
		Test ¹		Control ¹		1X oil		10X oil	
		1 ²	2	1	2	1	2	1	2
P1	0	1	0	1	0	0	0	0	0
	1	4	1	2	1	3	0	0	0
	2	4	0	10	0	3	0	0	4
	3	8	3	3	1	12	5	33	15
P2	0	1	1	1	1	0	0	30	0
	1	5	0	0	1	2	5	7	0
	2	11	10	1	0	3	0	2	6
	3	6	5	0	8	6	0	4	3
P3	0	2	1	2	1	4	1	0	0
	1	5	0	4	10	2	8	8	0
	2	1	1	1	0	8	0	12	3
	3	8	9	1	14	1	10	3	1
P4	0	1	0	1	0	21	0	0	0
	1	5	0	0	0	5	0	16	1
	2	15	7	0	0	1	5	8	0
	3	21	18	1	1	3	8	10	7
C21	0	0	0	0	0	5	0	7	1
	1	2	0	1	0	9	4	53	0
	2	1	2	6	0	4	0	0	2
	3	0	0	7	0	0	3	1	4
C22	0	0	0	0	0	9	0	2	2
	1	0	1	0	2	1	0	0	0
	2	3	0	1	1	0	7	0	0
	3	0	2	0	0	3	10	5	3
C23	0	0	3	0	3	0	0	0	0
	1	0	0	1	0	1	0	4	7
	2	3	3	0	0	0	0	0	0
	3	1	4	2	0	7	4	14	4
C24	0	3	0	3	0	0	0	1	1
	1	19	0	2	0	1	12	0	0
	2	1	0	2	2	1	0	0	0
	3	2	11	7	15	13	15	28	18

¹Zero time "Test" and "Control" values obtained from one reaction vessel per replicate

²Replicate

TABLE 58. CO₂ produced from cellulose (expressed as percent CO₂-C/Total C) in systems inoculated with sediment collected during Cruise II

Site	Incubation Time (weeks)	Percent CO ₂ Produced							
		Test ¹		Control ¹		1X oil		10X oil	
		1 ²	2	1	2	1	2	1	2
P1	0	0	0	0	0	0	0	0	0
	1	3	1	0	0	1	1	4	3
	2	5	2	1	0	3	5	4	4
	3	11	13	1	3	16	11	9	13
P2	0	0	0	0	0	0	0	0	0
	1	1	1	0	0	0	1	1	1
	2	4	3	2	1	2	1	1	1
	3	7	4	1	1	6	4	6	4
P3	0	0	0	0	0	0	0	0	0
	1	0	1	0	0	0	2	1	1
	2	2	4	1	1	4	1	1	0
	3	4	5	2	1	2	4	4	2
P4	0	0	0	0	0	0	0	0	0
	1	1	1	1	0	1	1	3	2
	2	3	2	2	1	3	2	2	5
	3	11	7	1	1	8	3	8	8
C21	0	0	0	0	0	0	0	0	0
	1	1	2	0	0	1	1	3	4
	2	1	1	1	1	2	2	1	2
	3	11	7	2	3	10	8	15	12
C22	0	1	1	1	1	1	2	2	1
	1	5	4	1	3	4	7	4	6
	2	5	4	1	1	1	5	6	3
	3	12	11	2	2	12	11	4	9
C23	0	0	0	0	0	0	0	0	0
	1	0	1	0	0	1	3	1	2
	2	4	4	1	0	1	3	2	1
	3	7	5	1	1	6	8	11	8
C24	0	1	0	1	0	1	0	1	2
	1	4	3	1	3	2	5	6	3
	2	4	4	1	1	4	4	7	6
	3	1	7	1	1	5	5	8	6

¹Zero time "Test" and "Control" values obtained from one reaction vessel per replicate

²Replicate

TABLE 59. CO₂ produced from cellulose (expressed as percent CO₂-C/Total C) in systems inoculated with sediment collected during Cruise III

Site	Incubation Time (weeks)	Percent CO ₂ Produced							
		Test ¹		Control ¹		1X oil		10X oil	
		1 ²	2	1	2	1	2	1	2
P1	0	0	0	0	0	0	1	0	0
	1	0	1	0	0	1	2	1	2
	2	8	12	0	9	9	10	6	10
	3	7	6	1	1	10	5	8	19
P2	0	0	0	0	0	0	0	0	0
	1	1	1	0	0	2	1	1	4
	2	4	6	1	1	6	1	4	5
	3	3	3	0	0	4	6	4	6
P3	0	0	0	0	0	0	0	0	0
	1	1	1	0	1	0	1	1	2
	2	2	3	3	0	3	1	2	2
	3	2	3	0	0	10	7	5	3
P4	0	0	0	0	0	0	0	0	0
	1	2	3	1	1	1	2	2	2
	2	2	7	0	1	1	4	2	3
	3	5	3	1	0	10	4	9	5
C21	0	0	0	0	0	0	0	0	0
	1	2	0	0	0	2	3	5	3
	2	7	10	1	1	14	7	6	7
	3	11	11	0	0	12	11	13	6
C22	0	0	0	0	0	0	0	0	0
	1	1	2	0	0	2	4	1	3
	2	5	11	1	1	10	4	8	5
	3	5	3	0	0	12	3	5	23
C23	0	0	0	0	0	0	0	0	0
	1	1	1	0	1	2	1	2	2
	2	6	5	1	1	4	3	4	6
	3	9	4	1	0	12	5	10	5
C24	0	0	0	0	0	0	0	0	0
	1	1	1	1	0	1	1	3	2
	2	5	5	1	1	8	6	2	3
	3	6	1	0	0	11	3	12	4

¹Zero time "Test" and "Control" values obtained from one reaction vessel per replicate

²Replicate

TABLE 60. CO₂ produced from bovine serum albumin (expressed as percent CO₂-C/Total C) in systems inoculated with sediment collected during Cruise I and tested for proteolysis

Site	Incubation Time (weeks)	Percent CO ₂ Produced							
		Test ¹		Control ¹		1X oil		10X oil	
		1 ²	2	1	2	1	2	1	2
P1	0	0	13	0	13	3	14	0	17
	1	9	26	0	19	28	1	9	29
	2	89	87	4	38	57	50	46	87
	3	51	15	2	26	78	71	54	54
P2	0	3	4	3	4	8	2	14	9
	1	1	3	2	4	14	1	20	1
	2	72	69	53	27	52	49	68	56
	3	54	14	3	23	68	56	91	78
P3	0	0	1	0	1	0	2	0	2
	1	5	4	1	1	6	7	2	3
	2	55	81	24	43	34	28	56	26
	3	28	27	10	11	24	61	69	56
P4	0	1	1	1	1	0	0	0	3
	1	16	10	0	1	10	6	4	14
	2	26	22	30	34	32	34	35	51
	3	42	6	2	12	17	19	5	4
C21	0	8	0	8	0	1	13	18	1
	1	4	66	1	55	5	2	21	4
	2	85	60	72	0	74	76	73	92
	3	98	76	89	21	86	98	69	91
C22	0	2	3	2	3	4	4	0	6
	1	12	6	0	5	0	13	5	48
	2	84	57	22	3	66	82	68	0
	3	31	21	42	0	93	65	95	72
C23	0	0	9	0	9	0	9	0	1
	1	17	14	1	1	28	40	41	2
	2	90	66	71	0	78	1	64	66
	3	50	6	8	4	76	57	67	85
C24	0	6	28	6	28	1	4	10	0
	1	22	10	12	0	9	6	18	14
	2	42	89	54	1	52	88	85	69
	3	65	60	8	2	58	87	80	61

¹Zero time "Test" and "Control" values obtained from one reaction vessel per replicate

²Replicate

TABLE 61. CO₂ produced from bovine serum albumin (expressed as percent CO₂-C/Total C) in systems inoculated with sediment collected during Cruise II and tested for proteolysis

Site	Incubation Time (weeks)	Percent CO ₂ Produced							
		Test ¹		Control ¹		1X oil		10X oil	
		1 ²	2	1	2	1	2	1	2
P1	0	5	3	5	3	0	0	0	0
	1	5	5	0	0	2	6	2	6
	2	42	56	2	24	18	43	9	16
	3	50	45	9	6	26	20	53	41
P2	0	1	0	1	0	0	1	0	0
	1	1	2	0	1	1	2	1	2
	2	20	16	7	5	6	4	17	20
	3	29	16	11	4	22	20	14	8
P3	0	0	0	0	0	1	1	0	0
	1	1	2	1	0	3	4	2	1
	2	16	4	2	3	6	28	18	8
	3	54	32	4	8	11	21	21	5
P4	0	0	0	0	0	1	0	0	0
	1	5	3	1	0	3	6	5	2
	2	31	29	5	2	36	7	10	17
	3	60	36	8	10	12	13	36	17
C21	0	4	0	4	0	0	0	1	0
	1	9	2	1	2	1	3	9	5
	2	60	32	5	2	48	58	53	51
	3	49	87	38	33	79	30	88	77
C22	0	24	9	24	9	19	10	23	7
	1	41	17	14	7	32	19	37	23
	2	66	45	16	11	68	28	58	50
	3	19	54	17	10	28	28	27	25
C23	0	0	0	0	0	0	2	1	0
	1	1	7	1	1	3	2	7	2
	2	48	46	0	0	13	36	9	5
	3	86	70	35	8	28	57	66	65
C24	0	16	6	16	6	11	10	7	8
	1	14	7	9	6	15	7	10	18
	2	49	18	4	4	35	33	25	24
	3	17	25	5	5	30	20	30	20

¹Zero time "Test" and "Control" values obtained from one reaction vessel per replicate

²Replicate

TABLE 62. CO₂ produced from bovine serum albumin (expressed as percent CO₂-C/Total C) in systems inoculated with sediment collected during Cruise III and tested for proteolysis

Site	Incubation Time (weeks)	Percent CO ₂ Produced							
		Test ¹		Control ¹		1X oil		10X oil	
		1 ²	2	1	2	1	2	1	2
P1	0	0	0	0	0	0	0	0	0
	1	4	3	2	1	5	2	5	3
	2	13	7	5	1	6	22	18	15
	3	58	41	0	0	39	37	32	49
P2	0	0	0	0	0	0	0	0	0
	1	2	1	0	0	2	2	3	2
	2	5	4	0	1	12	10	11	12
	3	18	30	0	0	37	44	36	47
P3	0	0	0	0	0	0	0	0	0
	1	2	2	0	0	2	1	2	1
	2	8	4	0	0	7	3	6	3
	3	25	27	0	0	20	33	31	29
P4	0	0	0	0	0	0	0	0	0
	1	3	4	0	0	8	4	9	2
	2	5	4	0	0	9	10	15	7
	3	31	27	0	0	40	41	39	33
C21	0	0	0	0	0	0	0	0	0
	1	11	3	0	0	5	4	3	6
	2	12	11	0	0	3	13	8	32
	3	37	44	0	0	44	34	46	54
C22	0	0	0	0	0	0	0	0	0
	1	2	2	0	0	3	2	2	2
	2	9	10	0	0	4	7	3	7
	3	27	25	0	0	40	24	25	33
C23	0	0	0	0	0	0	0	0	0
	1	1	1	0	0	1	1	1	1
	2	4	10	0	0	7	6	3	8
	3	23	15	0	0	22	30	24	26
C24	0	0	0	0	0	0	0	0	0
	1	1	1	0	0	1	1	1	1
	2	4	9	0	0	3	7	3	8
	3	15	24	0	0	16	33	20	31

¹Zero time "Test" and "Control" values obtained from one reaction vessel per replicate

²Replicate

TABLE 63. Impact of oil on metabolic activity and photosynthesis of *Nostoc muscorum* ATCC # 27347

System	Replicate	Metabolic Activity $\mu\text{g O}_2$ consumed/mg cells	Photosynthesis $\mu\text{g O}_2$ produced/mg cells
Test	1	2.3	158
	2	12.5	163
	3	11.7	156
	4	0.0	168
	5	0.0	150
		5.3 ± 6.3	159 ± 7
Control	1	0	0
	2	0	0
	3	0	0
	4	0	0
	5	0	0
		0 ± 0	0 ± 0
1X oil	1	9.4	183
	2	9.7	169
	3	9.4	179
	4	14.1	167
	5	0	168
		8.5 ± 5.2	173 ± 7
10X oil	1	18.8	133
	2	11.7	156
	3	11.7	149
	4	14.0	114
	5	14.0	136
		14.0 ± 2.9	138 ± 16

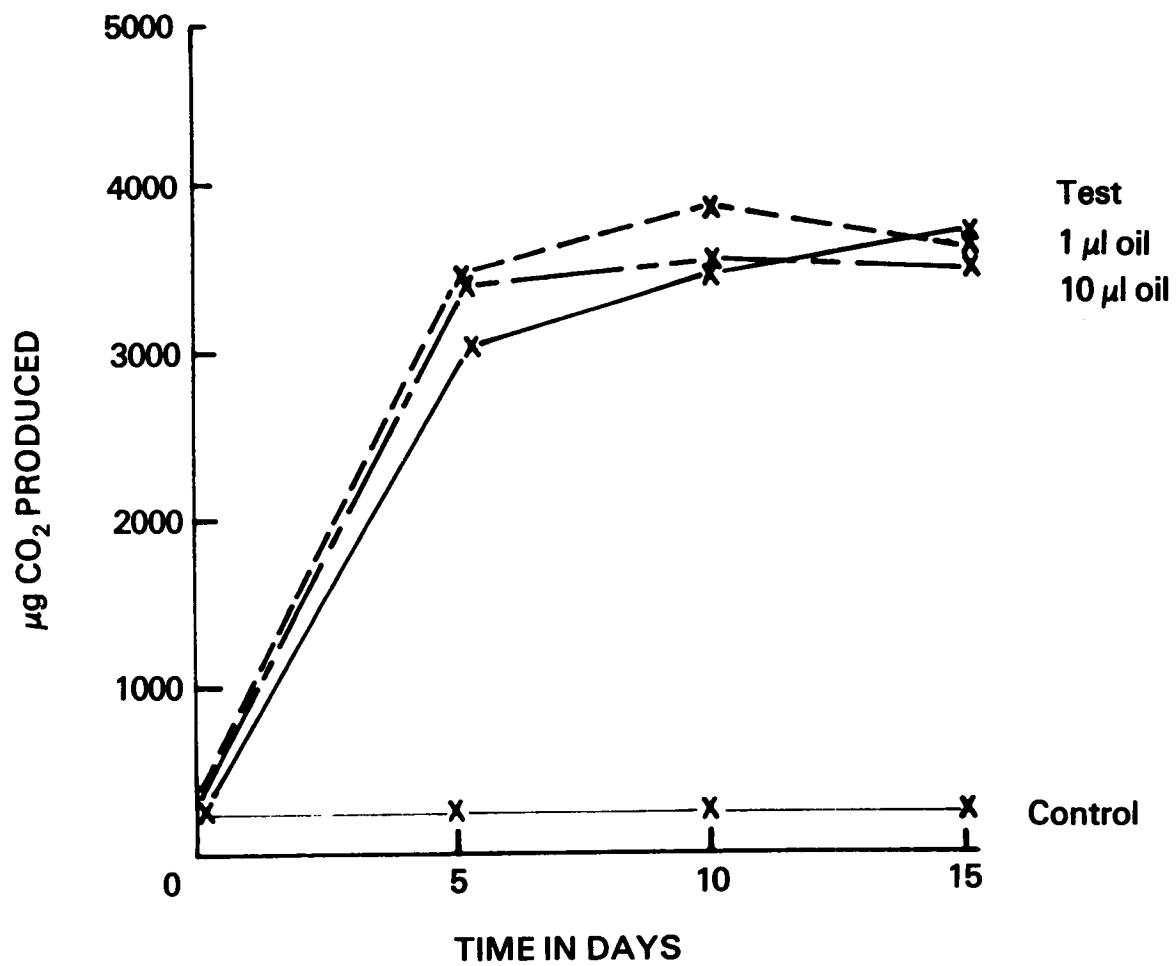


FIG. 27. Impact of oil on the rate of CO₂ produced from proteolytic activity by *Pseudomonas* sp 3.

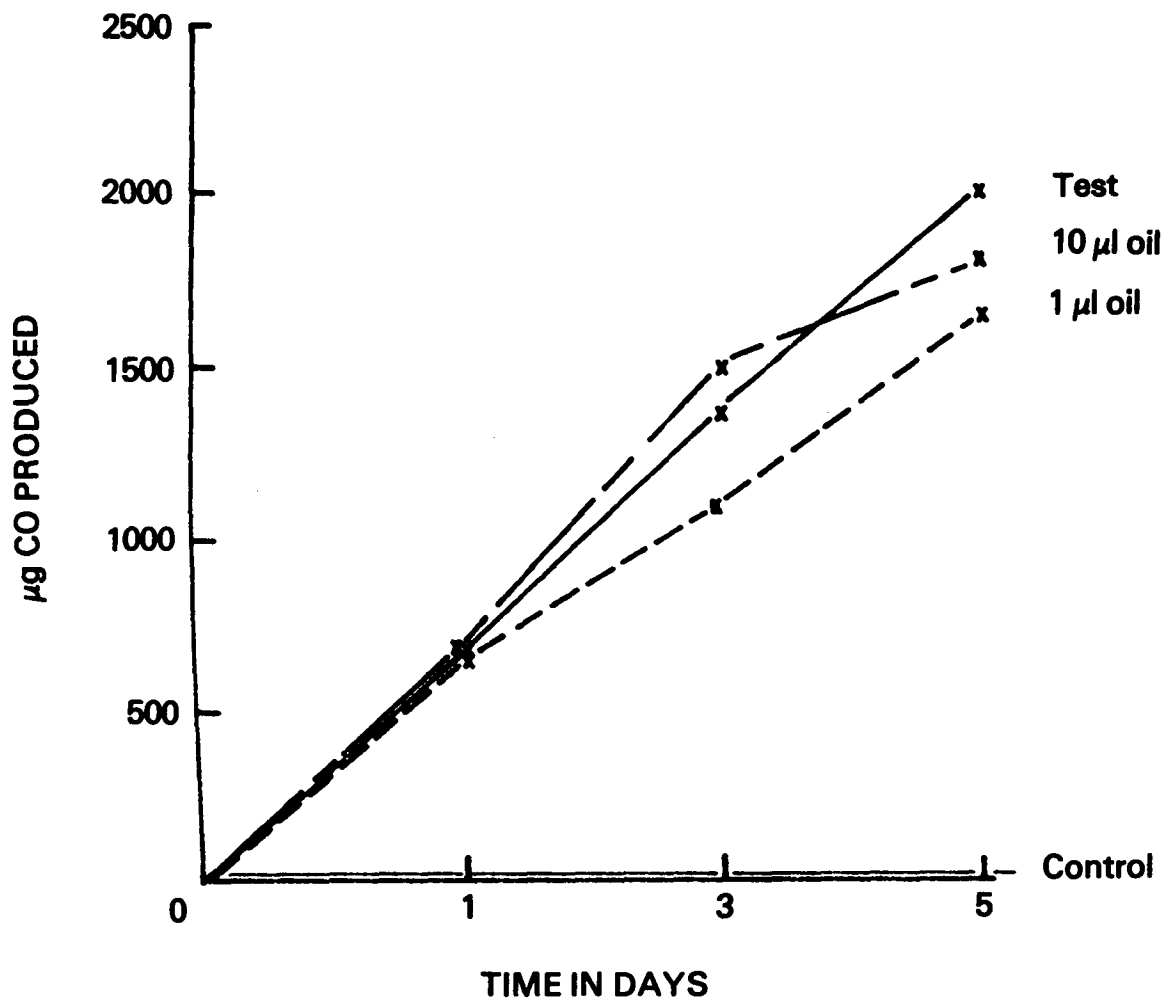


FIG. 28. Impact of oil on the heterotrophic activity of *Enterobacter* sp 1 as determined by measuring CO₂ using GLC.

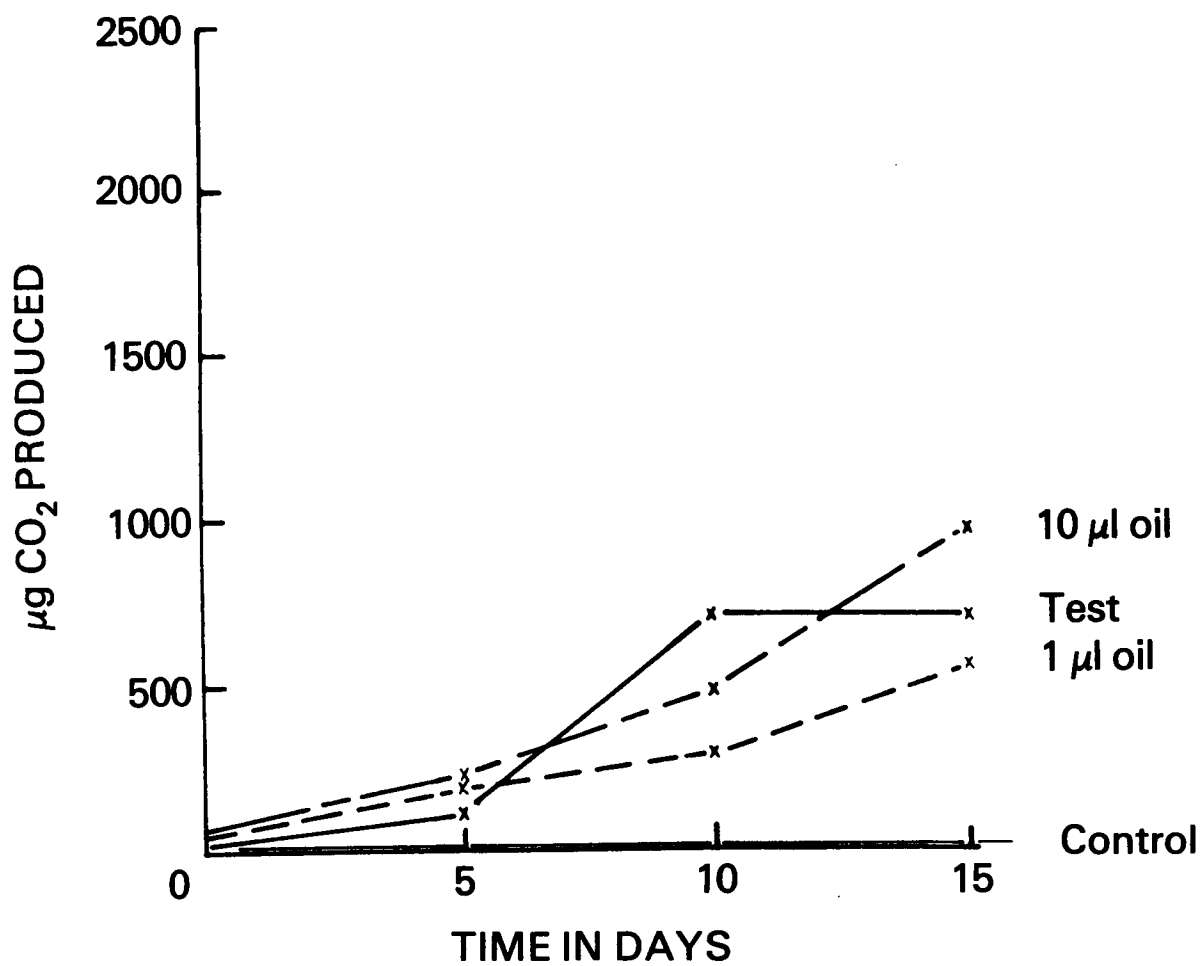


FIG. 29. Impact of oil on cellulose utilization by measuring $^{14}\text{CO}_2$ produced by *Cellulomonas* sp 1.

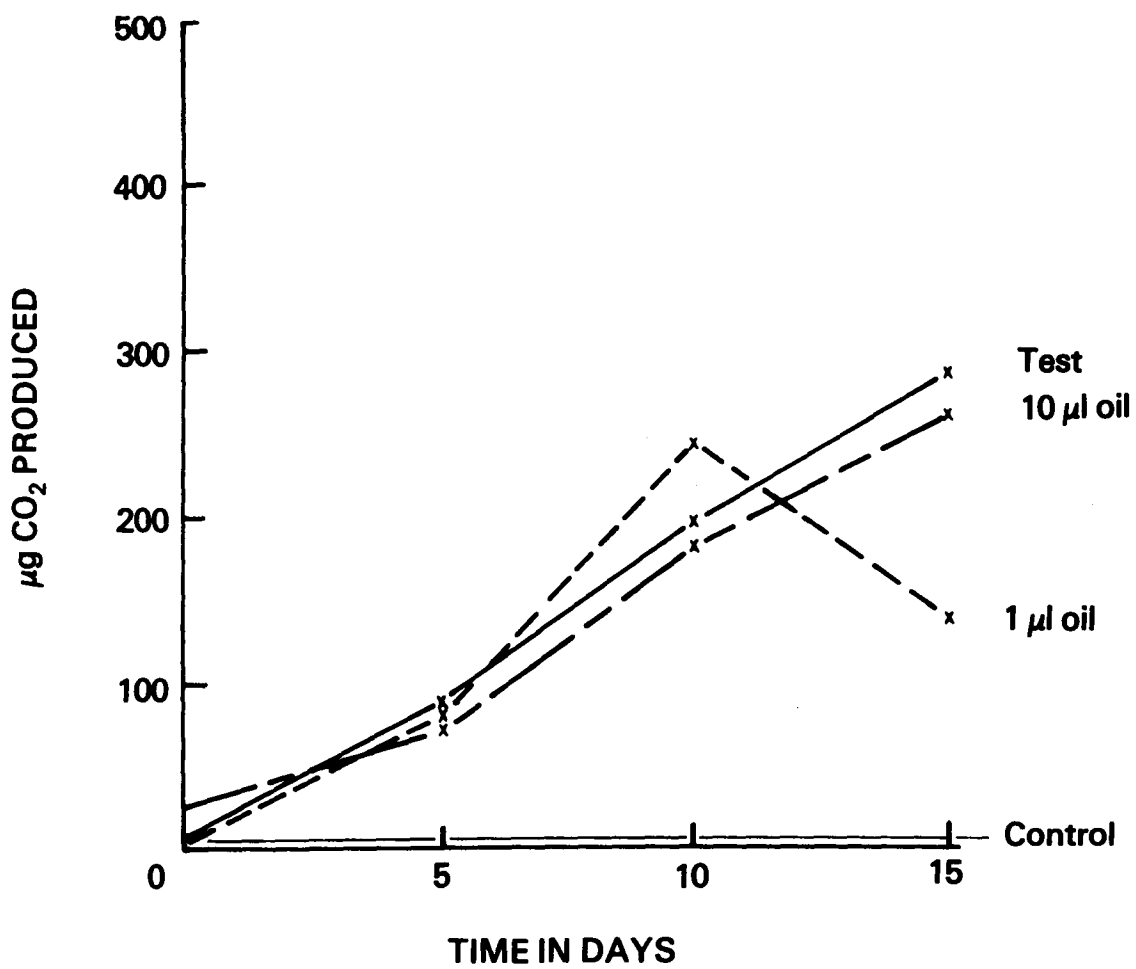


FIG. 30. Impact of oil on the rate of ¹⁴CO₂ produced during lipolysis by *Pseudomonas* sp 5.

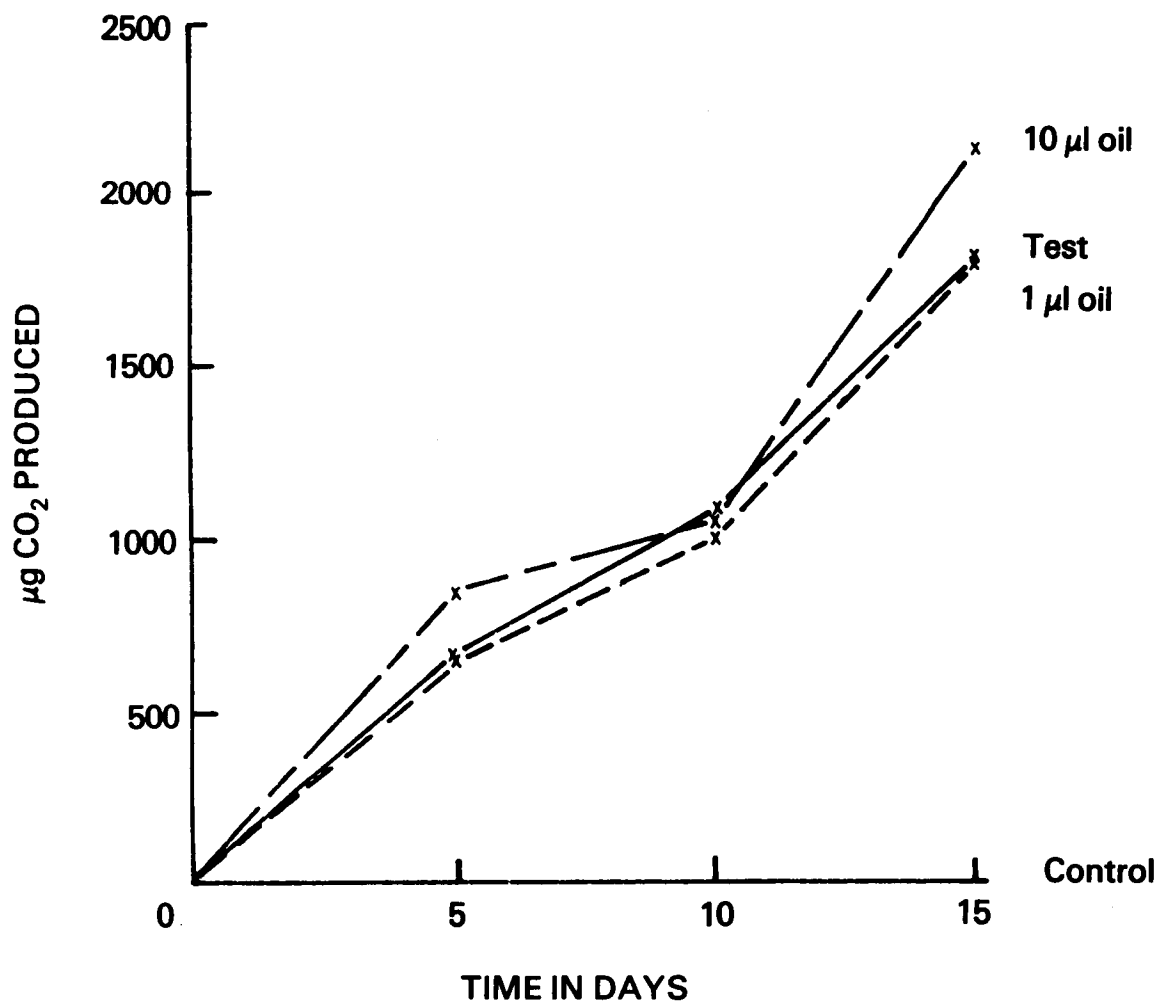


FIG. 31. Impact of oil on chitin utilization by *Pseudomonas* sp 6.

IV. DISCUSSION

A. Analysis of Microbial Populations

1. Aerobic Heterotrophic Bacteria

Numbers of aerobic heterotrophs at control sites decreased from an average of $ca. 1 \times 10^6$ (per ml of sediment) during Cruise I (May) to an average of $ca. 6 \times 10^4$ during Cruise II (August-September). The average increased to $ca. 6 \times 10^5$ during Cruise III (January), suggesting a seasonal trend possibly due to a lack of a sufficient nutrient(s) during the summer.

Average numbers of aerobic heterotrophs for primary platforms ($ca. 4 \times 10^6$) were higher during Cruise I than during Cruise II as shown in Table 9. Average numbers of aerobic heterotrophs for Cruise III ($ca. 8 \times 10^5$) were in a numerical range between those for Cruises I and II, as was previously observed for aerobic heterotrophs at control sites (Tables 8 and 9). The ratio of numbers of aerobic heterotrophs at primary platforms for Cruises I:II:III was 50:1:10, suggesting the same seasonal trend previously observed at control sites, where the ratio was 20:1:10. The average number of aerobic heterotrophs at the secondary platforms (sampled during Cruise II) was 8×10^4 (Table 10), which was similar to the numbers found at the primary platforms and control sites during that Cruise. Thus, although numbers of heterotrophs at the primary platforms were significantly higher than at the corresponding controls for Cruise I, this was not the case for the other two cruises. Furthermore, all sites seemed to demonstrate the same seasonal trends in variation.

As was suggested earlier, one of the possible explanations for the large decrease in population during the August cruise (Cruise II) is a lack of sufficient nutrient(s) during the summer. Walker and Colwell (1976a) have reported similar results for aerobic heterotrophs in control site sediments of Chesapeake Bay, *viz.* lower numbers of sediment microorganisms in August, 1977, due, in part, to oxygen depletion. Although examination of measured levels of DO did not confirm it as a limiting factor during this study, most of these DO readings were not taken at the water-sediment interface and possibly do not reflect the true situation in the sediment. Since all plate counts were conducted under aerobic conditions, a shift in environmental conditions toward anaerobiosis would likewise cause a shift in the microbial population from aerobes to anaerobes. Therefore, if the DO number in the sediment was lower, the obligate aerobes would decline in number, and the corresponding increase in obligate anaerobes would not have been detected. Overall, no correlations were observed between counts and DO, salinity, or inorganic sediment nutrients (see Section B below). No direct correlations were observed between population numbers and sediment type as determined by grouping sites P1, P4, C23, and C24 as the clay-silt group, and sites P2, P3, C21, and C22 as the sand-silt group (Huang, 1981). Furthermore, no consistent correlations were observed when numbers of aerobic heterotrophs were compared with total organic carbon (TOC), pyrene content, or total hydrocarbon (TH) content (data from Volume I, Part 8). Although analysis of the data for pyrene, TOC, TH, and aerobic heterotrophs suggests that for sites P2, P3,

C22, and C24 sampled during Cruises I (most pronounced effect), II, and III, hydrocarbons may have had a negative impact on aerobic heterotrophs, such is not the case for sites P1, P4, C21, and C23.

There are many other factors that may influence numbers of aerobic heterotrophic bacteria that are detected in marine sediments. These include: current patterns; river influence (Mississippi); age, size and development of platforms; number, type, and size of spills; and other factors that are described below. Cruise II provided an opportunity to examine possible relationships between aerobic heterotrophic bacteria and possible factors that might influence their numbers at a significant number of platforms and control sites (Table 64). When these factors are examined it appears that age and degree of development do not correlate with numbers of aerobic heterotrophs. Of the factors that appeared to correlate with numbers of aerobic heterotrophs, *viz.*, depth, distance from shore and distance from the Mississippi River delta, the latter appeared to have the greatest influence on numbers of aerobic heterotrophic bacteria, especially for those sampling sites located within 40 km of the delta (see Fig. 3). Although this trend is not demonstrated by Duncan multiple range analysis (Table 65), it is clearly shown using a correlated t-test (Fig. 4).

It should be noted that (as per the conditions of the contract) all of the preceding results were based on colony-forming units (CFU) per ml of sediment. However, even when values were compared after transformation to CFU per gram of sediment, essentially the same conclusions could be drawn as before.

The marine agar used to enumerate aerobic heterotrophs in this study, ZoBell's 2216 (ZoBell, 1941) has been reported to be one of the most efficient media for quantifying heterotrophic bacteria from the marine environment (Simidu, 1974). The spread-plate technique used in this study appears to be superior to the pour-plate method because the latter method causes die-off of autochthonous organisms unable to survive exposure to the temperature of molten agar (Buck and Cleverdon, 1960).

The samples and subsamples obtained in this study were frozen after they were collected and analyzed after 7-14 days. Subsamples removed after 7 days and plated on Marine Agar revealed an average (statistically insignificant) 10% decrease in aerobic heterotrophs (Table 11). The numbers of aerobic heterotrophs that survived frozen storage appeared to decrease exponentially, reaching 50% after 45 days frozen storage and 18% after 90 days frozen storage (Fig. 5). Although it appears that approximately 10% of the aerobic heterotrophs might not have survived frozen storage of 7-14 days, counts of aerobic heterotrophs were not corrected for these losses because the decrease was statistically insignificant within this time frame. These losses partially contradict the conclusions of Anthony (1963) who stated that freezing and then thawing after 6 months does not significantly change the microbial counts of sediments, but support the validity of the data for this report, and confirm the report of Walker and Colwell (1975) who reported an 83-90% reduction in sediment

TABLE 64. Rank of primary platforms (P1-P4), secondary platforms (S5-S20) and control sites (C21-C24) sampled during Cruise II

Distance from Mississippi River Delta	Distance from Shore	Site rank by		Degree of Development
		Depth	Age of Platform	
S6(25) ^a	P2(5) ^a	C21(3) ^b	P2(1954) ^c	S6(24) ^d
S5(30)	S5(6)	S20(18)	S20(1954)	P2(24)
P1(30)	S12(11)	S19(6)	S10(1955)	S13(24)
S13(30)	S19(27)	S5(9)	S8(1957)	S15(21)
S7(40)	C21(9)	P2(12)	S11(1957)	S17(18)
C21(40)	C22(10)	P1(18)	P1(1962)	S16(18)
S9(40)	P1(19)	S12(17)	P3(1968)	S12(17)
S8(60)	S10(20)	C24(18)	S5(1962)	P3(16)
P2(80)	S11(21)	S10(20)	S6(1965)	S10(16)
S12(80)	S8(27)	S11(20)	S7(1965)	P1(15)
C22(90)	S13(41)	C22(21)	S9(1965)	S20(13)
P3(90)	S20(15)	S18(25)	S12(1965)	S18(13)
S10(100)	S6(42)	S8(27)	P4(1964)	S19(12)
S11(100)	P3(42)	S14(29)	S19(1966)	S14(12)
P4(110)	P4(53)	P4(46)	S13(1968)	S11(12)
C20(120)	C23(32)	C23(37)	S18(1970)	S7(12)
C23(130)	S18(52)	P3(35)	S16(1971)	P4(12)
S19(180)	S7(56)	S16(45)	S17(1972)	S8(10)
S16(200)	C24(39)	S13(51)	S14(1973)	S9(7)
C24(200)	S9(64)	S6(52)	S15(1974)	S5(1)
S14(200)	S14(68)	S7(65)		
S15(230)	S16(97)	S17(75)		
S18(240)	S15(115)	S9(85)		
S17(250)	S17(120)	S15(98)		

^aKilometers
^bMeters

^cYear installed
^dNumber of wells

TABLE 65. Rank and Duncan subsets of sites as established by microbial enumerations¹

Site	MA ²	ABOA ³	RB ⁴	MRNHM ⁵	MRSHM ⁶
P1	4A ¹	14ABCD	13A	18A	9ABC
P2	7A	8DE	19A	13A	13ABC
P3	10A	17AB	12A	5A	15AB
P4	19A	21ABC	21A	1A	10ABC
S5	2A	1G	18A	14A	5BCD
S6	3A	12ABCDE	16A	10A	3CD
S7	5A	7DE	20A	11A	1D
S8	12A	10BCDE	11A	8A	7ABC
S9	9A	5EF	6A	6A	6BCD
S10	20A	15ABCD	10A	9A	23AB
S11	11A	16ABC	4A	12A	12ABC
S12	14A	6EF	15A	7A	4BCD
S13	6A	2F	2A	2A	2CD
S14	22A	22AB	5A	15A	22AB
S15	23A	11ABCDE	14A	21A	21AB
S16	17A	9CDE	23A	23A	20AB
S17	21A	3EF	3A	24A	19AB
S18	13A	23AB	17A	17A	18AB
S19	24A	24A	22A	20A	24A
S20	8A	18ABC	24A	19A	17AB
C21	1B	4EF	1B	22A	8ABC
C22	15A	20AB	8A	4A	14ABC
C23	18A	19AB	9A	16A	11ABC
C24	16A	13ABCD	7A	3A	16AB

¹Numbers reflect ranking of colony-forming units/ml of sediment ranging from '1' (high) to '24' (low). Letters reflect statistical subsets based on Duncan's Multiple Range Test, with 'A' being low.

²Total aerobic plate count on Marine Agar at 20 C

³Aerobic hydrocarbonoclasts on Oil Agar at 20 C

⁴Aerobic yeast and mold count on Cooke Rose Bengal Agar at 20 C

⁵Nitrate-reducing hydrocarbonoclasts on Rosenfeld's Medium at 20 C

⁶Sulfate-reducing hydrocarbonoclasts on Rosenfeld's Medium at 20 C

aerobic heterotrophs after sediments were frozen for 6 months. The findings also agree with those of Stewart and Marks (1978) who found no decrease in bacterial populations from sediments frozen for seven days.

A number of investigators have performed bacteriological surveys in the Gulf of Mexico. Thus, it is useful to examine the results of these studies to determine how they compare with the results obtained in the present study. At least two of these studies cannot be compared to the present investigation because bacteriological populations of water, not sediment, were examined. ZoBell (1954) examined the aerobic heterotrophic bacteriological populations from Gulf of Mexico sediments, but made no attempt to quantify these organisms. Oppenheimer, Miget, and Kator (1974), in GURC's OEI, examined the association between hydrocarbons in seawater and the ability of microorganisms in the water column to oxidize hydrocarbons. Oujesky et al. (1979) examined the bacteriological populations in seawater of the South Texas Outer Continental Shelf (STOCS). Sizemore and Olsen (1980) examined the aerobic heterotrophic bacteriological populations at a control site five miles north of the Buccaneer oil platform, and Schwarz et al. (1980) examined a number of stations on the STOCS. Both groups reported numbers of aerobic heterotrophs similar to those found in this study. Neither Sizemore nor Schwarz, however, reported a decrease in aerobic heterotrophs in the late summer or early fall.

2. Aerobic Bacteria on Oil Agar

Numbers of aerobic bacteria on oil agar (ABOA) were obtained after incubating plates at 20 C for 5-7 days. A similar medium without oil was used as a control. Very few, if any, colonies were observed on the medium without oil, possibly because of the short incubation period. A longer incubation period of 14-21 days might have permitted detection of colonies on this control medium, but these slow-growing organisms would not have affected the results anyway. Few of the colonies of ABOA were tested for their ability to use Empire Mix crude oil, so that the ABOA can be described as having only a presumptive ability to use oil.

At control sites numbers of ABOA decreased from an average of $ca. 9 \times 10^3$ per ml sediment during Cruise I to an average of $ca. 3 \times 10^3$ during Cruise II, and increased to $ca. 1.8 \times 10^5$ during Cruise III (Table 12). However, unlike numbers of aerobic heterotrophs on marine agar, numbers of ABOA did not show a significant seasonal trend because of larger standard errors (Fig. 6).

Average numbers of ABOA for primary platforms during Cruise I ($ca. 2 \times 10^6$) and Cruise III ($ca. 7 \times 10^5$) were higher than during Cruise II ($ca. 5 \times 10^3$) as shown in Table 13. The ratio of numbers of ABOA at primary platforms for Cruises I:II:III was 400:1:185, suggesting a seasonal trend previously observed for aerobic heterotrophs at control sites and primary platforms. This trend was less pronounced for ABOA at control sites where the ratio was 3:1:60. As discussed previously, this seasonal trend possibly reflects low DO levels during Cruise II (August-September, 1978). The average number of ABOA at secondary platforms sampled during Cruise II (1×10^4) was similar to that recorded for controls and platforms during Cruise II (Table 13). Thus, it appears that average numbers of

ABOA were higher at platforms than at controls for Cruises I and III, but not for Cruise II when all numbers were quite low.

As discussed above for aerobic heterotrophic bacteria, there are many factors that can influence numbers of ABOA recorded for sediment samples collected during Cruise II. Ranking of the sites sampled during Cruise II by numbers of ABOA did not appear to correlate with any of the factors listed in Table 64, except for proximity to the Mississippi River delta, as demonstrated by Duncan's Multiple Range Test (Table 65 and Fig. 7). Nor were any correlations observed between ABOA and salinity, inorganic sediment nutrients, or dissolved oxygen, although (as mentioned previously) most of the DO readings were not taken at the water-sediment interface and possibly do not reflect the true situation in the sediment.

As discussed by Walker and Colwell (1976 b), it is often useful to express presumptive or confirmative estimates of oil-degrading microorganisms as a percentage of the heterotrophic population, as was done for ABOA (Table 15, Figs. 8 and 9). The ABOA expressed as a percentage of the aerobic heterotrophic bacterial population can be averaged for each site, ranked (Table 66) and compared to various physical, chemical, and geographic factors. Again, there did not appear to be a correlation between ABOA as a percentage of the aerobic heterotrophic bacterial population and any of those factors except for proximity to the River delta. In a recent review Atlas (1981) states that the level and proportion of hydrocarbon utilizers appears to be a sensitive index of hydrocarbons in the environment. In oil polluted areas the hydrocarbon utilizers may constitute up to 100% of the microbial population, while in unpolluted areas they may only be 0.1% of the total population. Based on the above it would be concluded that the entire area investigated in the current study has been subjected to hydrocarbons, albeit of unknown origin.

TABLE 66. Rank of primary platform and control sites for Cruises I, II, and III from highest to lowest based on ABOA as a percentage of the total aerobic heterotrophic bacterial population

Site rank for Cruise		
I	II	III
P3(73) ¹	P2(15)	P2(124)
P2(69)	C21(14)	P4(98)
P4(48)	C24(13)	P3(93)
P1(41)	C23(9)	P1(79)
C21(7)	P1(7)	C21(72)
C22(1)	P3(7)	C22(56)
C23(0.1)	P4(7)	C24(3)
C24(0.1)	C22(5)	C23(2)

¹ ABOA expressed as a percentage of the total aerobic heterotrophic bacterial population for ABOA and aerobic heterotrophs enumerated in samples from control sites and samples collected at 500 m N, E, W, and S of primary platforms

In addition to the factors listed in Table 64, it might be possible to correlate numbers of ABOA with

sediment pyrene content, TOC, or TH. When this was attempted no correlation was found between ABOA (a presumptive measure of oil-degrading bacteria) and sediment hydrocarbon content. It is worth pointing out that Walker and Colwell concluded that there is a "threshold" concentration of oil in the environment—or a percentage of petroleum-degrading microorganisms in the microbial population of the environment—below which there is little correlation between the two, and these findings have been substantiated elsewhere (Brown, 1980). The fact that statistically significant differences in numbers of hydrocarbonoclastic organisms did occur at various locations in the study area suggests that the population has been exposed to levels of oil above this "threshold," but the origin of this oil is unknown. In fact, the effects of natural environmental factors are magnified greatly due to the fact that most of the microbial activity in the sediments occurs in the top two or three centimeters, which in this area of the Gulf is of an extremely transient nature. Consequently, even though platform sites yielded consistently higher populations of ABOA than did the control sites, conclusions on cause-and-effect relationships regarding platform activity alone should be drawn with extreme caution due to the influence of other forces in the area—predominantly the Mississippi River, which has been shown to introduce more than 12,000,000 cubic feet of hydrocarbons into the Gulf of Mexico annually (Murisawa, 1968).

In the attempt to rank platform and control sites based on ABOA as a percentage of the heterotrophic population (Table 66), it was assumed that pyrene, TOC, or TH might be representative of sediment hydrocarbons, and that these hydrocarbons might be petrogenic in origin and possibly have resulted from discharge at platforms and accumulation in platform sediments. If these assumptions are not valid (*i.e.* pyrene, TOC, and TH do not provide the most sensitive indicators of sediment petroleum hydrocarbons) and we examine percents of ABOA, the consistently higher ranking of primary platforms over controls becomes quite noticeable (Table 66). The methods we have selected to quantify hydrocarbons or hydrocarbon-degrading bacteria may; therefore, have to be refined.

3. Yeasts and Fungi

ZoBell (1954) summarized some reports of fungi in the marine environment as they related to the Gulf of Mexico, but made no attempt to quantify yeasts or fungi. Ahearn, Meyers, and Standard (1971) described the ability of yeast isolates from Louisiana marshlands and the Gulf of Mexico to grow in the presence of oils and hydrocarbons. They also reported that numbers of yeasts in marshland sediments ranged from 5×10^2 to 9×10^4 per ml sediment, but they did not report numbers of yeasts in Gulf of Mexico sediment. Ahearn and Meyers (1972) described some genera of yeasts and fungi that were isolated from Barataria Bay, Louisiana and Gulf of Mexico waters. Brown, Light, and Minchew (1980) did quantify the yeasts and molds in a number of sediment samples obtained from the Gulf of Mexico. Thus, this study appears to be the second attempt to quantify yeasts and fungi in the Gulf of Mexico sediments.

Numbers of yeasts and fungi were obtained after incubating plates of PDA and RB at 20 C for 5-7 days. Counts on PDA were one to two orders of magnitude

lower than counts on RB. The reason for the lower counts on PDA (not included in the results) is unknown. No attempts were made to distinguish yeasts from filamentous fungi when these microorganisms were enumerated and subsequently both will be discussed as fungi. In general, numbers of fungi were much more variable from sample to sample than were numbers of heterotrophic bacteria. This phenomenon may result from greater potential patchiness of sediment heterotrophic fungi than heterotrophic bacteria, or from problems resulting from inconsistent breakup/cohesion of the mycelia during sample preparation—a problem commonly encountered during enumeration of fungi. When average counts of fungi were determined, no attempts were made to distinguish and remove outliers before calculating the average because of the inability to distinguish outliers from "normal" numbers of the microorganisms, except for grab sample #2, Control Site C21, Cruise II, where 29,900 CFU per ml was considered an outlier.

At control sites, most of the counts for fungi were similar within each cruise, but different between cruises as was previously observed for aerobic heterotrophic bacteria and ABOA at control sites. Exceptions were counts of fungi at Sites C21 and C24 during Cruise I which were significantly different from counts of fungi at Sites C22 and C23. During Cruises I, II, and III, counts of fungi at control sites ranged from average of 50-2,250 CFU per ml sediment, 430-1,450 CFU per ml sediment and 60-675 CFU per ml sediment, respectively (Table 16). Counts of fungi at control sites were 0-3 and 2-4 orders of magnitude less than counts of ABOA and aerobic heterotrophic bacteria, respectively. It is difficult to determine whether these counts actually represent *in situ* fungal populations at control sites, because of the variability of counts between grab samples (*e.g.* counts from individual grab samples at Site C23 during Cruise I ranged from 50 to 4,400 CFU per ml sediment). Thus, it is difficult to determine if statistically significant seasonal changes in fungal populations occur because of sample to sample and site to site variability within each cruise, as could be determined for aerobic heterotrophic bacteria and ABOA for control sites. Similarly, it is difficult to correlate any expected seasonal trend with changes in DO. If seasonal changes correlated with potentially low DO during Cruise II were predicted, it might be estimated that numbers of these eucaryotic organisms (which are obligate aerobes) would have decreased during Cruise II, as was previously observed for aerobic heterotrophic bacteria and ABOA at control sites. This; however, was not the case.

At primary platforms, most of the fungal counts were similar within each cruise but different between cruises, as was previously observed for aerobic heterotrophic bacteria and ABOA at primary platforms. During Cruises I, II, and III counts of fungi at primary platforms ranged from 8,834-45,000 CFU per ml sediment, 587-1,081 CFU per ml sediment and 544-1,261 CFU per ml sediment, respectively (Table 17). Counts of fungi at primary platforms were 1-3 and 2-3 orders of magnitude less than counts of ABOA and aerobic heterotrophic bacteria, respectively. As discussed above for control site fungal populations, it is difficult to determine whether these counts represent actual *in situ* fungal populations because of the variability of counts between

grab samples (e.g. fungal counts from individual grab samples at the East transect of Primary Platform P2 ranged from 50 to 45,000 CFU per ml sediment during Cruise I). Thus, it is difficult to determine if there are statistically significant differences in fungal counts at primary platforms from season to season. However, if the average fungal counts are examined, the data suggest that fungal populations at primary platforms were higher during Cruise I than during Cruises II and III.

The average number of fungi detected in sediments at secondary platforms was similar to numbers detected at control sites and primary platforms during Cruise II (Table 18). Numbers of fungi in sediments at secondary platforms were 0-2 and 1-3 orders of magnitude less than numbers of ABOA and aerobic heterotrophic bacteria, respectively. Thus, sediment fungal populations for control sites and platforms sampled during all three cruises appeared to be similar, except for Cruise I when the fungal populations appeared to be higher at primary platforms than at control sites.

As discussed above for aerobic heterotrophic bacteria and ABOA, there are many factors that can influence numbers of sediment fungal populations (Table 64). Ranking of the sites sampled during Cruise II by numbers of heterotrophic fungi did not appear to correlate with any of the factors mentioned previously, viz., distance from the Mississippi River, distance from shore, depth, age or degree of development, DO, salinity, inorganic sediment nutrients, TOC, TH, pyrene content, or sediment type.

Colwell, Walker, and Nelson (1973) reported 20 fungi per ml of Eastern Bay sediment (pristine Chesapeake Bay shellfish-harvesting sediment) during November, 1972. Walker and Colwell (1974) reported 500-600 fungi per ml of Eastern Bay sediment during February and April 1973. Walker et al. (1976) reported $<10^1$ fungi per ml of Georgia Bight sediment at a station southeast of Cape Lookout, N.C. on the Atlantic OCS at a depth of 60 m in June, 1974. Colwell et al. (1976) reported $<1-60$ fungi per ml of Georgia Bight sediment at several stations along the coasts of Florida, Georgia, South Carolina and North Carolina on the Atlantic OCS at depths ranging from 65 to 530 m in November, 1974. Colwell et al. (1976) also examined sediments on the Blake Plateau and reported 20-30 fungi per ml of sediment at seven stations east of Jacksonville, Florida, with depths ranging from 120-770 m. Walker et al. (1977) examined the effects of sample preparation and incubation time on counts of yeasts and fungi from estuarine and marine sediment. They determined that sediment dilutions filtered through cellulose or polycarbonate membranes produced higher counts of yeasts and fungi than did spread-plating of minimally diluted sediment, and that 7-14 days was an optimal incubation time without producing overgrowth. They reported 50-300 fungi per ml of Eastern Bay sediment from filtered samples. Thus, it appears that most of the sediments examined in this study contained higher fungal populations than those described above. This may be a function of differences in medium, differences in sediment TOC, or actual higher population counts in the Gulf of Mexico.

At sites likely to be contaminated with oil, Colwell, et al., (1973) reported 1,000 fungi per ml from Colgate Creek sediment (oil-laden sediment in Baltimore Harbor) during November, 1972. Walker and Colwell

(1974) reported 1,000-7,500 fungi per ml Colgate Creek sediment during February and April, 1973. Walker et al. (1977) reported 1,000-2,000 fungi per ml sediment from plate and filter samples of Colgate Creek sediment, respectively. Thus, it appears that sediments likely to be contaminated with oil (primary and secondary platforms) which were examined in this study contained populations of heterotrophic fungi similar in number to those determined by others using different media and incubation conditions, except for primary platforms examined during Cruise I for which numbers were higher than previous reports.

4. Microorganisms from Rosenfeld's Nitrate-Hydrocarbon Medium (MRNHM)

Numbers of MRNHM were obtained by inoculating MPN tubes of oil-containing, nitrate-containing medium (see Tables 2-4) with 1/10, 1/100, and 1/1000 dilutions of sediment and incubating the tubes at 20 C for three weeks. No attempts were made to confirm the hydrocarbon-utilizing capability of the microorganisms cultured in this medium. Thus, these microbes cannot be referred to definitely as nitrate-reducing, hydrocarbon-using microorganisms. Counts of MRNHM (Tables 19-21) appeared to be as variable as counts of fungi. Obvious outliers, i.e. numbers 50-100 times greater than an average value, were eliminated before any means were calculated. To the authors' knowledge, only limited studies have been performed to quantify microorganisms that may reduce nitrate and oxidize hydrocarbons; thus, only limited discussion of these microorganisms is possible. In truth it cannot be stated with certainty that the organisms could utilize hydrocarbons since the crude oil employed in the medium contains non-hydrocarbon carbonaceous matter.

Unlike previous observations for aerobic heterotrophic bacteria, ABOA and sometimes for fungi, numbers of MRNHM were different at individual control sites within each cruise. The variability of the MRNHM counts made it difficult to detect differences at control sites between cruises, although MRNHM counts at Control Sites C23 and C24 for Cruise III appeared to be higher than MRNHM counts at other control sites during any cruise (Table 19). Thus, it was difficult to estimate the effect of season on MRNHM counts. During Cruises I, II, and III counts of MRNHM at control sites exhibited averages of 8-16 per ml sediment, 2-50 per ml sediment and 25-190 per ml sediment, respectively (Table 19). Counts of MRNHM at control sites were 0-3, 2-5, and 3-5 orders of magnitude less than the corresponding counts of fungi, ABOA and aerobic heterotrophic bacteria.

At primary platforms, most of the MRNHM counts (see Table 20) were similar within any given cruise, but different between cruises, as was previously observed for aerobic heterotrophic bacteria, ABOA and fungi. An exception was Primary Platform P1 which appeared to have higher MRNHM counts than the other primary platforms during Cruise III, but which also had a coefficient of variation $>10\%$. During Cruises I, II, and III MRNHM counts ranged from 21-91 per ml sediment, 5-6 per ml sediment and 11-204 per ml sediment, respectively. Counts of MRNHM at primary platforms were 0-3, 3-5, and 3-5 orders of magnitude less than the corresponding counts of fungi, ABOA and aerobic heterotrophic bacteria. These counts suggest an effect of

season, but not necessarily an effect of decreased DO, because an increase, not a decrease, in MRNHM counts would have been anticipated if DO were lower during Cruise II, since MRNHM should be anaerobes. However, while the exact carbon source for microbial growth is not definitely known, it is certain that nitrate reduction did occur, because nitrites were found in the medium.

The average number of MRNHM detected in sediments at secondary platforms was similar to numbers detected at control sites and primary platforms during Cruise II (Table 21). Numbers of MRNHM in sediments at secondary platforms were 2-3, 2-4, and 3-4 orders of magnitude less than numbers of fungi, ABOA, and aerobic heterotrophic bacteria, respectively. Thus, MRNHM counts appeared to be similar at controls and platforms during all three cruises.

Variability in numbers of MRNHM among the three cruises makes it difficult to comment on the effect of season on these microorganisms. However, if anaerobic nitrate-reducing, hydrocarbon-utilizing microorganisms were cultured in Rosenfeld's medium, and if it is valid to assume that DO decreased during Cruise II, then numbers of MRNHM possibly would have increased during Cruise II; this was not the case.

As discussed above, there are many factors that can influence numbers of MRNHM. Ranking of sites sampled during Cruise II by numbers of MRNHM did not appear to correlate with any of the factors described in Table 64, except for proximity to the Mississippi River delta (Fig. 32). Although this correlation is not supported by Duncan analysis (see Table 65) it is strongly indicated by a correlated t-test analysis. Numbers of MRNHM did not appear to be correlated with sediment pyrene content, TOC, TH, sediment inorganic nutrients, DO, salinity, or sediment type.

5. *Microorganisms from Rosenfeld's Sulfate-Hydrocarbon Medium (MRSHM)*

Numbers of MRSHM were obtained by inoculating MPN tubes of oil-containing, sulfate-containing medium (see Tables 3-5) with 1/10, 1/100, and 1/1000 dilutions of sediment and incubating the tubes at 20 C for three weeks. As was the case with MRNHM, no attempt was made to confirm hydrocarbon degradation, so this is merely a presumptive test for organisms with this capability. Again, it cannot be stated with certainty that the organisms could utilize hydrocarbons, since the crude oil employed in the medium contains non-hydrocarbon carbonaceous matter. It is certain; however, that sulfate reduction did occur since sulfides were found in the spent medium.

Counts of MRSHM at the control sites (see Table 22) ranged from 3-20 per ml sediment (Cruise I), 2-5 per ml sediment (Cruise II), and 5-35 per ml sediment (Cruise III). At primary platforms, most of the MRSHM counts (see Table 23) were similar within any given cruise, but different between cruises (as was previously observed for aerobic heterotrophic bacteria, ABOA, fungi, and MRNHM). During Cruises I, II, and III, MRSHM counts ranged from 12-48 per ml sediment, 3-4 per ml sediment, and 4-18 per ml sediment, respectively. The average number of MRSHM detected in sediments at secondary platforms was similar to those detected at control sites and primary platforms during Cruise II (see Table 24).

Overall, these counts suggest the same seasonal effect mentioned for other microbial types, but the cause of this trend cannot be determined using the data available due to a lack of correlation between numbers and sediment inorganic nutrients, DO, salinity, sediment type, TOC, TH, pyrene content, or any physical factors associated with the platforms. However, a Duncan multiple range analysis of the data from Cruise II (with the availability of 24 sites for comparison) shows an effect of proximity to the Mississippi River delta more clearly than it does for any other microbiological parameter (see Table 65 and Fig. 10).

B. Sample Analysis of Chemical Nutrients

The chemical parameters studied showed a high degree of variability between sites with few definite consistent trends (see Tables 25-31). Ammonia-nitrogen concentrations demonstrated the only clear-cut seasonal trend, with Cruise I values (160 $\mu\text{g-atoms per kg sediment}$ at the control sites and 190 $\mu\text{g-atoms per kg sediment}$ at the primary platforms) being definitely lower than those from Cruise II (1300 and 1100 $\mu\text{g-atoms per kg sediment}$, respectively) or Cruise III (1100 $\mu\text{g-atoms per kg sediment}$ at both platforms and controls). Conversely, total Kjeldahl nitrogen values were somewhat higher during Cruise I (30,000 and 26,000 $\mu\text{g-atoms per kg sediment}$ for control sites and primary platforms, respectively) than during the other two cruises (14,000 and 13,000 for Cruise II; 16,000 and 9300 for Cruise III). Nitrate-nitrogen concentrations showed the least variability of any chemical parameters. Nitrate-nitrogen values (in $\mu\text{g-atoms per kg sediment}$) for the control sites during Cruises I, II, and III were 540, 690, and 630, respectively. The corresponding values for the primary platforms were 420, 730, and 570. At no time were detectable levels of nitrite-nitrogen observed.

Ortho-phosphate-phosphorus values showed the only statistically significant differences between platforms and controls, but the results were contradictory between cruises, with platforms having more phosphate than controls for Cruise I (140 vs. 4.6 $\mu\text{g-atoms per kg sediment}$), and less phosphate than controls for Cruise II (62 vs. 250 $\mu\text{g-atoms per kg sediment}$). Cruise III data showed no significant differences for phosphate (66 vs. 90 $\mu\text{g-atoms per kg sediment}$ for primary platforms and control sites, respectively) or for any other chemical parameter. Total phosphorus values were somewhat (statistically insignificant) lower at control sites than at primary platforms for Cruise I (17,200 vs. 20,000 $\mu\text{g-atoms per kg sediment}$) and Cruise II (9300 vs. 12,000), but again, this trend did not hold true during Cruise III where both values were 13,000 $\mu\text{g-atoms per kg sediment}$. For all six chemical parameters studied, concentrations at secondary platforms were similar to values obtained at the primary platforms during Cruise II.

Brown, et al. (1980) reported concentrations of ammonia-nitrogen, total nitrogen, and phosphate-phosphorus in the northern Gulf of Mexico. Their values are similar to those obtained in this study.

It might be assumed that variations in chemical nutrients would affect numbers of microorganisms in the system. As shown by Duncan's multiple range analysis, ammonia-nitrogen concentrations did demonstrate a certain degree of east-west variation, with the highest concentrations being found in the area nearest the Mississippi River delta (Fig. 33). However, as has already

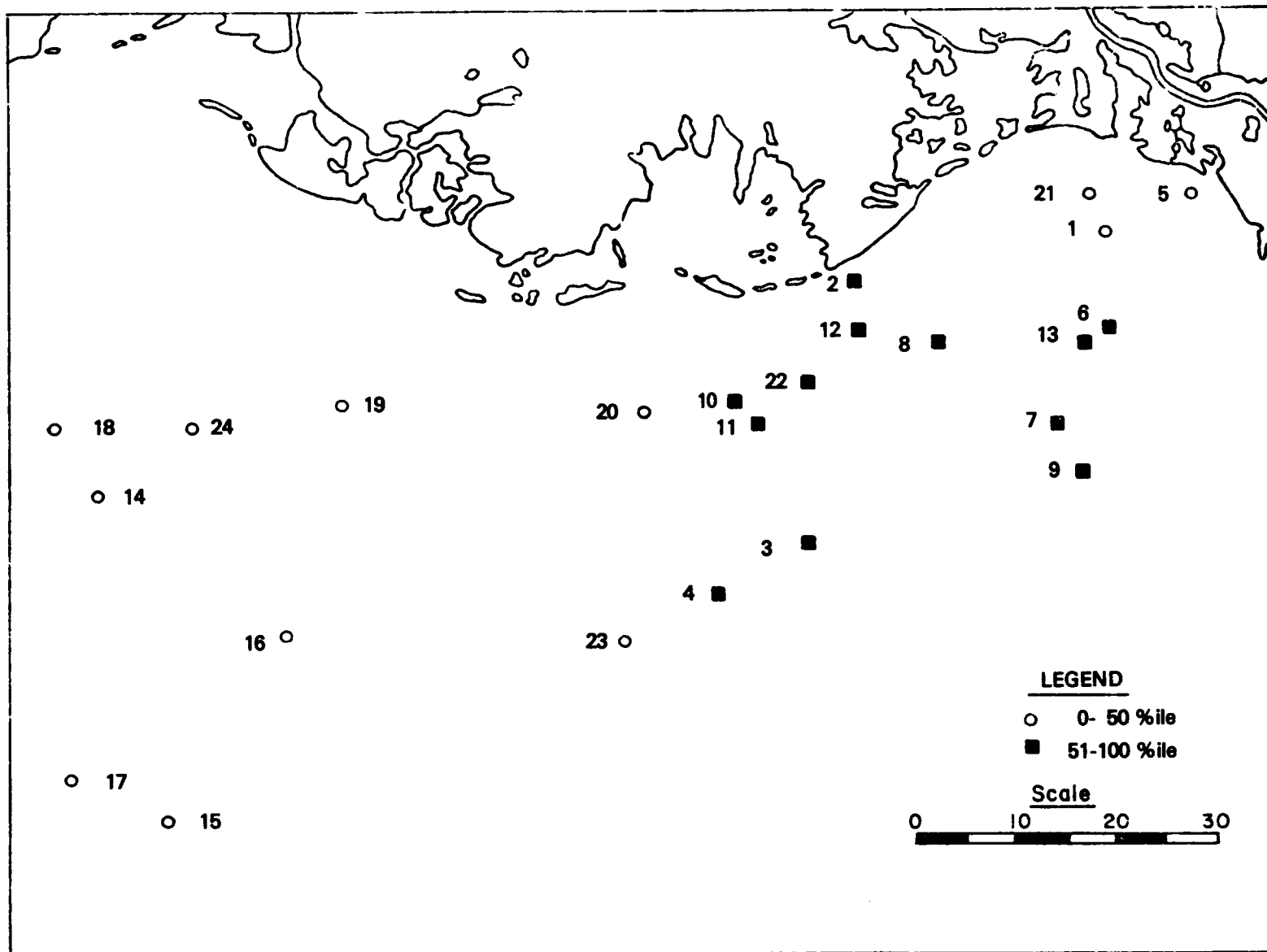


FIG. 32. Nitrate-reducing, hydrocarbon-utilizing bacteria.

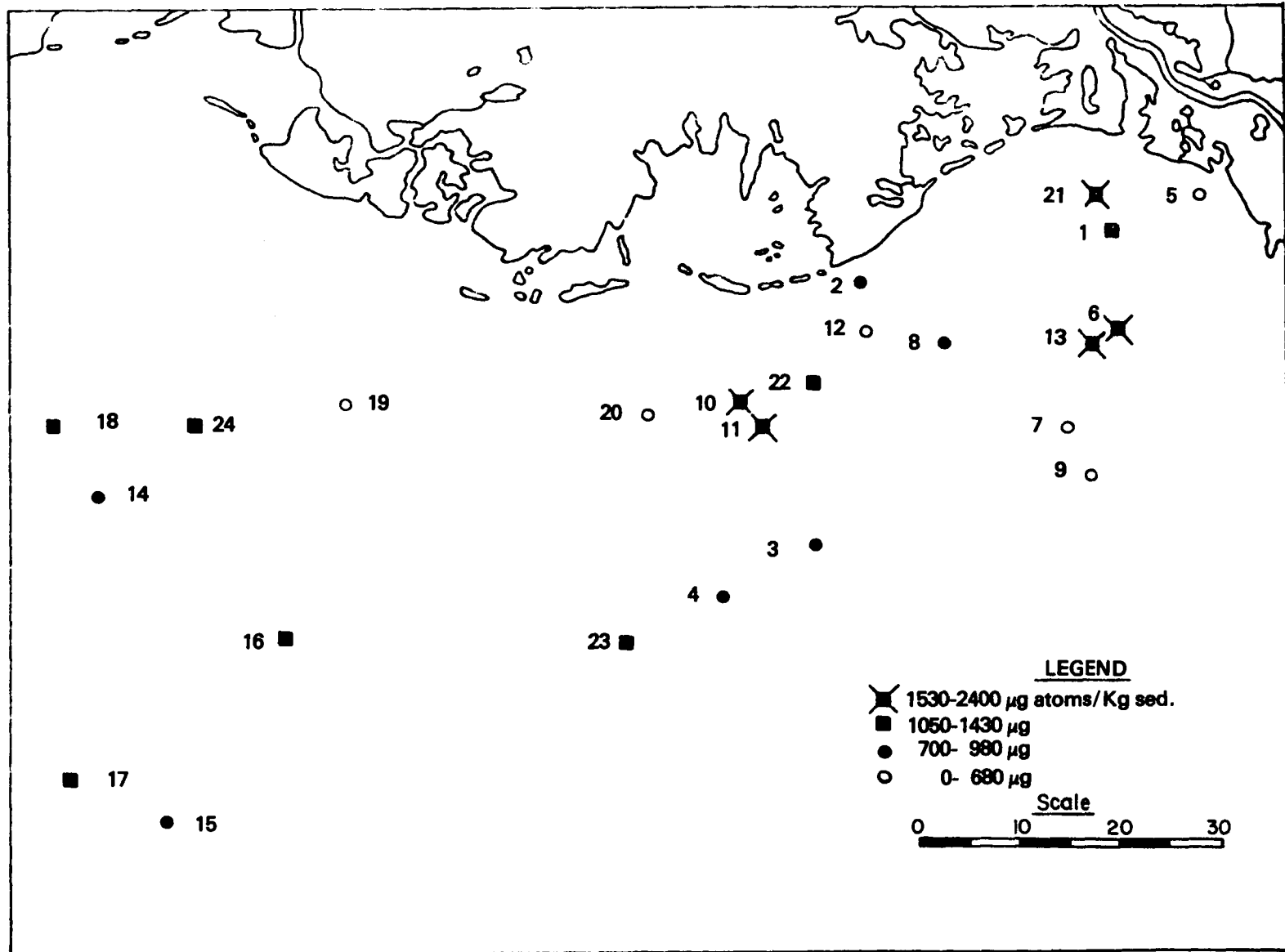


FIG. 33. Subsets of ammonia-nitrogen concentration based on Duncan's Multiple Range Test.

been discussed for each of the microbiological groups above, no direct chemical/microbial correlations were discernible for ammonia or any other chemical parameter. Although the concentrations of all these chemical nutrients might be considered somewhat low to support the microbial populations reported, it must be pointed out that in this area the nutrients are constantly replenished by the influx from rivers.

C. Oil Degradation

One of the data products required for this contract was an estimation of the oil-degrading potential of the sediments. In this connection it is interesting to note that Caparelo and Larock (1975) found that the hydrocarbon-oxidizing potential of environmental samples reflects the hydrocarbon burden of the area and the ability of the indigenous microorganisms to utilize hydrocarbons. Similarly, Walker and Colwell (1976a) reported greater rates of uptake and mineralization for samples collected from an oil-polluted area than from a relatively unpolluted area.

There are a number of ways in which the oil-degrading potential may be calculated. For example, loss of hydrocarbon from the system has been employed by Oppenheimer, et al. (1980) while Caparelo and Larock (1975) and Walker and Colwell (1976) used ^{14}C -radiolabeled hydrocarbons. Johnston (1970) estimated oil-degrading potential of sand columns containing Kuwait crude on the basis of oxygen consumption.

In the present study, the system designed to estimate the oil-degrading potential of the sediment yielded erratic results in terms of oil utilization. The fact that hydrocarbons were produced by microorganisms during the metabolism of the oil precluded determining the exact amount of oil degraded. Therefore, the method employed in this report for estimating the oil-degrading potential of the sediments was based on the amount of oxygen required to oxidize hydrocarbon-carbon to carbon dioxide. For these calculations a respiratory quotient of 0.67 was assumed on the basis of an oil having 70% saturated aliphatic alkanes (calculated as pentane) and 30% aromatics (calculated as benzene). For this investigation, the maximum rate of oxygen utilization occurred in sediments obtained from the control sites during Cruise II using a temperature of 15 C. In this case, the estimated oil-degrading potential of the sediments was 56 μg of hydrocarbon carbon converted to carbon dioxide per milliliter of sediment per day. Calculations for this estimate were as follows:

$$\begin{aligned} & (\text{ml O}_2/\text{ml sed.}/\text{day}) (\text{RQ}) (\text{at. wt. of C}) \\ & = \mu\text{g hydrocarbon carbon}/\text{ml sed.}/\text{day} \end{aligned}$$

Thus

$$(6.9) (0.67) (12) = 56 \mu\text{g hydrocarbon carbon}/\text{ml sed.}/\text{day}$$

It is generally conceded that the rate limiting factor in the degradation of the oil in the sediments is the oxygen supply. This certainly seems to be the case in the present investigation since the addition of phosphate and nitrogen (ammonium or nitrate ions) did not enhance oxygen utilization in the system, nor did the availability of an increased amount of oil.

If the oil-degrading potential were calculated on the basis of data obtained in the experiments conducted on the Warburg apparatus, much higher potentials would be generated - due in part to the increased availability of oxygen brought about by the shaking action of the system. It is believed; however, that the value of 56 μg hydrocarbon carbon/ml sed. per day is more realistic.

The pattern of hydrocarbon utilization by mixed and pure cultures conformed to the pattern shown by many previous workers (Wyman and Brown, 1975). For example, in the present study the straight-chain aliphatics were the first class of compounds attacked by the microflora, followed by the branched-chain aliphatics and aromatics. In the case of the aromatics, the two-ring compounds were degraded faster than the three-ring aromatics. A major portion of the carbonaceous material in oil was converted to carbon dioxide.

D. Magnitude of and Impact of Oil upon Microbial Processes

The actual impact of microorganisms in the environment is a function of their activity rather than their numbers. A major portion of this study, therefore, was devoted to the examination of twelve sediment microbial processes, and the impact of oil on these processes. As for the processes which did not exhibit demonstrable activity (sulfate reduction, phosphate uptake, nitrification, denitrification, nitrogen fixation, and photosynthesis), it must be stressed that experimental conditions could have been established such that metabolic potentials could be determined. However, the purpose of this project was to ascertain *in situ* reaction rates, and; therefore, natural environmental conditions were adhered to in so far as was possible.

Similarly, the studies concerning the impact of oil were designed to determine the effect of chronic (not massive) levels of oil pollution. The concentrations of oil used (20 and 200 μg oil/ml sediment) were deemed sufficient for this purpose, but the findings should not be extrapolated to imply similar results due to higher concentrations following a major oil spill.

1. Nitrification

None of the composite sediment samples demonstrated detectable nitrification activity (see Table 41). In order to determine if the lack of activity might be due to an effect of freezing the samples prior to analysis, four of the eight composites tested during Cruise III were fresh (never frozen). Again; however, no detectable activity was demonstrated. The cause of this lack of activity is; therefore, most probably the lack of a sufficient carbon source, although activity in the sediments would also be inhibited by the relatively low levels of dissolved oxygen since nitrification is a highly aerobic process.

Because the sediments exhibited no nitrification, there could be no inhibitory effect due to the presence of oil. However, although both a pure culture (*Nitrosomonas europaea*) and a terrestrial enrichment culture demonstrated perceptible nitrifying activity, neither of the oil-containing systems (40 μg or 400 μg of Empire mix crude oil) exhibited nitrification, which implies that oil either inhibits or masks the process under the experimental conditions used.

2. Nitrogen Fixation

None of the composite sediment samples demonstrated detectable nitrogen fixation (see Table 42). In

order to determine if the lack of activity might be due to an effect of freezing the samples prior to analysis, four of the eight composites tested during Cruise III were fresh (never frozen). Again; however, no detectable activity was demonstrated. The cause of this lack of activity is; therefore, most probably the lack of a sufficient carbon source. Because the sediments exhibited no nitrogen fixation, there could be no inhibitory effect due to the presence of oil.

3. Denitrification

None of the composite sediment samples demonstrated detectable denitrification activity (see Table 43). In order to determine if the lack of activity might be due to an effect of freezing the samples prior to analysis, four of the eight composites tested during Cruise III were fresh (never frozen). Again; however, no detectable activity was demonstrated. As was pointed out in the discussion of MRNHM, it is known that microorganisms capable of reducing nitrates are present in the sediment, using either components of the crude oil or the agar as a carbon source. The cause of the lack of activity in this study is; therefore, most probably the lack of a sufficient carbon source. Because the sediments exhibited no denitrification, there could be no inhibitory effect due to the presence of added oil.

4. Sulfate Reduction

Most of the composite sediment samples demonstrated no detectable sulfate reduction. In order to determine if the lack of activity might be due to an effect of freezing the samples prior to analysis, four of the eight composites tested during Cruise III were fresh (never frozen). Again; however, no detectable activity was demonstrated. As was mentioned in the discussion of MRSHM above, it is known that microorganisms capable of reducing sulfates are present in the sediment, using either components of the crude oil or the agar as a carbon source. The lack of activity in this study is; therefore, most probably due to the lack of a sufficient carbon source. Due to the overall lack of activity, there could be no inhibitory effect of oil. However, some of the composites containing oil (replicate 2 of Control Site C22 from Cruise I containing 400 μg oil and both replicates from Primary Platform P2 from Cruise II with both 40 and 400 μg oil) demonstrated some degree of sulfate reduction. Since all samples received identical treatment, this anomaly could be explained either by chance distribution of a small number of sulfate-reducing bacteria, or by the utilization of one or more compounds in the oil as a carbon source. The exact cause cannot be ascertained with certainty, but it should be noted that the two sites in question (C22 for Cruise I and P2 for Cruise II) did not show especially high numbers of MRSHM as discussed above.

5. Sulfur Oxidation

Unlike the previously mentioned metabolic processes, sulfur oxidation activity was definitely demonstrated in all composites tested, with an average of 844 μg of sulfur oxidized per ml of sediment per day (see Table 45 and Fig. 20). This was to be expected due to high concentrations of sulfur found in the sediment (Huang, 1981), and rates would not be inhibited by the lack of a carbon source (other than CO_2) since sulfur oxidation is an autotrophic process. There was no

observable impact of oil (20 μg or 200 μg oil per ml sediment). However, while site C22 demonstrated the least activity of the eight sites, sites C21 and C23 were the most active, contributing to the observation (statistically significant but probably biologically insignificant) that control sites were somewhat more active than were platforms.

In order to determine if the rates of activity might have been affected by the freezing of the samples prior to analysis, four of the eight composites tested during Cruise III were fresh (never frozen). Similar results were obtained from both sets of samples (fresh and frozen), yielding the conclusion that freezing of the sediments for a period of seven days did not affect the magnitude or occurrence of sulfur oxidation.

6. Photosynthesis

None of the 48 composite samples demonstrated detectable photosynthetic activity (see Table 46), most probably due to a lack of algae brought about by an insufficient quantity of light reaching the sediments. Because of this total lack of activity, there could be no inhibitory effect due to the presence of oil. Likewise, there was no variation between sites or cruises. It should be noted again that all photosynthesis experiments were carried out on-board ship immediately after sample retrieval, and; therefore, the samples were never frozen.

7. Heterotrophic Activity

Most of the 48 composite samples demonstrated rapid heterotrophic activity (defined as the conversion of ^{14}C -glucose to $^{14}\text{CO}_2$) as was seen in Tables 47-49. Heterotrophism showed the highest rate of activity (an average of 556 μg CO_2 per day, with values as high as 1345 $\mu\text{g}/\text{day}$) although the samples reached apparent stationary phase rather quickly (ca. 5-6 days with 14-30% of the glucose used) probably due to low useable nitrogen concentrations. Glucose utilization showed little variability among sites, with platform activities not significantly different from those of control sites. Cruise III activity rates were much higher than those of the other two cruises, showing a lack of correlation with mere numbers of heterotrophic organisms which were largest for Cruise I. The presence of oil did not significantly affect activity rates by either the composites or by the pure culture (*Enterobacter* sp.) isolated from Primary Platform P1 during Cruise II (Fig. 21).

8. Phosphorus Uptake

None of the composite sediment samples demonstrated detectable phosphorus uptake (see Table 50). In order to determine if the lack of activity might be due to an effect of freezing the samples prior to analysis, four of the eight composites tested during Cruise III were fresh (never frozen). Again; however, no detectable activity was demonstrated. The cause of this lack of activity is; therefore, most probably the lack of a sufficient carbon source. Because the sediments exhibited no phosphorus uptake, there could be no inhibitory effect due to the presence of added oil.

9. Lipolysis

Most of the 48 composite samples demonstrated low-level but statistically significant rates of lipolytic activity, with an average of 93 μg of tripalmitin being converted to CO_2 per ml of sediment per day, and a

maximum of 269 $\mu\text{g}/\text{day}$ (see Tables 51-53). Platform P1 and Control Site C21 demonstrated the highest rates of activity, while Platform P2 showed the lowest. There was no detectable difference among cruises, but lipolysis was the only one of the carbonaceous processes in which platform sites as a whole showed less activity than did control sites. The addition of oil to the test systems had no significant effect on the rate of lipolysis. Similar results were obtained with both of the pure cultures (*Pseudomonas* sp. 5 and 7) isolated during Cruise II (Fig. 30).

10. Chitinolysis

Chitinolysis was the least consistent of the processes studied, with much variation among sites and among cruises, as was shown in Table 54-56. An average of 82 μg of chitin was converted to CO_2 per ml of sediment per day, with a maximum observed rate of 414 $\mu\text{g}/\text{day}$. Chitinolysis was the only one of the carbonaceous processes which demonstrated an impact of oil, with depressed activity as a result of exposure to oil observed during Cruise I. However, there was no significant impact of oil detected during the other two cruises, and oil did not affect chitinolysis by either of the two pure cultures (*Pseudomonas* sp. 6 and 8) isolated during Cruise II (see Fig. 31).

11. Cellulolysis

Most of the 48 composite samples demonstrated low-level but statistically significant rates of cellulolytic activity, with an average of 55 μg of cellulose being converted to CO per ml of sediment per day (with a maximum of 172 $\mu\text{g}/\text{day}$) as was shown in Tables 57-59. There were no significant differences between platform and control sites or among cruises, but Control Site C24 consistently showed the lowest level of activity. Although all rates were low, there was no indication that oil had any effect on cellulolysis by the composites or by either of the two pure cultures (*Cellulomonas* sp. 1 and 2) isolated during Cruise II (see Fig. 29).

12. Proteolysis

All of the 48 composite samples demonstrated definite proteolytic activity (see Tables 60-62), averaging 320 μg protein per ml sediment per day. Although this rate is lower than that for glucose utilization, the proteolytic cultures did not appear to reach stationary phase until virtually all (>90%) of the protein had been degraded to CO_2 . This is most probably due to the fact that the protein also served as a source of nitrogen, which was the probable rate-limiting nutrient for heterotrophic activity. Platform Site P1 was consistently the most active site, but in general there was no significant difference between platforms and controls or among cruises. The addition of oil had no effect on the amount of CO_2 produced from protein by the composite samples or by either of the two pure cultures

(*Pseudomonas* sp. 3 and 4) isolated during Cruise II (see Fig. 27).

E. Recommendations for Further Studies

We recommend a continuation of the existing program for a 2-year period with the following modifications:

1. Redesign studies to take river flow into account. This should include some secondary platforms wherein background data are in hand. Sample replication can be increased and stations can be expanded by eliminating directional stations at each site.
2. Study vertical profile of sediments and near bottom water. Since surficial sediments are in a constant state of flux, deeper cores might show correlations between microbial populations, hydrocarbon profile, and other parameters such as trace elements. Obviously more meaningful comparisons of data may be made and might reflect on the history of the area.
3. Analyze samples for total bacterial numbers, sulfate-reducing hydrocarbonoclasts and nitrate-reducing hydrocarbonoclasts and eliminate microbial processes and nutrient chemistry. Most of the processes do not occur at levels which would yield statistical or biological significance, and we have obtained sufficient data on the ones that do occur.
4. Expand program to include evaluation of fish, sediments, and near-bottom water samples for enumeration of *Vibrio* species. Existing literature has suggested that indirect effects of crude oil may make *Vibrio* diseases more prevalent (Giles et al., 1978). Fin rot has been observed in fish around the oil platforms and this study might yield information on chronic effects of low levels of oil in the area.
5. Evaluate microbial populations in terms of changes in a variety of types based on carbon utilization as monitored by replicate plate techniques.
6. Analyze sediments for ability to support growth via bioassay techniques. Sediments deficient in nutrients and/or toxic material could be detected by introducing a known amount of a specific microbial species in the particular sediment and monitoring the decrease or increase in total numbers. This study might indicate ways of accelerating treatment of oil spills.

V. CONCLUSIONS

At times there were statistically significant differences between the microbial population of the sediment at platform sites and control sites, while at other times no differences were found. Essentially no difference was found between the chemical nutrients at the control sites as compared to the platform sites. However since most of the microbial activity takes place in the upper portion of the sediments, it is easily influenced by currents, storms, etc. Therefore, while microbial differences in the surficial sediments may be brought about by man's activity, nature redistributes the sediments to such an extent that meaningful cause and effect relationships are obscured. The impact of river flow and currents were clearly indicated in the results obtained from Cruise II in which statistically significant differences in microbial populations were correlated with geographic location. These considerations lead to the conclusion that nature's activity masked man's activity. Since only the top one to two centimeters of sediment were analyzed in this

investigation, the impacts of river flow and currents were magnified.

Experiments to determine the magnitude of, and the impact of oil on, microbial processes in the sediments were designed to reflect conditions closely approximating the natural environment. No evidence was obtained to indicate that any of the reasonably active microbial processes were adversely affected by low levels of oil.

There was essentially no difference between the oil-degrading potential of the sediments from the control sites as compared to the platforms. While the laboratory data indicated a maximum oil-degrading potential of 56 μg hydrocarbon-carbon oxidized to carbon dioxide per ml of sediment per twenty-four hours, the actual rate *in situ* might be less at certain times due to lack of adequate oxygen.

It was concluded that freezing sediment samples immediately after collection did not adversely affect the microbial enumeration results, provided that the frozen samples were analyzed within seven days.

VI. ACKNOWLEDGEMENTS

We express our appreciation to Dr. Tom Kellogg, Director of the Mississippi State University Radioisotope Center, for his technical assistance with radioisotopic procedures; to Dr. Ray Heiser of The Mississippi State University Department of Statistical Services for his assistance in the statistical analysis of the data; to Ms. Mary Jane Coign for the chemical analysis of oil in various test systems; to Ms. Susan Brown, Ms. Annette Benton, Ms. Susan Benton, Ms. Mary Randolph, Ms. Ann Ardahl, Ms. Page Coggin, Dr. Hazel Aranha, Dr. Doug Minchew, Mr. Geoff Frank, Mr. Kenneth Swan, Mr. Kenny Jeanes, Mr. Bill Greenleaf, Mr. Melford

Smith, Mr. John Burczak, Mr. Tom Saunders, and Mr. Carlos Dobbel for their aid in the microbial enumerations, sediment chemistry, and microbial processes; to Mr. E.E. Bailey and Mr. Ken Parish of the Mississippi State Chemical Laboratory for analyzing Kjeldahl nitrogen and total phosphorus in the sediments; to Ms. Jane Benyo for typing this manuscript; and finally Mr. Wayne Reed and the Purchasing Department of Mississippi State University need special acknowledgement for the expedient and professional manner in which they handled some extraordinary demands placed on their department.

VII. LITERATURE CITED

- Ahearn, D.G., and S.P. Meyers. 1972. The role of fungi in the decomposition of hydrocarbons in the marine environment, pp. 12-18. *In* Biodeterioration of materials. Vol. 3. Applied Science Publishers, London, England.
- Ahearn, D.G., and S.P. Meyers [ed.] 1973. The microbial degradation of oil pollutants. Center for Wetland Resources. LSU Publ. No. LSU-SG-73-101. 322 p.
- Ahearn, D.G., S.P. Meyers, and P.G. Standard. 1971. The role of yeasts in the decomposition of oils in marine environments. *Ind. Microbiol.* 12:126-134.
- American Public Health Association. 1975. Standard methods for the examination of water and wastewater. 14th edition. American Public Health Association, New York.
- Anonymous. 1969. Conference on prevention and control of oil spills. American Petroleum Institute and Federal Water Pollution Control Administration. American Petroleum Institute, Washington, D.C.
- Anonymous. 1971. Conference on prevention and control of oil spills. Environmental Protection Agency and U.S. Coast Guard. American Petroleum Institute, Washington, D.C.
- Anonymous. 1973. Conference on prevention and control of oil pollution. American Petroleum Institute, Environmental Protection Agency and U.S. Coast Guard. American Petroleum Institute, Washington, D.C.
- Anonymous. 1974. Research needed to determine chronic effects of oil on the marine environment-workshop proceedings. Exxon, Houston, Texas. 43 p.
- Anonymous. 1975. Conference on prevention and control of oil pollution. American Petroleum Institute, Environmental Protection Agency and U.S. Coast Guard. American Petroleum Institute, Washington, D.C.
- Anonymous. 1976. Sources, effects and sinks of hydrocarbons in the aquatic environment. American Institute of Biological Sciences, Washington, D.C. 578 p.
- Anonymous. 1977. Conference on prevention and control of oil pollution. American Petroleum Institute, Environmental Protection Agency and U.S. Coast Guard. American Petroleum Institute, Washington, D.C.
- Anonymous. 1979. Conference on prevention and control of oil pollution. American Petroleum Institute, Environmental Protection Agency and U.S. Coast Guard. American Petroleum Institute, Washington, D.C.
- Anonymous. 1981. Conference on prevention and control of oil pollution. American Petroleum Institute, Environmental Protection Agency and U.S. Coast Guard. American Petroleum Institute, Washington, D.C.
- Anthony, E.H. 1963. Regional variability of bacteria in North Atlantic sediments, pp. 532-552. *In* C.H. Oppenhiemer [ed.] Symposium on marine microbiology. Charles C. Thomas, Springfield, Mass.
- Atlas, R.M. 1981. Microbial degradation of petroleum hydrocarbons: an environmental prospective. *Microbiol. Rev.* 45:180-209.
- Atlas, R.M., and R. Bartha. 1973. Fate and effects of polluting petroleum in the marine environment. *Residue Rev.* 49:49-85.
- Blumer, M., P. Blokker, E.B. Cowell, and D.F. Duckworth. 1972. Petroleum, p. 19-. *In* E.G. Goldberg [ed.] A guide to marine pollution. Gordon and Breach, New York.
- Blumer, M., and J. Sass. 1972a. Oil pollution: persistence and degradation of spilled fuel oil. *Science* 176:1120-1122.
- Blumer, M., and J. Sass. 1972b. The West Falmouth oil spill II. Chemistry. Woods Hole Oceanographic Institution, Woods Hole, Mass. 90 p.
- Boesch, D.F., C.H. Hershner, and J.H. Milgram. 1974. Oil spills and the marine environment. Ballinger Publishing Co., Cambridge, Mass. 114 p.
- Bourne, W.R.P. 1968. Effects of oil pollution on bird populations, p. 99-128. *In* J.D. Carthy and D.R. Arthur [ed.] The biological effects of oil pollution on littoral communities, Field Studies Council, London.
- Brown, L.R. 1980. Fate and effect of oil in the aquatic environment Gulf Coast Region. Final Rep. on Contract No. 68-01-0745. U.S. Environmental Protection Agency. 101 p.
- Brown, L.R., M. Light, and C.D. Minchew. 1980. Geographic distribution of petroleum hydrocarbons in surficial bottom sediments of the Gulf of Mexico, pp. 181-205. *In* R.A. Geyer [ed.] Marine environmental pollution, 1: Hydrocarbons. Elsevier Scientific Publishing Co., Amsterdam, The Netherlands.
- Brown, L.R., and C.D. Minchew. 1978. The analysis of sediment samples for hydrocarbons. Final Rep. for Contract No. DOT-CG-81-76-1476. October 30, 1978. (A Scientific Study to Develop One or More Practical Methods for the Accurate Assessment of an Oil Spill Cleanup).
- Brown, L.R., W.E. Phillips, G.S. Pabst, and C.M. Ladner. 1969. Physical, chemical, and microbiological changes occurring during degradation of oil in aquatic and brackish water environments. *In* Proc. of ASME Winter Annual Meeting. Los Angeles, California.
- Buck, J.D., and R.C. Cleverdon. 1960. The spread plate as a method for the enumeration of marine bacteria. *Limnol. Oceanogr.* 5:78-80.
- Caparello, D.M., and P.A. Larock. 1975. A radioisotope assay for the quantification of hydrocarbon biodegradation potential in environmental samples. *Microbiol. Ecol.* 2:28-42.
- Carthy, J.D., and D.R. Arthur. 1968. The biological effects of oil pollution on littoral communities. Field Studies Council, Institute of Petroleum, London.

- Coler, R.A., and H.B. Gunner. 1969. Microbial populations as determinants in protozoan succession. *Water Res.* 3:149-156.
- Colwell, R.R., and J.D. Walker. 1977. Ecological aspects of microbial degradation of petroleum in the marine environment. *CRC Critical Rev. Microbiol.* 4:423-445.
- Colwell, R.R., and J.D. Walker, and J.D. Nelson, Jr. 1973. Microbial ecology and the problem of petroleum degradation in Chesapeake Bay, pp. 185-197. *In* D.G. Ahearn and S.P. Meyers [ed.] *The microbial degradation of oil pollutants*. Center for Wetland Resources. LSU Publ. No. LSU-SG-73-01.
- Colwell, R.R., J.D. Walker, B.F. Conrad, and P.A. Seesman. 1976. Microbiological studies of Atlantic Ocean water and sediment from potential off-shore drilling sites. *Develop. Ind. Microbiol.* 17:269-282.
- Conrad, B.F., J.D. Walker, and R.R. Colwell. 1976. Utilization of crude oil and mixed hydrocarbon substrate by Atlantic Ocean microorganisms. *Dev. Ind. Microbiol.* 17:283-292.
- Cowell, E.B. [ed.] 1971. *The ecological effects of oil pollution on littoral communities*. Applied Science Publishers, Ltd., Essex, England. 248 p.
- Crow, S.A., S.P. Meyers, and D.G. Ahearn. 1974. Microbiological aspects of petroleum degradation in the aquatic environment. *La Mer* 12:37.
- Davis, J.B. 1967. *Petroleum microbiology*. Elsevier, New York.
- D'Emidio, J.A. 1972. Moderator, Proc. Navy Oil Spill Conf. Naval Ordnance Laboratory, White Oak, Maryland. 273 p.
- Dubos, R. 1928. The decomposition of cellulose by aerobic bacteria. *J. Bacteriol.* 15:223-234.
- Freide, J., P. Guire, R.K. Gholson, E. Gandy, and A.F. Gandy, Jr. 1972. Assessment of biodegradation potential for controlling oil spills on the high seas. Rep. No. 4110.1#3.1, U.S. Coast Guard Office of Research and Development, Washington, D.C. 130 p.
- Giles, R.C., L.R. Brown, and C.D. Minchew. 1978. Bacteriological aspects of fin erosion in mullet exposed to crude oil. *J. Fish. Biol.* 13:113-117.
- Hada, H.S., and R.K. Sizemore. 1981. Incidence of plasmids in marine *Vibrio* spp. isolated from and oil field in the northwestern Gulf of Mexico. *Appl. Environ. Microbiol.* 41:199-202.
- Hepple, P. [ed.] 1971. *Water pollution by oil*. Applied Science Publishers, London. 399 p.
- Holmes, R.W., and F.A. Dewitt, Jr. 1970. *Santa Barbara Oil Symposium*. University of California, Santa Barbara.
- Hoult, D.P. 1969. Oil on the sea. Proc. Symposium on the scientific and engineering aspects of oil pollution of the sea. Plenum Press, New York. 114 p.
- Huang, W.H. 1981. Ecological investigations of petroleum production platforms in the central Gulf of Mexico. Vol. I: Pollutant fate and effects studies. Part 2 1/2 Sediment physical characterization. Final Rep. Bureau of Land Management. Southwest Research Institute, Houston, Texas.
- Johnston, R. 1970. The decomposition of crude oil residues in sand columns. *J. Mar. Biol. Ass. U.K.* 50:925-937.
- Marx, W. 1971. *Oilspill*. Sierra Club, San Francisco, California.
- Miles, D.H., M.J. Coign, and L.R. Brown. 1977. A liquid chromatographic fluorescence technique for estimating crude oil in water, sediment, and biological material. *In* Proceedings 1977 Oil Spills Conference, March 8-10, 1977, New Orleans, LA.
- Moulder, D.S., and A. Varley. 1971. A bibliography on marine and estuarine oil pollution. *Marine Biology Assoc., U.K.* 129 p.
- Moulder, D.S., and A. Varley, 1975. A bibliography on marine and estuarine oil pollution, supplement. *Marine Biology Assoc., U.K., N.Y.* 152 p.
- Murisawa, M. 1968. *Streams, their dynamics and morphology*. McGraw-Hill Book Company, N.Y.
- National Academy of Sciences. 1975. Petroleum in the marine environment. Proc. of workshop on inputs, fates and the effects of petroleum in the marine environment, May 21-25, 1973. Ocean Affairs Board, National Research Council. 107 p.
- Nelson-Smith, A. 1973. *Oil pollution and marine ecology*. Plenum Press, New York, N.Y. 260 p.
- OCS Environmental Assessment Program. 1979. Environmental assessment of the Alaskan continental shelf V. Receptors 1/2 Microbiology, contaminant baseline. NOAA and BLM, Washington, D.C. 698 p.
- Olsen, K.D., and R.K. Sizemore. 1981. Effects of an established offshore oil platform on the autochthonous bacterial community. *Develop. Ind. Microbiol.* In press.
- Oppenheimer, C.H., R. Miget, and H. Kator. 1974. Hydrocarbons in seawater and organisms and microbiological investigations. *Gulf Univ. Res. Consortium, Offshore Ecol. Invest., Project No. OE73HJM*.
- Oppenheimer, C.H., S. Siegel, L. Day, and C. Duncan. 1980. Distribution of hydrocarbon-oxidizing bacteria on the Georgia Shelf area and oil-degrading activities, pp. 265-290. *In* R.A. Geyer [ed.] *Marine environmental pollution, 1: Hydrocarbons*. Elsevier Scientific Publishing Co., Amsterdam, the Netherlands.
- Oujesky, H., W. Brooks, B. Smith, and B. Hamilton. 1979. Water column bacteriological studies of the South Texas Outer Continental Shelf. *Develop. Ind. Microbiol.* 20:695-703.
- Samson, A.L., J.H. Vandermuelen, P.G. Wells, and C. Moyse. 1980. A selected bibliography on the fate and effects of oil pollution relevant to the Canadian marine environment. Report No. EPS3-EC-80-5, Environment Canada, Ottawa. 191 p.
- Schwartz, J.R., S.K. Alexander, C.S. Giam, and H.S. Chan. 1980. Analysis of benthic bacteria, pp. 438-468. *In* R.W. Flint and N.N. Rabalais [ed.] *Environmental studies, South Texas Outer Continental Shelf, 1975-1977. Vol. III*.

- Contract No. AA551-CT8-51, BLM, Department of Interior, Washington, D.C.
- Seesman, P.A., J.D. Walker, and R.R. Colwell. 1976. Biodegradation of oil by marine microorganisms at potential off-shore drilling sites. *Dev. Ind. Microbiol.* 17:293-298.
- Sharpley, J.M. 1966. *Elementary petroleum microbiology*. Gulf Publishing, Houston, Texas.
- Simidu, U. 1974. Improvement of media for enumeration and isolation of heterotrophic bacteria in seawater, pp. 249-257. *In* R.R. Colwell and R.Y. Morita [ed.] *Effect of ocean environment on microbial activities*. University Park Press, Baltimore MD.
- Sizemore, R.K., and K. Olsen. 1980. Bacterial communities. Vol. IV. *In* W.B. Jackson and E.P. Wilkins [ed.] *Environmental assessment of Buccaneer gas and oil field in the Northwestern Gulf of Mexico, 1978-1979*. Nat. Mar. Fish. Serv., Southeast Fish. Center.
- Skerman, V.B.D. 1967. A guide to the identification of the genera of bacteria. The Williams and Wilkins Company, Baltimore, MD.
- Stewart, J.E., and L.J. Marks. 1978. Distribution and abundance of hydrocarbon-utilizing bacteria in sediments of Chedabutto Bay, Nova Scotia, in 1976. *J. Fish. Res. Board Can.* 35:581-584.
- Templeton, W.L., E.A. Sutton, R.M. Bean, R.C. Arnett, J.W. Blaylock, and R.E. Wildung, 1975. Oil pollution studies on Lake Maracaibo, Venezuela, pp. 489-496. *In* Conference on Prevention and Control of Oil Pollution. American Petroleum Institute.
- Texas Instruments. 1979. South Atlantic OCS Benchmark Program. Vol. 3. BLM, Washington, D.C. 474 p.
- University of Texas Marine Science Institute. 1979. Environmental studies South Texas OCS. Bureau of Land Management, Washington, D.C.
- Walker, J.D., and R.R. Colwell. 1975. Factors affecting enumeration and isolation of actinomycetes from Chesapeake Bay and Southeastern Atlantic ocean sediments. *Mar. Biol.* 30:193-201.
- Walker, J.D., and R.R. Colwell. 1976a. Petroleum: Degradation by estuarine microorganisms, pp. 197-204. *In* *Biodeterioration of materials*. Vol. 3. Applied Science Publishers, London, England.
- Walker, J.D., and R.R. Colwell. 1976b. Enumeration of petroleum-degrading microorganisms. *Appl. Environ. Microbiol.* 31:198-207.
- Walker, J.D., J.J. Calomiri, T.L. Herbert, and R.R. Colwell. 1976. Petroleum hydrocarbons: Degradation and growth potential for Atlantic Ocean bacteria. *Mar. Biol.* 34:1-9.
- Walker, J.D., and R.R. Colwell. 1974. Microbial degradation of model petroleum at low temperatures. *Microbial Ecol.* 1:63-95.
- Walker, J.D., P.A. Seesman, T.L. Herbert, and R.R. Colwell. 1976. Petroleum hydrocarbons: degradation and growth potential of deep-sea sediment bacteria. *Environ. Pollut.* 10:89-99.
- Walker, J.D., B.F. Conrad, P.A. Seesman, and R.R. Colwell. 1977. Comparison of membrane filter counts and plate counts on heterotrophic and oil agar used to estimate populations of yeast, fungi and bacteria. *In* R.H. Bordner, C.F. Frith, and J.A. Winter [ed.] *Symposium on recovery of indicator organisms employing membrane filters*. U.S. E.P.A., Cincinnati, Ohio.
- Walton, S. 1981. Troubled waters: Academy looks again at petroleum in the marine environment. *BioScience* 31:93-96.
- Ward, C.H., M.E. Bender, and D.J. Reish [ed.] 1979. The offshore ecology investigation. Effects of oil drilling and production in a coastal environment. *Rice Univ. Stud.* 65(4½5):1-589.
- Wyman, J.F., and L.R. Brown. 1975. Microbial degradation of Empire Mix crude oil. *Dev. Ind. Microbiol.* 17:321-320.
- Zafiriou, O., M. Blumer, and J. Myers. 1972. Correlation of oils and oil products by gas chromatography. Report No. 72-55. Woods Hole Oceanographic Institution, Woods Hole, Mass. 110 p.
- ZoBell, C.E. 1941. Studies on marine bacteria. 1. The cultural requirements of heterotrophic aerobes. *J. Mar. Res.* 4:42-75.
- ZoBell, C.E. 1954. Marine bacteria and fungi in the Gulf of Mexico. *Fish. Bull. Fish and Wild. Serv.* 55:217-222.
- ZoBell, C.E. 1969. Microbial modification of crude oil in the sea, pp. 317-326. *In* Joint Conf. on Prevention and Control of Oil Spills. American Petroleum Institute, Washington, D.C.

VIII. PUBLICATIONS RESULTING FROM THIS STUDY

A. Published

1. Ardahl, A. 1979. Studies on the biological oxidation of ammonium to nitrite. Masters Thesis. Mississippi State University.
2. Brown, L.R., K.L. Jeanes, W.F. Greenleaf, and G. Frank. Oil-degrading potential of surficial sediments near oil-producing platforms, pp. 725-730. *In Proc. 1981 Oil Spill Conference*, Atlanta, Ga. EPA, API, USCG.
3. Childers, G.W., R.W. Landers, H.G. Aranha, A.H. Ardahl, M.S. Randolph, and L.R. Brown. Effect of crude oil on microbial pro-

cesses in marine sediments, pp. 731-735. *Proc. 1981 Oil Spill Conference*, Atlanta, Ga. EPA, API, USCG.

B. In Preparation

1. Effect of freezing on total bacterial numbers in marine sediments.
2. Comparisons of the microbial ecology and sediment chemistry found at selected petroleum platform sites and non-petroleum-producing areas in the north-central Gulf of Mexico.



The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.



The Minerals Management Service Mission

As a bureau of the Department of the Interior, the Minerals Management Service's (MMS) primary responsibilities are to manage the mineral resources located on the Nation's Outer Continental Shelf (OCS), collect revenue from the Federal OCS and onshore Federal and Indian lands, and distribute those revenues.

Moreover, in working to meet its responsibilities, the **Offshore Minerals Management Program** administers the OCS competitive leasing program and oversees the safe and environmentally sound exploration and production of our Nation's offshore natural gas, oil and other mineral resources. The MMS **Minerals Revenue Management** meets its responsibilities by ensuring the efficient, timely and accurate collection and disbursement of revenue from mineral leasing and production due to Indian tribes and allottees, States and the U.S. Treasury.

The MMS strives to fulfill its responsibilities through the general guiding principles of: (1) being responsive to the public's concerns and interests by maintaining a dialogue with all potentially affected parties and (2) carrying out its programs with an emphasis on working to enhance the quality of life for all Americans by lending MMS assistance and expertise to economic development and environmental protection.