Research Report on

STUDY OF EFFECTS OF OIL DISCHARGES AND DOMESTIC AND INDUSTRIAL WASTEWATERS ON THE FISHERIES OF LAKE MARACAIBO, VENEZUELA

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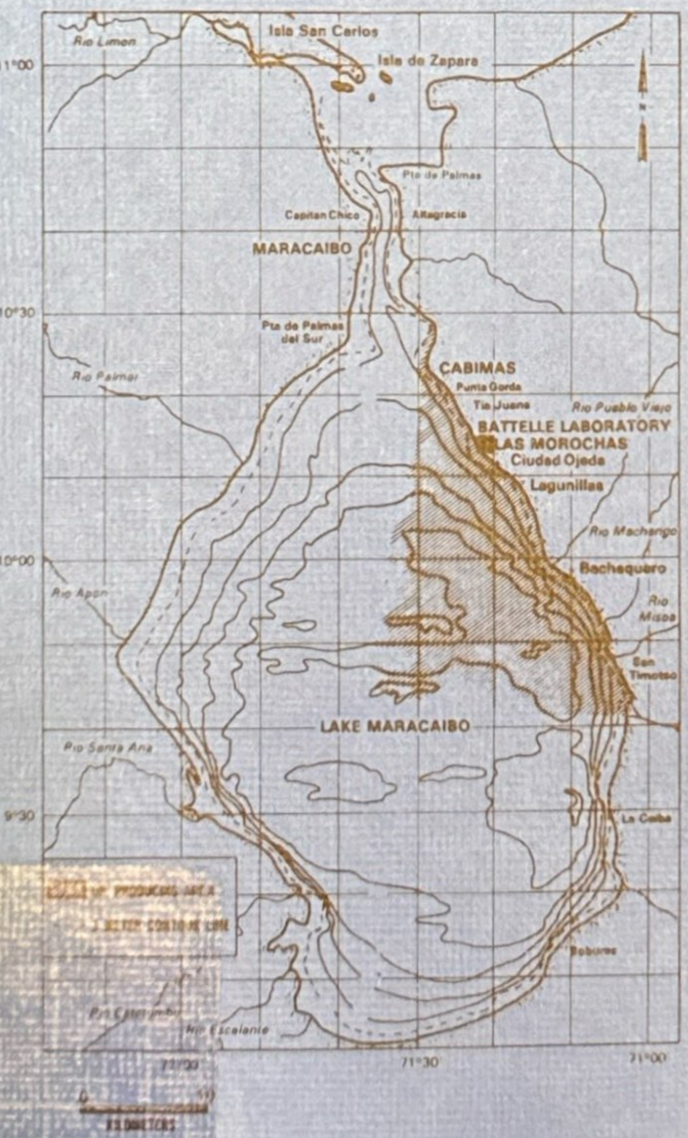
VOLUME I Ecological Characterization and Domestic and Industrial Wastes

Volume II Fate and Effects of Oil

To Creole Petroleum Corporation
Caracas, Venezuela

October 1974



Battelle

Pacific Northwest Laboratories
Richland, Washington 99352

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RICHLAND, WASHINGTON 99352 U.S.A.

PREFACE

This report, published in two volumes, presents the findings and conclusions of a "Study of Effects of Oil Discharges, Domestic and Industrial Wastewaters on the Fisheries of Lake Maracaibo, Venezuela", conducted by Battelle Memorial Institute, Pacific Northwest Laboratories, Richland, Washington 99352, U.S.A., for Creole Petroleum Corporation, Caracas, Venezuela, under Research Contract 212B00899.

Volume I contains the data on the ecological characterization of the lake and of domestic and industrial wastewaters. Volume II contains the data on the fate and effects of oil.

Creole Steering Committee

The program was conducted with the advice and guidance of the Creole Petroleum Corporation's steering committee, under the chairmanship of Mr. H. J. Moore, Creole Petroleum Corporation. Members of the committee were:

Mr. W. P. Redmond	Creole Petroleum Corporation
Mr. J. P. Gracey	Creole Petroleum Corporation
Mr. W. M. Cooper	Exxon Research and Engineering Company
Mr. G. P. Canevari	Exxon Research and Engineering Company
Dr. P. H. Monaghan	Exxon Production Research Company

Research Staff

The project was under the direction of Mr. William L. Templeton, Associate Manager of Ecosystems Department, Battelle Memorial Institute, Pacific Northwest Laboratories. The following members of Battelle-Northwest staff and Oficina Nacional de Pesca, Ministerio de Agricultura y Cria (MAC), were the principal research contributors to the studies reported.

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Mr. Paul Fujihara	Specialist, Cellular and Molecular Biology
Mr. Ronald Arnett	Research Engineer, Water and Land Resources

We wish to thank particularly, Messrs. Ward Swift and Alfredo Riviere for their advice and help during the early stages of this project, as well as other support staff members in the areas of purchasing, logistics and contract accounting.

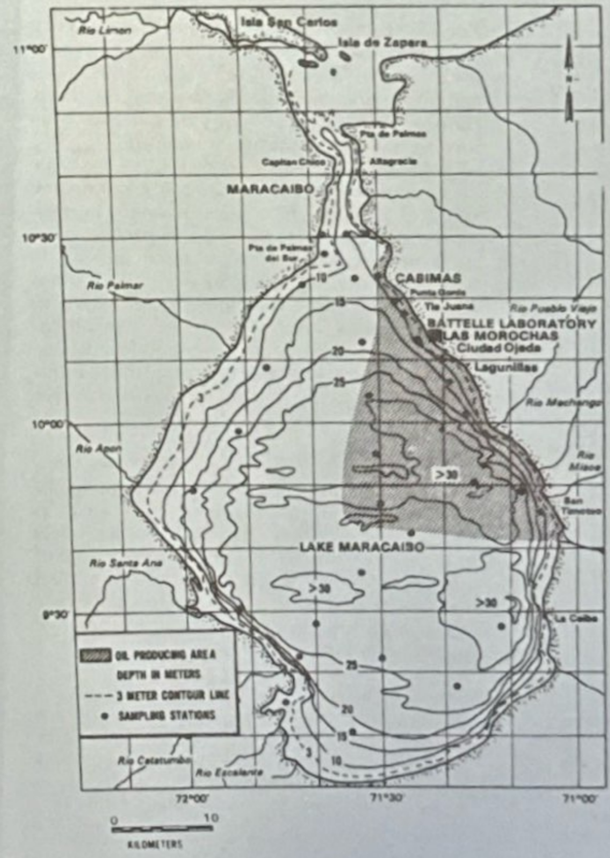
LAS MOROCHAS LABORATORY ON LAKE MARACAIBO

EXECUTIVE SUMMARY

This program, sponsored by Creole Petroleum Corporation, was designed to study the effects of oil discharges on the fishery resources of Lake Maracaibo, Venezuela. Although the assessment of the impact of the effects of oil was of primary concern, the design also included an evaluation of the potential problems associated with the discharge of domestic and industrial wastewaters. This was the first major ecological and pollution study conducted south of the Straits of Maracaibo.

The overall objectives of the program were broad, as indicated below:

- Acquisition of baseline data on water quality, primary productivity, macrofauna, sediments, and fishery resources of the lake to establish the ecological characteristics of the lake.
- Acquisiton of data on the major sources of industrial and domestic wastewaters discharged to the lake.
- Determination of low levels of oil and oil compounds in water, sediments, and organisms.
- Determination of toxicity of oil on selected aquatic organisms by laboratory bioassay.
- Assessment of the probable and potential impacts of oil, oil compounds, industrial and domestic waters on the fishery resources of Lake Maracaibo.



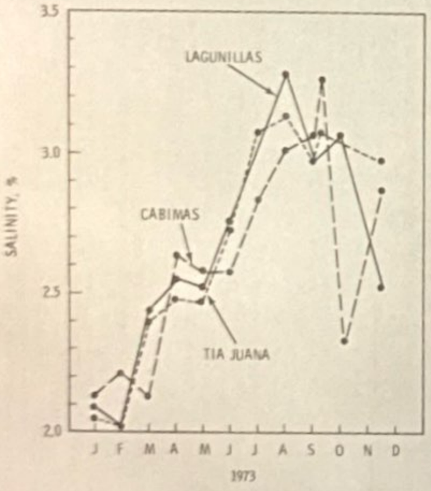
MAP OF LAKE MARACAIBO SHOWING OIL PRODUCING AREAS

ECOLOGY OF THE LAKE: IMPACT OF DOMESTIC AND INDUSTRIAL DISCHARGES

Ecological Characteristics of the Lake

Measurements of salinity confirmed the presence of a seasonal cyclic increase in surface waters from about 2.0 to 3.8 parts per thousand (ppt) over the period of January to August. A layer of high salinity water greater than 6.0 ppt was detected at 25 meters depth in an area southwest of Bachaquero. The concentration of nutrients increased in this layer during August through November, but was slowly eroded by the overlying fresh water. The release of these nutrients to the photosynthetic zone contributes to the algal bloom observed in late winter and early spring.

Water quality analyses demonstrated the presence of substantial concentrations of both phosphorous and nitrogen in the lake water. The highest concentrations of both nutrients in surface waters are located near the populated areas. Chlorophyll analyses have confirmed the observation that algae growth is most prolific in the same areas. The phosphorous concentrations in the lake water are several times higher in the surface waters than were measured in the mid 1950's.



SEASONAL INCREASE IN SALINITY ALONG BOLIVAR COAST IN 1973

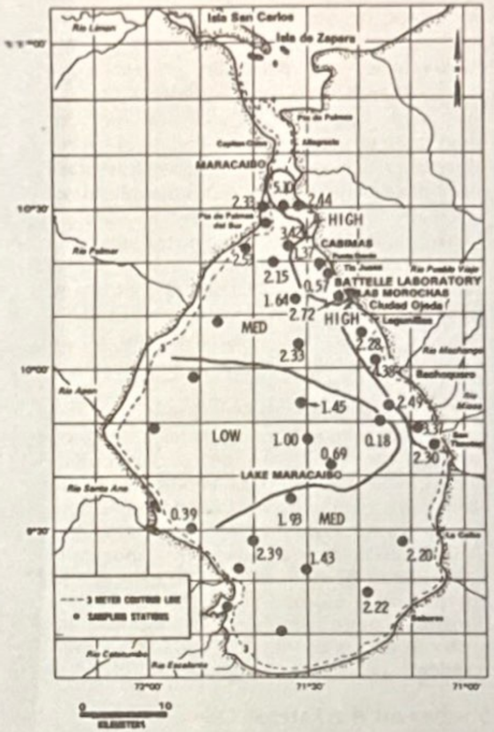
River water analyses showed that rivers discharge significant amounts of nutrients into Lake Maracaibo. Some of these nutrients result from domestic and industrial wastes entering the rivers upstream from Lake Maracaibo. Low dissolved oxygen levels were measured in several rivers. During the wet season, the river wastes are high in suspended solids. For example, the Catatumbo River plume can be detected many kilometers into the lake during the period of high runoff. Marked deltas exist at the mouth of the major rivers.

Benthic (bottom-living) flora and fauna are very sparse in the lake. Some polychaetes, snails, and clams are present. The fine, unconsolidated sediments and the periodic low oxygen conditions in the deeper regions of the lake offer limited habitats for benthic organisms.

The population of phytoplankton is dominated by the blue-green algae, *Anacystis cyanea*. At the primary level, the lake is probably the most productive body of water in the world with measured values greater than 5 grams carbon fixed per square meter per day. The concentrations and diversity of the phytoplankton community are lower and less diverse in the summer-fall than during the winter-spring period. Zooplankton populations are abundant but have a low diversity.

Stomach contents of a range of species were examined. Shrimp and small fish are the major food source for the carnivore fish, such as curvina *(Cynoscion maracaiboensis)*. Lisa *(Mugil spp.)* and bocachico *(Prochilodus reticulatus)* and sardina are plankton and detrital feeders. The major food sources for shrimp are algal, zooplankton and detrital material of organic and inorganic origin.

Fisheries data for commercial landings are only available for the past eight years, and are basically limited to total catch. These data indicate that there has been a continued increase in annual catch. There appears to be potential for increased landings. However, it is not possible, even on a qualitative basis, to state whether the fish populations of the lake have markedly changed over the last three decades, either in total or by species. Even if there had been changes, the lack of standard detailed data used in fisheries stock assessment does not allow apportionment of changes between fishing intensity on the one hand and effects of pollutant load, be it oil, domestic, or industrial wastes, on the other.



AVERAGE PRIMARY PRODUCTIVITY (EXPRESSED AS GRAMS CARBON FIXED PER SQUARE METER PER DAY)

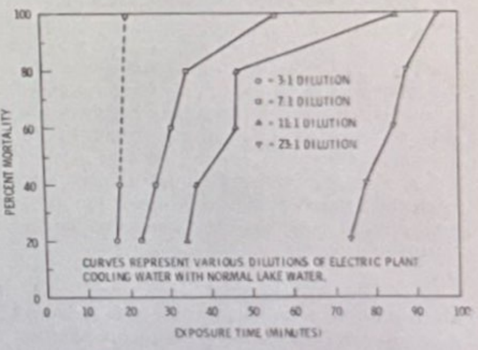
Impact of Domestic and Industrial Wastewaters

Domestic wastes from more than 1.5 million people in the lake basin are discharged directly or indirectly into the lake without treatment. As a result, bacteriological and oxygen demand problems occur near the populated areas. Limited bacteriological analyses indicated that in these areas fecal coliform concentrations exceed recommended U.S. standards.

The stimulation of algal growth by the addition of nutrients from these wastes, particularly phosphorous and nitrogen, appears to be a significant and growing problem. The concentrations of these nutrients are high with mean winter values in surface waters of 40 μg/ℓ phosphate phosphorous and 180μg/ℓ ammonia nitrogen. These values exceed those of the North American Great Lakes and suggest an accelerated rate of eutrophication.

Industrial wastes from tanneries, meat rendering plants, breweries, distilleries, dairy and food processing plants in the Maracaibo city area discharge substantial quantities of organic material and nutrients into the Straits of Maracaibo. To a lesser degree there is also discharge of toxic materials. Low oxygen zones were detected near the bottom in the Straits of Maracaibo, and the northern part of the lake. These zones appear to persist to some extent during the wet season. Temporary depletion of dissolved oxygen was also detected in the southeast area of the lake offshore of the indirect discharges of milk processing plants, an agricultural irrigation return water and a sugar processing plant. Low dissolved oxygen levels were also measured at the mouths of several important rivers discharging into the southern regions of the lake.

Thermoelectric power plants operating on the lakeside not only discharge a thermal effluent to the lake, but additionally, at least two of the plants use a toxic chemical, sodium pentachlorophenate as a biocide to reduce biological fouling in their cooling systems. Toxicity tests



TOXICITY OF BIOCIDE TO LISA

conducted with commercial species of fish and shrimp in effluent water showed this material to be extremely toxic, even after substantial dilution with lake water. To reduce the concentration in the discharge from a 250 megawatt plant to nontoxic levels (i.e., 0.2 ppm) would require dilution with 1/10 to 1/20 of the total water passing through the Straits of Maracaibo in a day.

FATE AND EFFECTS OF OIL

In the 60 years since the start of commercial petroleum operations in the Lake Maracaibo Basin, Venezuela has become one of the world's leading oil producers. The production available from Lake Maracaibo alone is estimated to be about 2,500,000 barrels per day. In view of the magnitude of these operations, some discharges of petroleum into the lake waters are inevitable from pipeline breaks and other accidents incidental to production and transport operations. There is no question that large spills of oil into aquatic environments can cause localized, adverse effects. This has been well documented by a number of investigations into the effects of large oil spills on marine coastal communities in other parts of the world. However, the more comprehensive problem of determining the long-term effects of petroleum discharges on the overall aquatic ecosystem of Lake Maracaibo is much more difficult. Long before exploration for oil was contemplated by man, the lake was exposed to petroleum contamination through natural seeps. In addition, there exists no relevant baseline chemical or biological data from early or preproduction eras against which to compare present day results.

Thus, it is not possible to quantitatively relate the influences of petroleum discharge to environmental change. However, it is highly desirable to at least qualitatively estimate the effects of petroleum on the lake ecosystem and to compare them with other factors only indirectly related to the petroleum industry, such as rapid industrial growth and population increase.

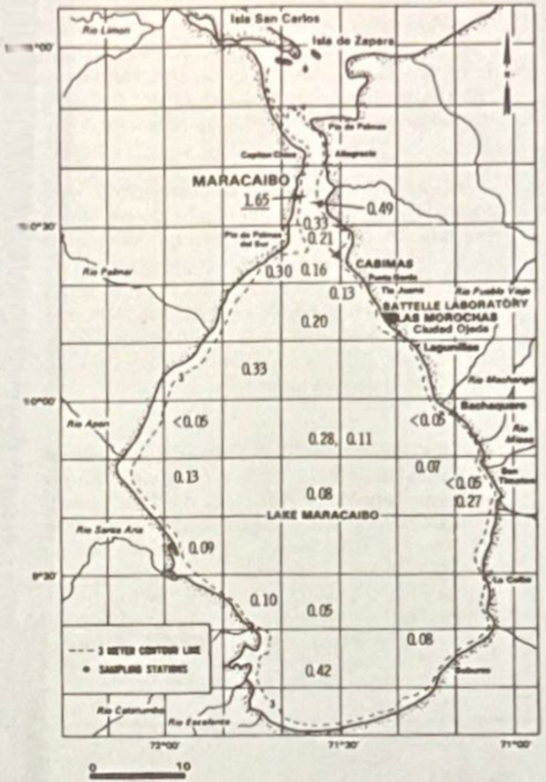
Two parallel investigations were conducted. The first was to determine the fate of petroleum discharged into lake waters; the second was to determine the possible effects of the petroleum discharges on the indigenous biota. The studies on the fate of oil were directed toward measuring levels of petroleum present in the water column, biota, and sediments of Lake Maracaibo. Since existing analytical methodology for determining trace quantities of hydrocarbons in environmental samples was often incomplete or inadequate, considerable effort was made to develop techniques. In addition, limited studies were conducted on the "weathering" characteristics of petroleum, on the potential for microbial utilization of petroleum components, and on the effectiveness of stable carbon isotope ratio measurements as an indicator of petroleum contamination.

Concurrent with the analytical measurements on field samples, laboratory studies designed to measure the effects of oil and oil components on lake biota of commercial importance to the Maracaibo region were undertaken. These studies were more comprehensive than standard bioassay methodology. They not only attempted to quantify, but also to identify and characterize the properties of the toxic components of petroleum in order to estimate the probable effects of these on the aquatic environment.

Studies on the Fate of Oil

Extractable Organic Material and Hydrocarbon in the Lake Maracaibo Water Column

The waters of Lake Maracaibo were periodically sampled over seven months from September 1973 to March 1974 at 27 locations. Samples taken at one meter depth and one meter from the lake bottom were extracted with carbon tetrachloride and the total extractable organic material determined using infrared spectrophotometry. More than 90 percent of the samples contained less than one part per million (ppm) of extractable organic material. Many of the deep samples having a higher content of extractable organics also contained suspended solids. Such contamination is attributed to the strong currents and generally unconsolidated sediments. Samples taken at one meter depth and containing more than 0.5 ppm extractable organic material were subjected to detailed investigation. Less than 15 percent of the extractable organic material could be classified as saturate or aromatic hydrocarbons, and no methylnaphthalenes or other aromatic materials were detected. Thus, the bulk of the organic material in these samples could not be identified as saturate or aromatic hydrocarbons of petroleum origin.



CONCENTRATIONS OF EXTRACTABLE ORGANICS IN LAKE WATER (SEPT-OCT 1973). 1m DEPTH CONCENTRATIONS IN PPM.

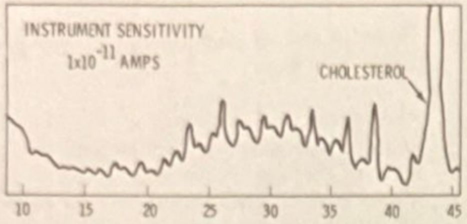
In a related study two samples of the blue-green algae *(Anacystis cyanea)* were analyzed for hydrocarbon content. One sample was taken from an oil producing area and the other from an area without oil production. The hydrocarbon compounds found in both samples were similar. The few hydrocarbons detected had properties consistent with alkanes normally found in these kinds of plants and which are believed to be formed biosynthetically. No other alkanes or naphthalenes of petroleum origin were found.

The results of investigations of the extractable organic material in the lake indicate that the bulk of the water column is largely free of hydrocarbons characteristic of petroleum, in spite of observed surface slicks, suspended bituminous particles, and bituminous material on the bottom. Investigations of the hydrocarbon composition of the blue-green algae, a predominant species in the lake, further served to illustrate that detectable hydrocarbon material in the water is not necessarily of petroleum origin. It is likely that most of the extractable organic material in these waters is derived from algal and other plant material.

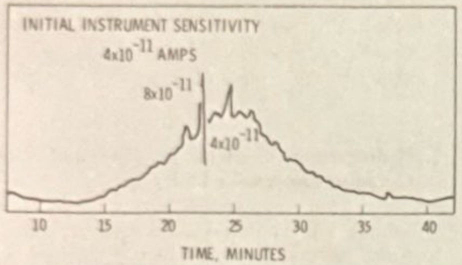
Hydrocarbon Content of Fish and Other Organisms from Lake Maracaibo

Samples of commercially important species were examined for hydrocarbon contents with two principal objectives: first, to determine if petroleum hydrocarbons were accumulated in the tissues of fish obtained from the lake, and second, to identify the types of hydrocarbons in the fish which normally would be consumed by the local population. Samples of fish muscle tissue representing six different species from the lake were analyzed for n-alkanes, methylnaphthalenes, total saturate hydrocarbons, and total aromatic hydrocarbons. The results obtained were compared with analyses of fish samples, both from other locations in Venezuela and from North American waters. In all, 24 fish from the lake were compared with 8 fish obtained from other areas which were relatively oil free.

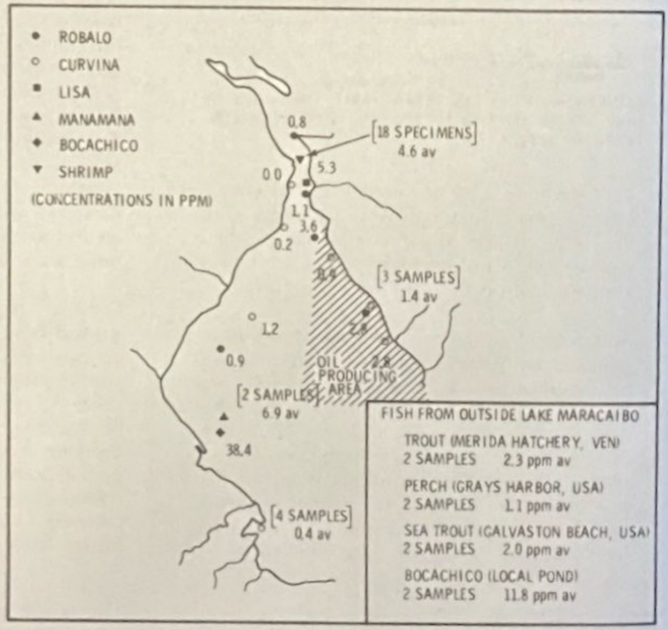
Curvina *(Cynoscion maracaiboensis)* was studied most intensively because of its relative abundance and commercial importance. This species proved to have the lowest hydrocarbon content, averaging less than 1.0 ppm total recoverable hydrocarbons. Other species from the lake, listed in order of increasing hydrocarbon content of the muscle tissue were robalo *(Centropomus ensiferus)*, lisa *(Mugil curema)*, white shrimp *(Penaeus schmitti)*, manamana *(Anodus laticeps)*, and bocachico



GAS CHROMATOGRAM OF THE SATURATE FRACTION FROM A CURVINA TISSUE STATION 2 (OFF LAGUNILLAS)



GAS CHROMATOGRAM OF THE SATURATE FRACTION FROM A SPOTTED SEA TROUT (GALVESTON BEACH, TEXAS)



TOTAL HYDROCARBON CONCENTRATIONS FOUND IN FISH MUSCLE TISSUE

(Prochilodus r. reticulatus). The high total hydrocarbon content of the bocachico (38 ppm) is of particular interest since this species of fish prefers fresh water and does not frequent the oil producing areas of Lake Maracaibo.

However, none of the samples examined were found to contain methylnaphthalene compounds. These compounds can be considered to be an indicator of contamination by petroleum; thus, the failure to detect them at levels below 10 parts per billion (ppb) suggests that these petroleum-derived hydrocarbon compounds are not accumulated in the muscle tissue of those samples examined.

On the other hand, significant quantities of individual saturated hydrocarbons were found in many samples of fish from the lake as well as from several samples obtained from outside the lake area. Invariably, the majority of this material had properties consistent with pristane, a naturally occurring hydrocarbon which has been previously reported to be present in the tissues of marine fish. Saturate hydrocarbons with properties corresponding to n-alkanes containing from 15 to 20 carbon atoms were also found in lesser amounts.

The data obtained indicates that there is little evidence for the accumulation of petroleum hydrocarbons in the fish samples examined. Although the number of samples is small and the species limited to the major commercial ones, it is significant that naphthalenes, indicative of oil contamination, were not found in any of the samples studied. Saturate hydrocarbons are thought to enter into the food web beginning with compounds naturally occurring in plants. Saturated hydrocarbons found in these tissues fall into a pattern consistent with this mechanism. The amounts and types of hydrocarbons found in the tissue samples appear to be more a function of the species of fish rather than the location from which they were obtained. This is in agreement with stable carbon isotope ratio data obtained on samples of tissue. The relative abundance of carbon-13 in the samples was more closely related to the species of fish than sample origin, again suggesting that carbon derived from petroleum has contributed little to the carbon cycle of the aquatic population.

Although an effort was made to determine the concentration of polynuclear aromatic hydrocarbons in samples of tissue from several species of fish, the sensitivities obtained using mass spectrometric methodology were disappointingly low. The results from a limited number of samples indicated no detectable hydrocarbons having structures consistent with polynuclear aromatics; however, the presence of less than 0.1 ppm of any specific type would not have been detected by the method.

Organic Composition and Hydrocarbon Content of the Sediments of Lake Maracaibo

Oil discharged into Lake Maracaibo is dissipated through evaporation, solubilization, and bacterial degradation. It is not unreasonable, however, to expect that the bituminous residues of petroleum discharges would eventually become part of the organic content of the lake bottom. It is therefore not surprising that observations of bituminous particles in the sediments of the lake were a frequent occurrence. In 15 dredge samples subjected to chemical characterization, two taken off the northeast shore were found to contain numerous bituminous particles ranging from 2-4 millimeters in diameter.

Lake Maracaibo sediments are rich in organic material. Samples have been examined which have extractable organic contents as high as 3 percent of the dry weight of the sediment, although many samples have lower amounts; on the order of about 1 percent. It is difficult, however, to assess the relative contribution of petroleum discharges to the total organic content of the lake sediments since it is precisely those areas of intensive oil production that also are affected by heavy plankton loads and by large volumes of industrial and domestic wastes.

In order to assess the contribution of petroleum hydrocarbons to the organic composition of the sediments, over 40 lake sediment core and dredge samples taken from widely separated geographical locations were characterized with respect to the organic composition. Quantitative measurements of the total organic extract were made as well as of the amount of saturate and aromatic hydrocarbons in the sediments. In addition, the organic fractions obtained were characterized by mass spectrometry according to abundance of component hydrocarbon types. Other measurements included determination of elemental composition, molecular weight, and total organic carbon.

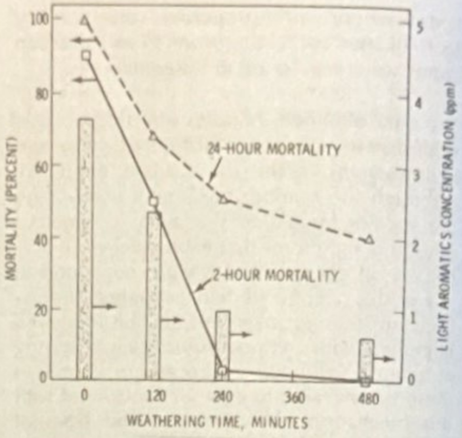
There seems to be little doubt that the area of the lake comprising the most intensive production activity has a sediment organic content distinctly different from other regions. Sediment samples from nonproduction locales had high relative concentrations of aromatic compound types; in contrast, aromatic hydrocarbons were relatively abundant in the sediment samples from producing areas, and their elemental compositions more consistent with a heavy aromatic petroleum fraction. The mass spectrometric data also suggest that some of the organic material of the sediments is petroleum residue. This can be deduced from the relative concentrations of saturate hydrocarbons in lake sediments, and from the consistency of the saturate compound-type distribution patterns with samples known to contain petroleum bitumen.

Studies on the Effects of Oil

Toxic Effects of Oil on Lake Maracaibo Fish, Shrimp, and Plankton

The toxicity studies conducted at the Las Morochas Laboratory were designed to answer a number of questions regarding the toxicity of petroleum to the aquatic biota of Lake Maracaibo. It was, of course, necessary initially to determine the concentration levels of crude oil required to induce mortality in commercially important species. Of perhaps equal importance, was the necessity of describing the factors which contribute to acute toxic effects, and of assessing the relative importance of these factors when actual discharges of oil into the lake occur. Thus, experiments designed to evaluate 24-hour median tolerance limits (TLm_{24}) of Maracaibo organisms, were accompanied by studies to determine the effects of "weathering" of crude oils on their toxic properties. In addition, laboratory comparisons between the toxicities of light and heavy crudes were made, and the long-term effects of repeated exposures to oil studied.

It was found that an important variable influencing the toxicity of oil in water was the type of agitation used to mix the oil with the water. Thus, a gentle agitation of an oil-water mixture at the level of 10,000 ppm induced no toxicity, whereas an identical mixture was highly toxic when agitated for 10 minutes with a magnetic stirrer. The viscosity of the test oil was also an important variable under the experimental conditions used. Tia Juana Light Crude mixed poorly with lake water giving erratic toxic responses, and resulting in no evident correlation between amount of oil added and toxicity. On the other hand, Tia Juana Heavy Crude mixed well with water, but imparted less toxicity than Tia Juana Medium Crude.



TOXICITY OF WEATHERED OIL TO SHRIMP

Most of the acute toxicity experiments were conducted using a static bioassay procedure in which a given amount of oil was agitated with lake water using vigorous mechanical stirring. This was followed by introduction of the test organisms into the separated water phase. The amount of oil contacted by the lake water is expressed in parts of oil per million parts of lake water (ppm). Thus, the values for oil toxicity are based on the amount of oil added to the water rather than the concentration of oil in the water column. Using this procedure, a 24-hour median tolerance limit (TLm_{24}) of white shrimp *(Penaeus schmitti)* for South Tia Juana Medium Crude was 1507 ppm, with 95% confidence limits of 1133 and 1667 ppm. For lisa *(Mugil curema)*, the value obtained was 2400 ppm (2325-2465). Robalo *(Centropomus ensiferus)* was about 10 times more resistant than shrimp to the toxic effects of South Tia Juana Medium. A dehydration chemical commonly employed in the processing of the crude oil did not increase the toxicity of oil, but apparently decreased the induction time for toxic response.

These TLm values do indicate the relative sensitivity of the organisms. Using the same agitation for each oil-water mixture, there is a good correlation between mortality and the total oil added to the system. Certain of the test waters were analyzed for total oil and for light aromatic hydrocarbons. These analyses revealed that mortality generally correlated poorly with total oil in the water, but reasonably well with the concentration of light aromatic hydrocarbons.

Experiments to determine the effects of weathering of surface films on resulting toxicity also showed a close correspondence between the quantity of light aromatics extracted into the water and mortality. Exposure of crude oil films to the atmosphere at the lakeside resulted in rapid losses of water soluble light hydrocarbons, with concomitant reduction in ability of the oil to induce mortality. After 24 hours, oil films on lake water approximately 3 millimeters in thickness lost all toxic properties. Thinner films approaching those which would be expected in an actual spill situation lost toxic properties in less than 90 minutes of exposure. Significantly, fish maintained in the weathering tanks during the oil film exposure tests did not experience mortality; rather, the fish tended to remain under areas covered by slicks.

Investigations of the properties of the toxic substances present in oil-water mixtures showed that sparging these test solutions with air rapidly rendered them less toxic to test organisms. Subsequent experiments were accompanied by analysis of the test solutions for concentrations of light aromatic hydrocarbons. These experiments demonstrated that even with the minimum amount of aeration required to maintain the test animals, light aromatic hydrocarbons were rapidly lost from solution. The disappearance of these hydrocarbons was approximately logarithmic with respect to time.

In addition to the acute toxicity testing, a long-term chronic study was carried out in which juvenile fish *(Mugil curema)* were exposed to oil spilled daily onto the surface of the test tanks through which lake water was flowing. During the course of the test (11 weeks), the growth rate of the exposed fish were monitored weekly, and at the end of the test, the hydrocarbon concentrations in the whole test fish were determined. There were no significant differences observed, either in growth rates or in detectable hydrocarbon concentration in the exposed fish when compared to the control fish.

In studies on the effects of oil on the phytoplankton community it was found that concentrations of up to 1000 ppm of crude oil enhanced the photosynthetic processes, as measured by ^{14}C uptake and oxygen evolution. At higher levels inhibition was found to take place.

Assessment of the Fate and Effects of Oil Discharges on the Biota of Lake Maracaibo

From the toxicity tests, it would appear that the probability of direct toxic action from a surface spill of oil on the fish of Lake Maracaibo is very low. Vigorous agitation is required to extract the toxic hydrocarbons from petroleum, and these compounds volatilize rapidly from a surface slick. A submarine leak may produce a localized toxic response, but there is insufficient data to assess the relative importance of such discharges to other potential sources of ecological stress throughout the lake.

Studies have further indicated that chronic exposure of fish and shrimp to "background" levels of oil in the lake are unlikely to result in mortality. Levels of extractable organic material in the lake waters are generally low, (~0.5 ppm) with good evidence that the total hydrocarbons in the water, at least below the

ANALYSIS OF LIGHT AROMATIC HYDROCARBONS IN 24-HOUR STATIC BIOASSAY OF SHRIMP

Oil[1]/lake water ratio[2] (ml/ℓ)	5.56	4.44	3.33	2.22	0
Benzene, mg/ℓ	2.10	1.85	1.38	0.84	
Toluene, mg/ℓ	5.08	3.42	4.27	2.04	
Et. Benzene, mg/ℓ	0.50	0.41	0.53	0.24	
m, p-Xylene, mg/ℓ	2.21	1.44	2.13	0.89	
o-Xylene, mg/ℓ	0.79	0.44	0.60	0.28	
"C_9^+" Aromatics, mg/ℓ	1.06	0.63	1.01	0.25	
Total Aromatics, mg/ℓ	11.74	8.19	9.92	4.54	
Total Oil (IR, mg/ℓ)	12.1	162.5	58.4	61.9	
Mortality, at 24 hrs. %	100	82	70	36	6

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[1] S. Tia Juana Light Crude Oil

[2] Oil stirred with lake water using a magnetic stirrer.

surface, is an order of magnitude lower. Further, there is no evidence for accumulation of petroleum hydrocarbons as represented by n-alkanes and methylnaphthalenes in the flesh of fish indigenous to the lake. What hydrocarbon material has been recovered from tissue samples appears to be of biogenic origin and more a function of species and diet.

There is evidence of the presence of significant quantities of bituminous residue in lake sediments obtained from areas of petroleum production. It is logical that spilled oil not removed from the water column by evaporation or bacterial degradation would become part of the sediments through sedimentation mechanisms similar to the accumulation of other organic debris. There are no data to indicate the rate of accumulation of bitumen in sediments, or whether the rate is increasing or decreasing. The probability that the bituminous material in the sediments has a significant impact on the lake ecosystem is low, however. Rapid sedimentation rates and the paucity of benthos throughout the lake to initiate transfer through the food chains give some assurance that the sediments represent a relatively permanent respository for this material.

CONCLUSIONS

There is no question that significant discharges of oil and oil compounds incidental to the production of petroleum in the Lake Maracaibo basin have occurred over the last four decades in addition to that material from natural seeps. However, the data obtained during the course of this program from both laboratory and field studies would indicate that present operations have not caused discernible damage. The rapid loss, in a few hours, of light hydrocarbons from surface films of oil to the atmosphere has been shown to reduce the toxicity to organisms significantly. The low concentrations of oil measured in lake water have not contributed to a detectable buildup of hydrocarbons in the muscle tissue of selected commercial species. The occurrence of bituminous residues in the sediments, particularly in the production areas, would suggest that the natural processes of volatilization, biodegradation and sedimentation are the major mechanisms for removing weathered oil from the biologically productive zone. Examination of the limited fisheries data available does not suggest that the resources are being depleted. Consideration of the potential impact of nonpetroleum wastes, both domestic and industrial, indicate that these nonpetroleum materials are contributing to the degradation of the water quality, which consequently may reduce the biological resources of the lake.

The Lake Maracaibo Basin is unmistakably a national resource and has the potential as a result of the existence of sizeable oil and gas reserves to develop into a major industrial center. In addition, the lake itself has great potential to support a managed fishery which can make a significant contribution to the protein and economic base of the country; and it clearly could be a major source of potable, irrigation, and industrial water for western Venezuela. However, this study indicates that even with the present expansion of urban areas and a wide variety of industries with limited waste treatment facilities, small but significant changes have occurred in the quality of lake water. Past trends and predictions for the future suggest that continued development is a virtual certainty, and it is suggested that without environmental protection the degradation of the lake system could continue to a stage when multiple use options of the resource will be severely reduced. The issues and problems arising from basic goals and conflicts of multiple use of the basin can be resolved through a well ordered master development plan of regional scope with local interests, needs, constraints, and opportunities carefully considered within a framework of national policy and objectives governed by the broad and long-range interests of the nation. Jurisdictional and legal factors including problems of disputed access or control of resources, the economics of profit oriented competition for access and use of lake resources, sociological and economic factors bearing on issues of environmental degradation and resources are major considerations that will need to be addressed by this plan.

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INTRODUCTION

Petroleum Production in Lake Maracaibo

According to Baptista (1966), the first reports about the existence of oil in Venezuela came in the sixteenth century from the Spanish conquistadores who observed seeps of oil along the coastline of Lake Maracaibo. Although there was some commercial activity related to petroleum in the region as early as the 1870's, the first significant progress for the Venezuelan oil industry is considered to be the discovery of the Mene Grande field in 1914. The tremendous economic potential of petroleum in the Maracaibo region was not fully realized, however, until 1922, when the Los Barrosos No. 2 well blew out. This well, located on the eastern shore of the lake south of Cabimas, gushed 100,000 barrels of oil per day until it could be brought under control.

By 1926, there were 73 oil companies operating in Venezuela. This year was marked by the discovery of the largest oil field in Venezuela; Lagunillas, on the eastern shore of the lake. Two years later, the field at Tia Juana, close to Lagunillas, was discovered. A third huge producer on the shore of Maracaibo is the field at Bachaquero, south of Lagunillas. The magnitude of the resources represented by these fields can be appreciated from their combined total production. In 1965, 671 million barrels of oil were produced from these three fields alone.

Today the oil industry is a major economic factor in the Lake Maracaibo area. Rodriguez (1973) reports that as of the end of 1970, a total of 5,200 wells were on the lake, with an additional 6,800 on the surrounding shores. A total of 15,100 km of pipeline (11,100 km in the lake) and over 200 flow stations existed to support a production of about 3 million barrels of oil per day. In addition to the production facilities, there were two refineries, 12 gas plants, and eight tanker terminals. The petroleum operations also support a number of related petrochemical industries and major thermoelectric plants in the Maracaibo area.

Discharges of Petroleum into Lake Maracaibo and Potential for Impact on Lake Ecology

In view of the magnitude of petroleum-related operations in Lake Maracaibo, it is perhaps inevitable that discharges of petroleum into the waters have occurred. Data regarding the sources, numbers and volumes of crude oil spilled have recently been made available through the Ministry of Mines and Hydrocarbons. Figure 1 gives a breakdown of the type of discharges and the relative frequency of accidents experienced with each type. Figures 2 and 3 give annual and monthly breakdowns of the number of spills since 1960, and Figure 2 also gives the total annual volumes spilled. Note from Figure 2, that although the volume spilled between 1965 and 1971 remained relatively constant, the number of reported spills increased dramatically. This suggests that the increase in reported spills is the result of more vigorous enforcement procedures. The data for 1972 may be exceptional due to two large spills totaling approximately 100,000 barrels of crude petroleum.

To put the annual volume of oil spilled into Lake Maracaibo into some perspective, it is instructive to relate the oil spilled in 1972 to the average annual inflow of fresh water to the lake of 54 x 10^9 cubic meters. One can calculate that there are more than three million times as much water inflow as there is petroleum.

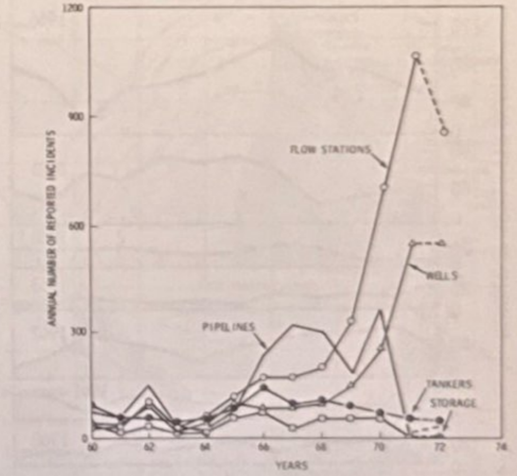

Figure 1
Number of annual discharges according to origin
(from Comision Coordinadora, 1973)

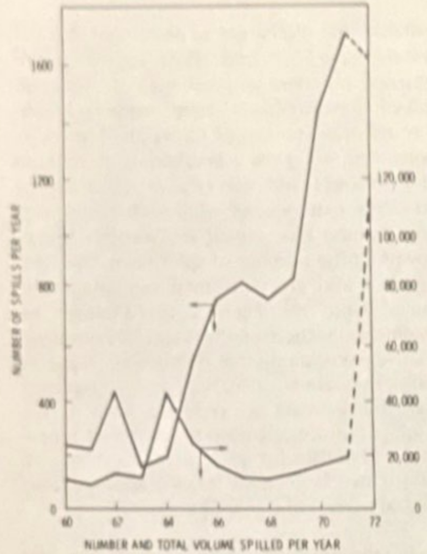

Figure 2
Annual volume of oil spilled and number of spills on Lake Maracaibo (1960-1972)
(from Comision Coordinadora, 1973)

This theoretical ratio of water to oil is unrealistically low from at least two points of view. First, most of the hydrocarbon compounds present in oil are extremely insoluble in water, and hence do not mix uniformly with lake water; second, much of the oil spilled on the lake surface evaporates into the atmosphere. Table 1 gives the losses of normal alkane hydrocarbons from a thick film of South Tia Juana Medium Crude Oil 36 hours after being spilled on lake water. The experiment, conducted at the Las Morochas Laboratory, was conducted at ambient lake water temperature of approximately 30°C. The analytical methodology employed was essentially that of Sivadier and Mikolaj (1973).

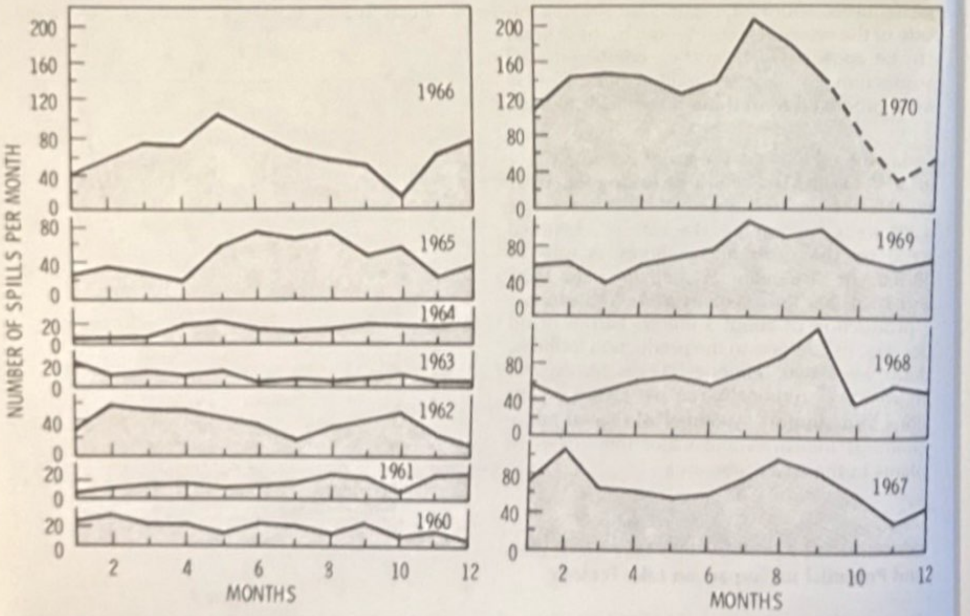

Figure 3
Monthly breakdown of reported oil spills (1960-1970)
(from Comision Coordinadora, 1973)

Table 1
Loss of paraffinic hydrocarbons
from an exposed oil film of
South Tia Juana Medium Crude Oil

Straight Chain n-Alkane Carbon (#)	Approximate Percent Loss 36-Hour Exposure
C 11	100
C 12	94.7
C 13	78.5
C 14	63.9
C 15	42.6
C 16	62.5
C 17	21.2
C 18	27.0
C 19	24.5
C 20	26.0
C 21	20.0
C 22	---
C 23	10.0
C 24	---

While the volume of oil spilled relative to the volume of the lake as a whole is small, there are a number of important considerations which make it quite possible to obtain much larger local concentrations of oil in water. First, the petroleum production is not uniformly distributed throughout the lake, but is heavily localized in the northeast quarter, and particularly along the lake shore from Cabimas in the north to San Timoteo toward the south (see map, Figure 4). Second, the generally counterclockwise current in the lake combines with prevailing westerly winds to confine much of the lake debris along the northeast shore, thus adding to the local impact of heavy oil production activity. Third, oil spilled at other locations in the lake forms films or slicks which can be rapidly transported by winds and currents to this same northeast quadrant of the lake. It is not impossible, therefore, that petroleum discharges into the lake could have significant local consequences and, as a result, bring about substantial changes in the general ecology of Lake Maracaibo.

In addition to the question of direct toxic effects to lake biota because of high local concentrations of petroleum hydrocarbons there is another aspect of petroleum pollution which needs to be considered. The concentration of certain organic residues by biotic tissue and the subsequent transfer and accumulation of these compounds through the food chain has been postulated as a mechanism for changes in the populations of a number of species of predator organisms near the top of the food chain.* Although there is, to date, no reported conclusive evidence for bioaccumulation of petroleum hydrocarbons and resultant decline of specific species, it is important that this subtle effect be explored in any general consideration of the influence of petroleum on the lake. This is particularly true when one considers that much of the commercial fisheries catch from Lake Maracaibo is represented by species of fish near the top of the food chain (see Volume I).

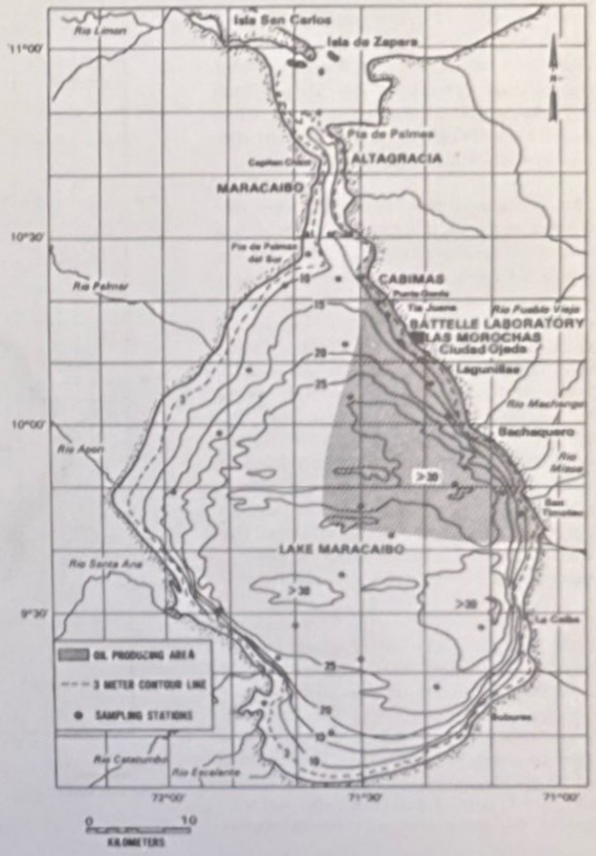

Figure 4
Map of Lake Maracaibo showing
oil producing areas

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*For a recent discussion of food-chain relationships of the pesticide, DDT, see Devlin (1974).

Basis of Approach to the Study of Oil in Lake Maracaibo

Ideally, when one considers a study of the influence of an allochthonous material on a specific environment, it is desirable to obtain a data base on the region prior to the introduction of the material. For a study of oil in the Maracaibo ecosystem, this is clearly impossible; the lake has been exposed to petroleum discharges through natural seepage for centuries. In addition, there exists no substance of relevant baseline biological or chemical data from preproduction eras against which to measure present day results.

Therefore, other approaches must be taken in order to formulate even a qualitative estimate of the effects of petroleum on the lake ecosystem. The study reported herein was constructed along two parallel lines of investigation; one directed toward determining the fate of petroleum discharged into the lake, and the other to determine the possible effects of the petroleum discharges on the indigenous biota.

The studies on the fate of oil were designed to assess the levels of petroleum and petroleum-derived hydrocarbons present in the water column, the sediments, and in the biota of Lake Maracaibo. For this purpose, 33 field stations were established along six transects for the collection of samples of water, biota and sediments. A map showing the station locations is shown in Figure 5 and on the inside cover of this report. In addition to detailed analyses of field samples for hydrocarbons representative of petroleum, this aspect of the work included determination of the stable carbon isotope ratio present in samples of biotic tissue and sediments.

Concurrent with the analytical work on field samples, laboratory studies were undertaken to measure the effects of oil and oil components on lake biota of commercial importance. The studies were more comprehensive than standard bioassay methodology, in that they not only attempted to quantify, but also to identify and characterize the properties of the toxic components of petroleum in order to obtain an estimate of the probable effects of these on the aquatic environment.

Although much of the data obtained from both the field and laboratory studies is quantitative, it is obvious that conclusions about the total environmental impact of petroleum spilled into the lake cannot be placed on a quantitative basis. The data presented in the following chapters have been used to formulate an estimate of the importance of oil discharge relative to other potential influences on the Lake Maracaibo ecosystem. This estimate, discussed in the Executive Summary, is based on both a careful assessment of the data generated, and the present understanding of the general ecology of Lake Maracaibo.

Figure 5
Sampling stations on Lake Maracaibo

FATE OF OIL IN THE LAKE MARACAIBO ENVIRONMENT

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FATE OF OIL IN THE LAKE MARACAIBO ENVIRONMENT

INVESTIGATION OF THE EXTRACTABLE ORGANIC AND HYDROCARBON CONTENT OF THE LAKE MARACAIBO WATER COLUMN

Introduction

An essential requirement for an assessment of the fate and effects of oil on the Lake Maracaibo system is to obtain an estimate of the average exposure of biota to petroleum. This task is not a straightforward one, since fluctuations in water movement, salinity, microbial activity, and primary productivity caused by seasonal and climatic variation can bring about corresponding variations in hydrocarbon distribution. It is therefore necessary to obtain analyses of lakewater samples derived from a number of locations and at time intervals spanning seasonal variations in order to obtain an estimate of overall exposure levels.

There were a number of practical problems related to obtaining and analyzing environmental samples of lake water. These included troublesome problems of unacceptable solvent purity, and of contamination of water extracts. Although these difficulties considerably shortened the time period over which valid data on lake water could be obtained, detailed investigations regarding contamination problems have resulted in the implementation of techniques permitting further characterization of the extractable organic content of lake water. These procedures have also provided an opportunity to investigate the validity of infrared methodology for analysis of petroleum residues in Maracaibo water samples. Table 2 shows a good correspondence between gravimetric and infrared methods. Many of the water extracts received early in the program, represented by the data in Table 2, were found to be contaminated with large quantities of colorless, refined, saturate hydrocarbon material. The authenticity of these samples as true environmental samples is therefore highly questionable. Our present methods have largely eliminated this difficulty.

Determination of Extractable Organic Material in Lake Water

The determined concentrations of extractable organic hydrocarbons in lakewater samples taken at depths of one meter and at one meter from the bottom over a period of seven months are given in Table 3. Procedural details of the infrared spectrophotometric method used (IR) are given in Appendix A. These data were obtained on samples which were frozen at the Las Morochas Laboratory and shipped frozen to the Richland laboratory. When possible, samples were shipped in triplicate as a precaution against breakage, and so that additional investigations could be made.

Table 2

Gravimetric and IR analysis of carbon tetrachloride extracts of Lake Maracaibo water samples

	Milligrams Per Liter of Extractable Organics	
Location by Station No.	IR Determination	Gravimetric Determination
25	52.5	58
32	52.2	53
30	40.0	35
1	7.4	10
12	2.9	3
13	2.4	3
34	3.0	5
8	23.2	28
4	5.7	7
24	1.4	3
21	6.5	11
22	1.2	2
27	0.8	1

In general, the values obtained for extractable organic material were quite low, considering the organic productivity of the lake. Over 90% of the 200 samples analyzed were found to contain less than 0.5 ppm extractable organics. In addition, a number of samples taken 1 meter above the lake bottom were observed to contain quantities of sedimentary material. These samples almost invariably gave values of well over 1 ppm. We can thus assume that many of the values over 1 ppm for the bottom samples result from extraction of organic compounds adsorbed on sedimentary material rather than from organic material actually in the water column.

In considering the data presented in Table 3, it is important to bear in mind the many classes of compounds known to be present in natural waters. Degans (1970) has reported that over 90% of organic matter in marine waters consists of insoluble polemeric nitrogenous com-

Table 3

Analysis of lakewater samples by infrared analysis of carbon tetrachloride extracts[1] at 2930 cm^{-1}
(Concentrations expressed as parts per million extractable organic in water)

		Month Sampled					
		September, 73	October, 73	November, 73		December, 73	
Station number	Depth (meters)	1 Meter	1 Meter	1 Meter	Bottom	1 Meter	Bottom
1	11			0.18	0.29	0.29	4.57
2	10			0.07		0.20	
3	15						
4	20	<0.05		0.26	0.17	0.46	0.13
5	27	<0.05		0.21	0.13		0.16
6	12	0.49		0.07	0.06,0.06	0.84	
7	15	0.16		0.71		0.84	0.45
8	22	0.20					
9	31			0.27	0.06	0.50	0.65
10	29	0.28	0.11	0.27	0.06	0.17	0.68
11	31		0.08	0.19	0.12	0.19	0.15
12	6	0.13		0.37	0.24	0.18	0.26
13	5			0.62	0.33	0.23	5.14
14	6	0.21	1.18			0.54	
16	6	0.33					
17	13	1.65					
18	13						
19	8	0.30					
20	14	0.27					
21	20					4.76	4.97
22	21		0.08			0.43	8.00
23	10		0.42				
24	33	0.07		0.11	0.07	3.78	1.89
25	33			0.11	0.54		0.23
26	34					0.12	0.18
27	23					6.05	0.52
28	16		0.10				
29	11						
30	29		0.05			0.13	0.23
31	24		0.09			0.32	0.14
32	12		0.13				
33	9	<0.05					
34	30	0.33					

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[1] For analytical methodology, see Appendix A
[2] Greenish emulsion formed during extraction
[3] Sediment observed in water sample

Table 3 (Continued)

		Month Sampled					
		January, 74		February, 74		March, 74	
Station number	Depth (meters)	1 Meter	Bottom	1 Meter	Bottom	1 Meter	Bottom
1	11	0.61	0.25	0.23	0.22	0.24	0.14
2	10	0.20	0.12	0.43	12.43[3]	0.40	0.09
3	15						
4	20	0.13	0.27		0.34	0.35	0.12
5	27						
6	12	0.94	3.83[3]	0.16	0.21	0.22	0.22
7	15	0.15	1.68[3]			0.17	0.17
8	22	2.04	0.26	0.15	0.33	0.13	0.22
9	31	0.59	0.14	0.16	0.23	0.15	0.11
10	29	0.55	0.18	0.06	0.15		
11	31	0.34	0.46	0.05	0.15	0.21	0.28
12	6	2.19[2]	0.25	0.29	0.23	0.26,0.29	0.42
13	5	0.30	0.47	0.19	0.70	0.23	11.74
14	6	0.18[2]	0.29[2]	0.83	0.27	0.29	0.27
16	6	0.72	2.50	0.31,0.13	3.52[3],4.35[3]	0.47	0.55
17	13						
18	13						
19	8						
20	14						
21	20	0.07	0.22	0.07	0.05		
22	21	0.28	0.08	0.11	0.15		
23	10						
24	33	0.14	0.15	0.17	0.09	0.28	4.20[3]
25	33	0.23	0.46	0.08	0.27	0.18	
26	34	0.08	0.60[3]	0.06	0.08	0.46	0.36
27	23	0.08	0.37	0.15	0.09	0.10	0.24
28	16						
29	11						
30	29	0.24	0.48	0.06	0.09	0.09	0.27
31	24	0.13	0.12	0.14	0.16	0.09	
32	12						
33	9						
34	30						

ponents derived from planktonic and microbial metabolism. Amino acids, fatty acids, and carbohydrates constitute the bulk of the remaining organic contents. Thus, one might expect considerable discrepancy between the total organic material in natural waters and the quantity which could be extracted using carbon tetrachloride. A comparison of the total organic carbon content found in Lake Maracaibo waters of 6-14 mg/ℓ (see Volume I) with the values for extractable organic matter given in Table 3 demonstrates a large difference between total organic content and extractable organics in this system.

The data, for extractable organics, when geographically plotted (see Figures 6 through 16) generally show few trends. Surface samples with high values of extractable organic material appear to be randomly distributed with the exception of the area of the Straits of Maracaibo. In this region, 8 of 24 surface samples examined were found to contain extractable organic material in excess of 0.5 ppm. This area is one of high population density, industrial activity, and tanker traffic. It is thus reasonable to find higher values for extractable organic hydrocarbon there.

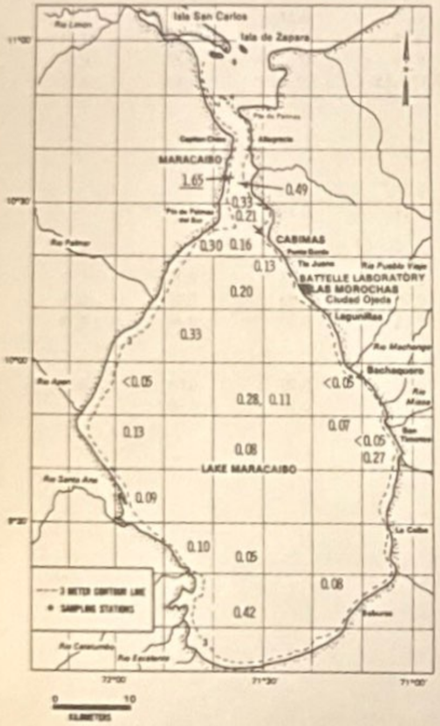


Figure 6
Analysis of water samples for extractable organics (September - October 1973) 1m depth, conc. in ppm

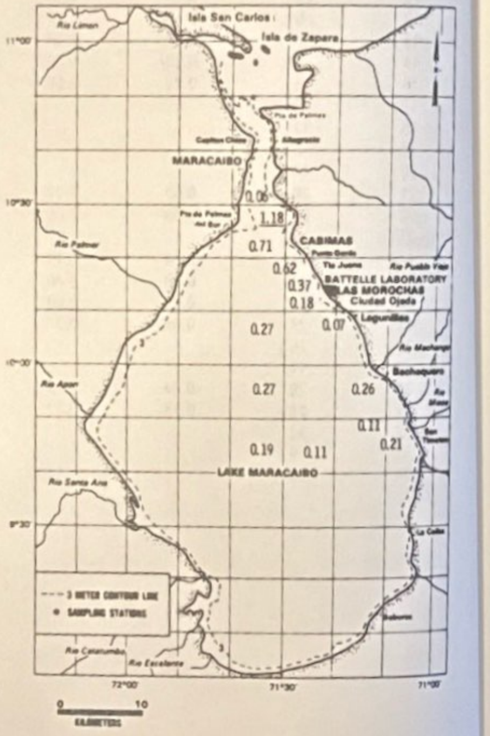


Figure 7
Analysis of water samples for extractable organics (November 1973) 1m depth, conc. in ppm

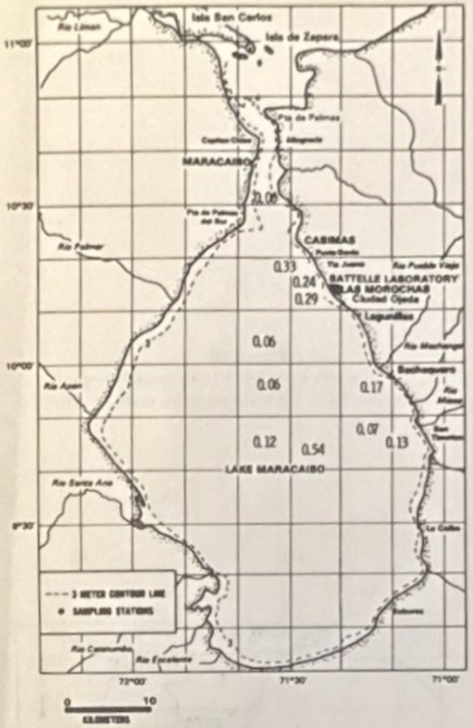
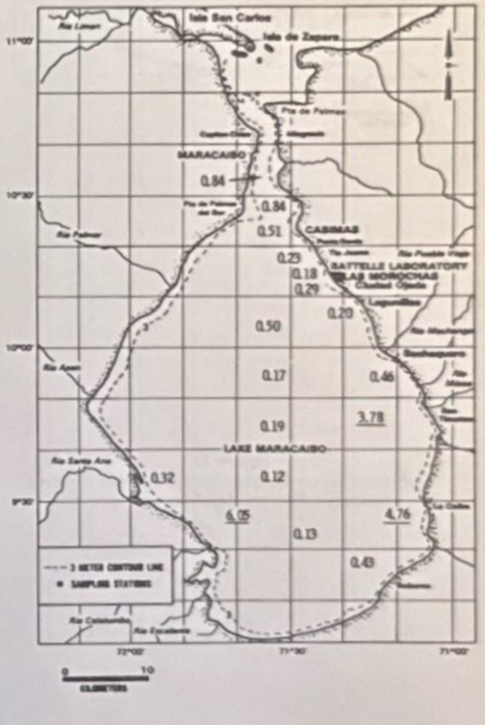


Figure 9
Analysis of water samples for extractable organics (December 1973) 1m depth, conc. in ppm

Figure 8
Analysis of water samples for extractable organics (November 1973) 1m from bottom, conc. in ppm



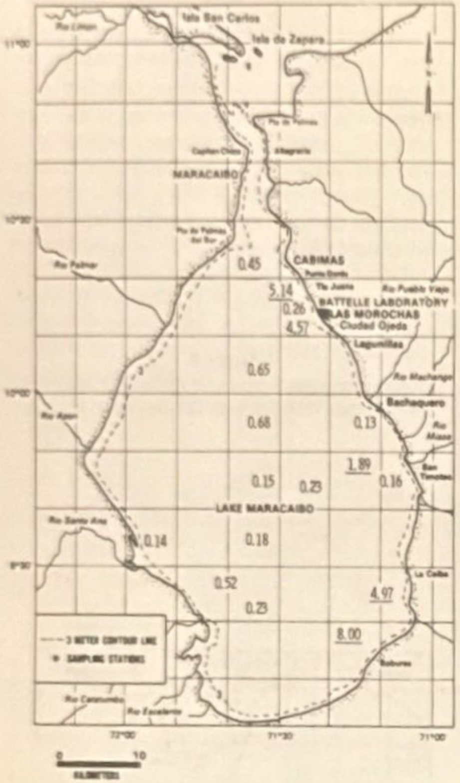


Figure 10
Analysis of water samples for extractable organics (December 1973) 1m from bottom, conc. in ppm

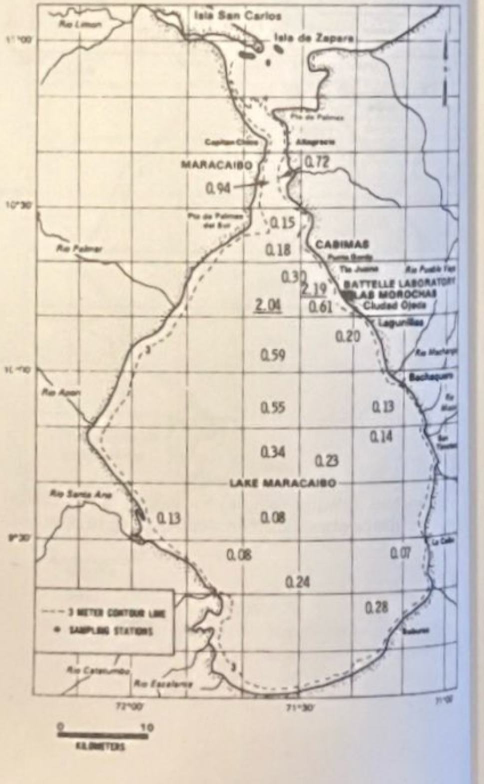


Figure 11
Analysis of water samples for extractable organics (January 1974) 1m depth, conc. in ppm

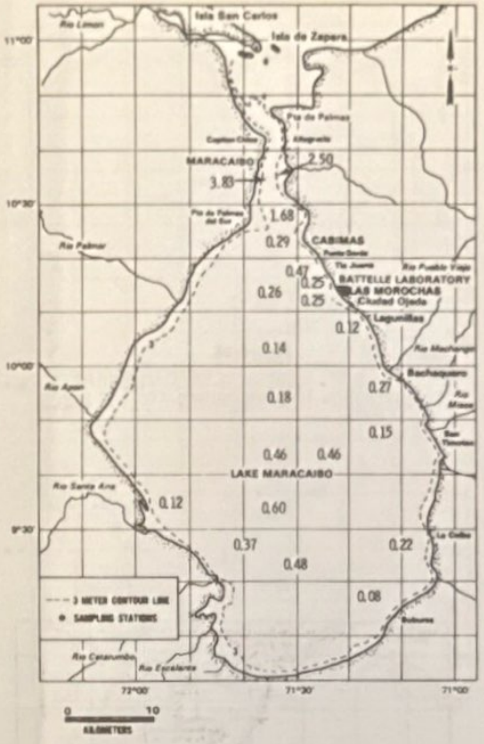


Figure 12
Analysis of water samples for extractable organics (January 1974) 1m from bottom, conc. in ppm

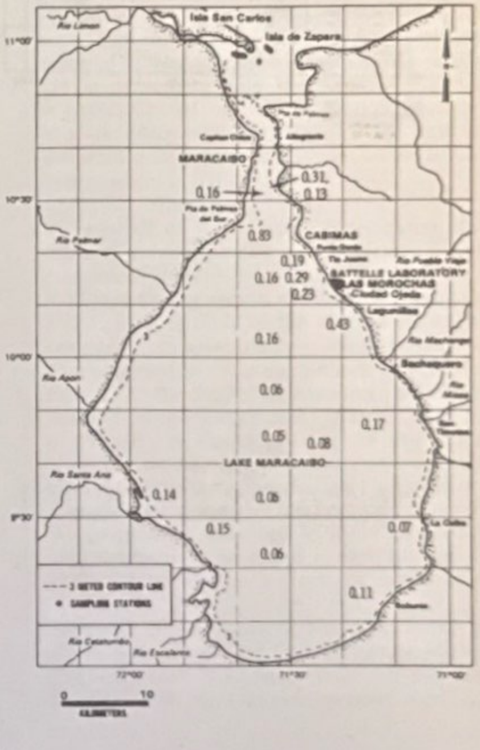


Figure 13
Analysis of water samples for extractable organics (February 1974) 1m depth, conc. in ppm

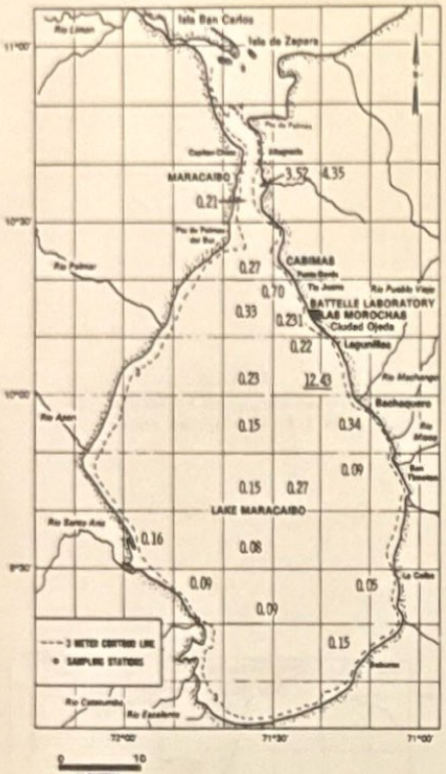


Figure 14
Analysis of water samples for extractable organics (February 1974) 1m from bottom, conc. in ppm

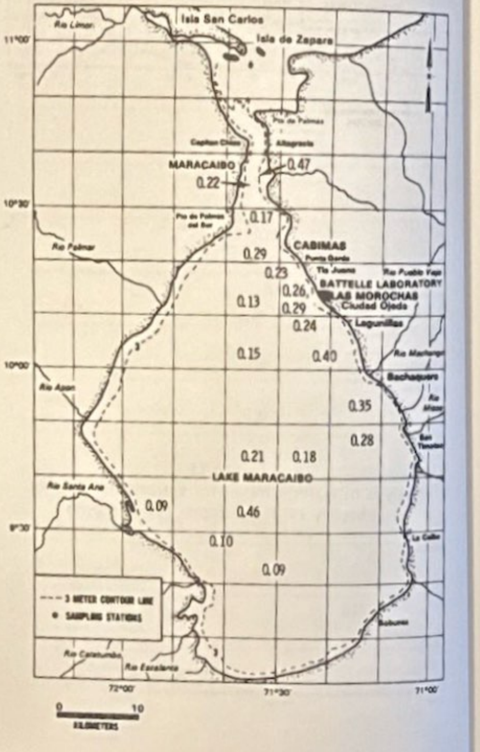


Figure 15
Analysis of water samples for extractable organics (March 1974) 1m depth, conc. in ppm

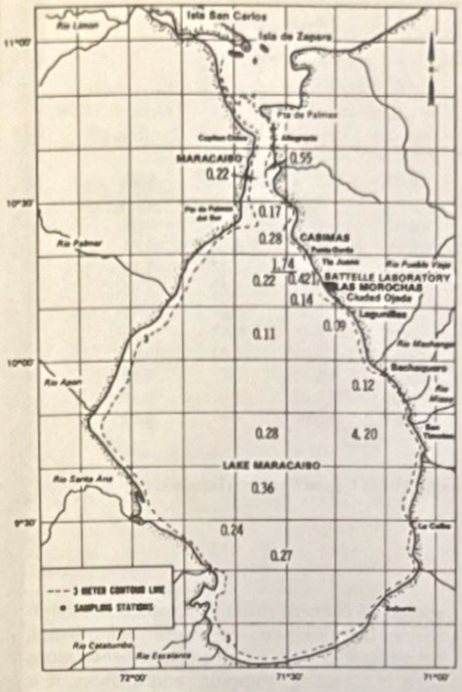


Figure 16
Analysis of water samples for extractable organics (March 1974) 1m from bottom, conc. in ppm

Examination of the Hydrocarbon Composition of Selected Lakewater Extracts

Several of the extracts having values greater than 0.5 ppm were subjected to more detailed examination of their organic composition. The procedure used, adapted from one given by Blumer (1970), was to chromatograph the extract on deactivated silica gel, followed by a gas chromatographic (GC) investigation of the separated fractions (see Appendix A).

Table 4 shows the quantitative results obtained from gas chromatographic investigation of 12 lakewater extracts separated with the silica gel column technique. An inspection of the data shows that there is a reasonable agreement between values for extractable organics as measured by IR, and the values for the total amount found in the separated fractions by GC, only in those cases where carbon tetrachloride has been used exclusively as the extracting solvent. When the extractable organic content by IR is compared with the organic material found by GC from hexane extracts of duplicate water samples, less material is found in the hexane extracts than in the carbon tetrachloride extracts. This is consistent with the increased polarity of carbon tetrachloride, which is a better solvent for nonhydrocarbons such as plant lipids. The existence of lipid material in several carbon tetrachloride extracts of lake water has been indicated by the identification of a band in the infrared spectrum at 1715 cm^{-1}, characteristic of carbonyl compounds.

The data obtained on the water extract fractions further show that the bulk of the material is more polar than either saturate or aromatic hydrocarbons since it requires methanol, a very polar solvent, to elute most of it from the gel column. In no case was the amount of saturate material recovered greater than 15% of the total, and there was no GC evidence of any material in the aromatic fractions. These results are in agreement with the findings of other investigations of extractable organic material in water (Brown et al., 1974) that the bulk of extractable organic material in water is not hydrocarbon in nature.

In one-half of the samples investigated, the material present in the saturate fraction was composed principally of an "envelope" of n-alkanes having carbon numbers between C_{20} and C_{27}. A portion of the chromatogram of saturates from a sample containing the highest concentration of these compounds is shown in Figure 17. The high "background" shown in this and subsequent chromatograms is because of "bleed" of liquid phase from the single SE-30 column used for these studies; thus only that material above the dashed line is representative of the sample. The envelope of paraffin compounds observed in these samples is very similar to those found in early samples of carbon tetrachloride extracts of lake water.

Although the quantity of n-alkanes found in these more recent samples is very much smaller (highest value: 0.22 mg/ℓ) than the previous ones (over 50 mg/ℓ in two samples), it still is a

Table 4

Gas chromatographic analysis of water extract fractions[1]

Month	Station Number	Extracting Solvent[1]	Hydrocarbon Concentration by GC (mg/ℓ): Saturate/Olefin Fraction	Aromatic Fraction	Polar Fraction	Total	Extractable Organic by IR (mg/ℓ)[1]
December, 73	27	CCl_4	0.22[2]	<0.01	5.78	6.00	6.05
December, 73	7	Hexane	0.10[2]	<0.01	0.29[3]	0.39	0.84
January, 74	6	Hexane	0.03[2]	<0.01	0.11[4]	0.14	0.94
December, 73	24	CCl_4	0.16[2]	<0.01	4.92[3]	5.08	3.78
December, 73	14	Hexane	0.12[2]	<0.01	0.05[3]	0.17	1.17
September, 73	17	CCl_4	0.06[2]	<0.01	2.76	2.82	1.65
January, 74	16	Hexane	<0.01	<0.01	0.06[4]	0.06	0.72
January, 74	10	Hexane	<0.01	<0.01	0.17[4]	0.17	0.55
January, 74	8	CCl_4	<0.01	<0.01	0.03[3]	0.03	2.04
January, 74	9	Hexane	<0.01	<0.01	<0.01	—	0.59
January, 74	27	Hexane	<0.01	<0.01	<0.01	—	0.59
January, 74	14	Hexane	<0.01	<0.01	<0.01	—	0.83

[1] For details of procedure, see Appendix A

[2] Saturate fraction almost entirely composed of n-alkanes ranging from C_{20} to C_{27} (see Figure 17)

[3] Two broad envelopes as in Figure 21

[4] Three broad envelopes as in Figure 20

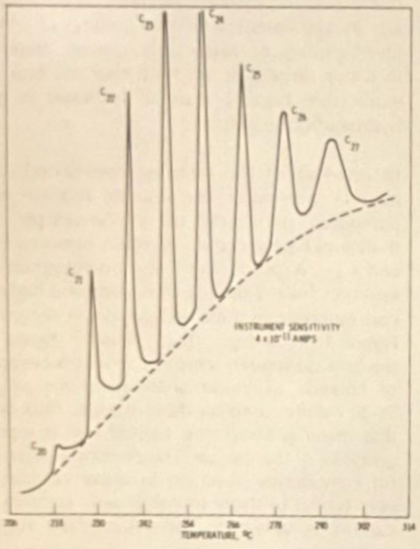


Figure 17

Gas chromatogram of fraction 1 (saturate/olefin) of lakewater sample - Station 27 (December) - extracting solvent: CCl_4

source of concern that this apparent contamination has not been completely eliminated, particularly in view of the extreme precautions used in sampling, shipping, and analysis. It is difficult to believe that the bulk of the hydrocarbon composition of Lake Maracaibo is composed of n-alkanes with carbon numbers of C_{20} to C_{27}, since these compounds are readily degraded by microorganisms and are extremely insoluble in water.

Gas chromatograms of polar fractions of lakewater extracts are shown in Figures 18 through 21. The chromatograms generally show the presence of a variety of high boiling material. The material in the sample from Station 17 (Figure 19) differs from that of Station 27 (Figure 18) only in an absence of material emerging between 194 and 230°C. A three-peak envelope appeared to be a characteristic of a number of samples; Figures 19 and 20 are representative of this type of sample. For the polar fractions of four of the samples inspected, two envelopes were detected, one in the region of 230°C and the other from 240 to 260°C. Figure 21 typifies this type of sample.

The data obtained on the polar material does not permit a definite conclusion as to its com-

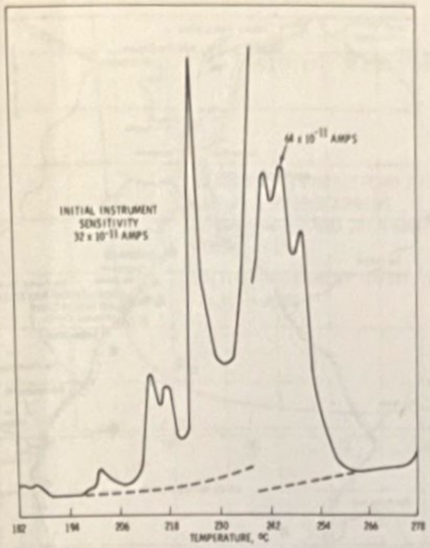


Figure 18

Gas chromatogram of fraction 3 (polar fraction) of lakewater sample - Station 27 (December) - extracting solvent: CCl_4

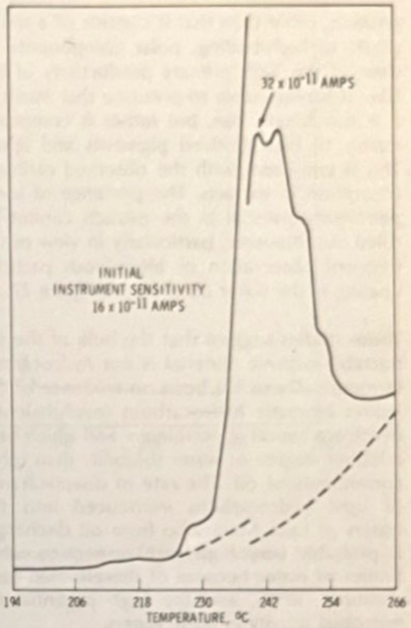


Figure 19

Gas chromatogram of fraction 3 (polar fraction) of lakewater sample - Station 17 (September) - extracting solvent: CCl_4

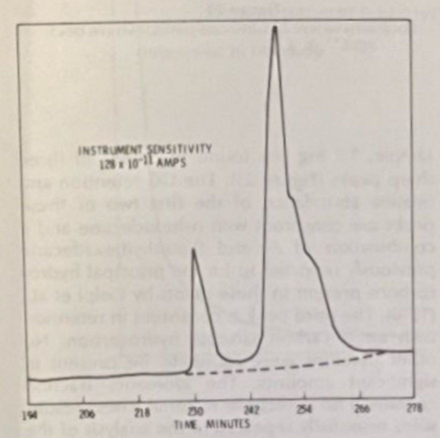


Figure 20

Gas chromatogram of fraction 3 (polar fraction) of lakewater sample - Station 10 (January) - extracting solvent: Hexane

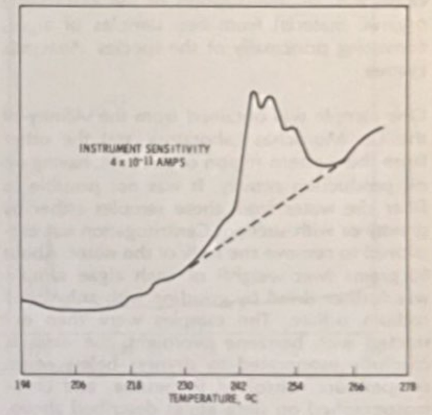


Figure 21

Gas chromatogram of fraction 3 (polar fraction) of lakewater sample - Station 24 (November) - extracting solvent: CCl_4

position, other than that it consists of a multiplicity of high-boiling, polar components. In view of the high primary productivity of the lake, it is reasonable to presume that much of it is not bituminous, but rather is composed mainly of plant-derived pigments and lipids. This is consistent with the observed carbonyl absorption in extracts. The presence of some petroleum material in the extracts cannot be ruled out, however, particularly in view of the frequent observation of bituminous particles floating in the water column (see Figure 22).

These studies suggest that the bulk of the extractable organic material is not hydrocarbon in nature. There has been no evidence of the lighter aromatic hydrocarbons (naphthalenes) which are typical of petroleum and which have a higher degree of water solubility than other constituents of oil. The rate of disappearance of light hydrocarbons introduced into the waters of Lake Maracaibo from oil discharges is probably very high with respect to other bodies of water because of the elevated temperature (30°C), and the high potential for microbial activity in these waters.

The Hydrocarbon Content of Blue-Green Algae *(Anacystis cyanea)*

The last of the analytical studies related to the organic content of the waters of Lake Maracaibo was an investigation of the extractable organic material from two samples of algae, consisting principally of the species *Anacystis cyanea*.

One sample was obtained from the vicinity of the Las Morochas Laboratory and the other from the western region of the lake, having no oil production activity. It was not possible to filter the water from these samples either by gravity or with suction. Centrifugation was employed to remove the bulk of the water. About 10 grams (wet weight) of each algae sample was further dried by grinding with anhydrous sodium sulfate. The samples were then extracted with benzene overnight, the extracts carefully evaporated to dryness below room temperature, dissolved in hexane, and chromatographed on silica gel as described above. Gas chromatograms of the saturate/olefin and polar fractions of these two samples are shown in Figures 23 through 26. Of the 29 milligrams of material extracted from the Las Morochas algae sample, 1.9 mg was found to consist of three sharp peaks (Figure 23). The GC retention and relative abundance of the first two of these peaks are consistent with n-hexadecane and a combination of 7- and 8-methylhexadecane previously reported to be the principal hydrocarbons present in these plants by Gelpi et al., (1970). The third peak is consistent in retention with an 18-carbon saturate hydrocarbon. No other paraffins were found to be present in significant amounts. The aromatics fraction contained no detectable material. These results were essentially repeated in the analysis of the saturate fraction of the algae taken from the western part of the lake, except that only 0.4 mg of the 21.1 mg extracted was composed of these compounds (Figure 24).

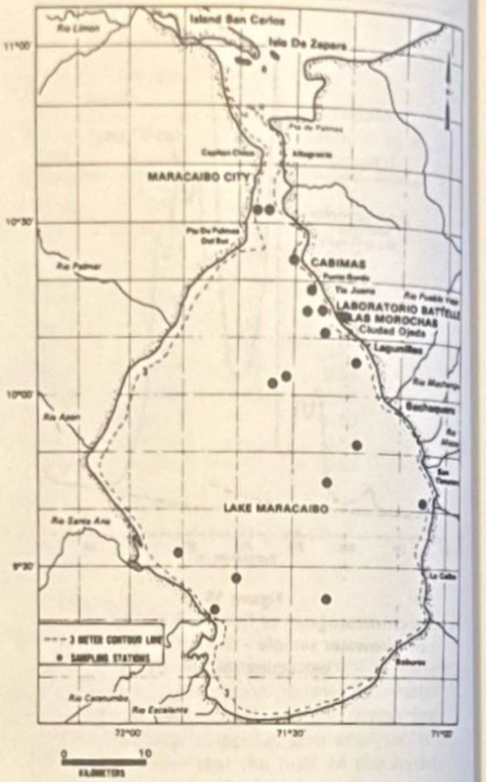


Figure 22
Locations where bituminous particles have been observed in lake water

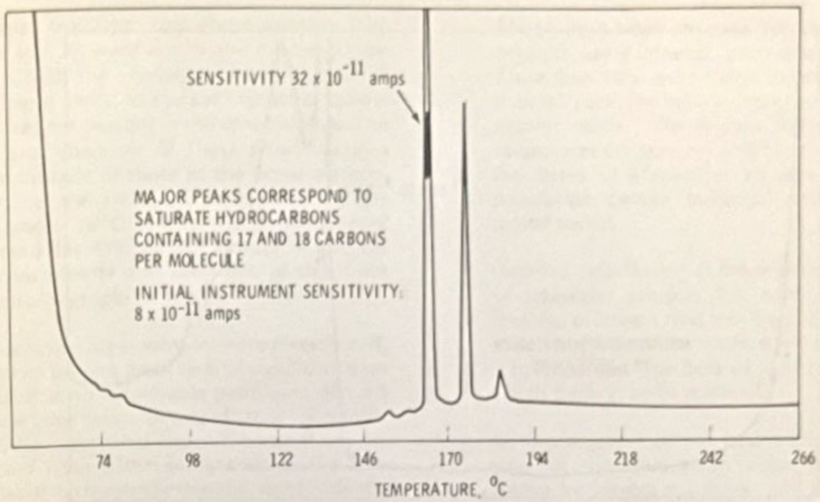


Figure 23
Gas chromatogram of saturate fraction of blue green algae *(Anacystis cyanea)* taken from oil producing region of lake

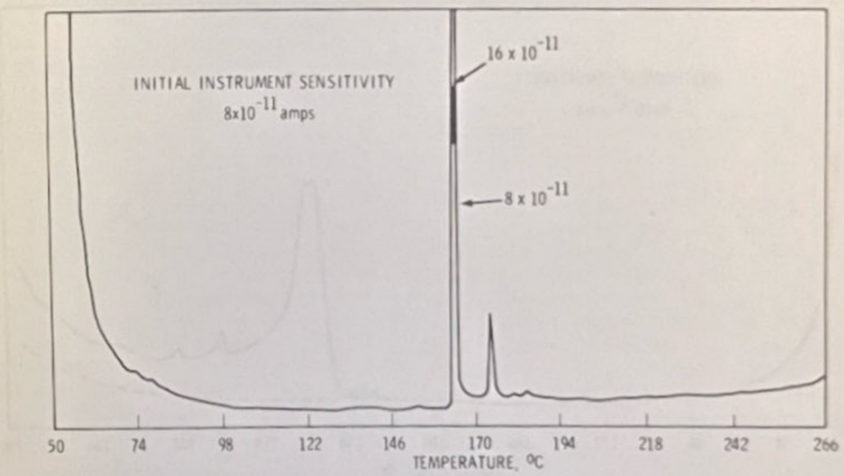


Figure 24
Gas chromatogram of saturate fraction of blue-green algae *(Anacystis cyanea)* taken from western (non-producing) region of lake

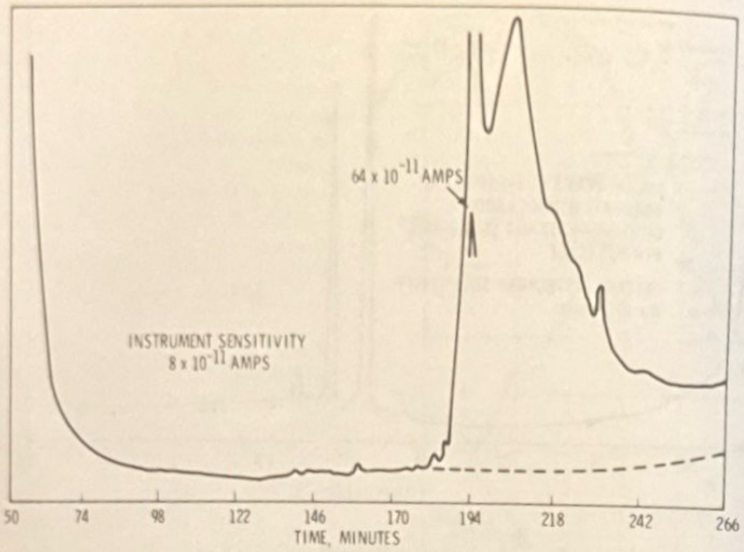


Figure 25
Gas chromatogram of polar fraction of blue-green algae *(Anacystis cyanea)* taken from oil producing region of lake

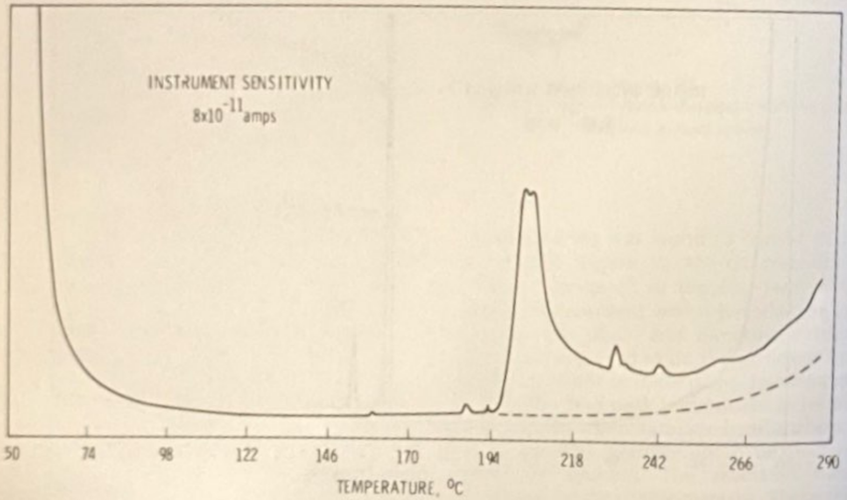


Figure 26
Gas chromatogram of polar fraction of blue-green algae *(Anacystis cyanea)* taken from western (non-producing) region of lake

The polar fractions from these samples (Figures 25 and 26) were similar and relatively nondescript, with the exception of one sharp peak emerging at 194°C in the Las Morochas sample which was not present in the other sample. The shape and character of these polar fractions are reminiscent of those of the water extracts, except that the average boiling point appears to be about 50°C lower. The polar material accounted for 67% of the extract of the Las Morochas sample and only 26% of that from the western sample.

Since only two algae samples were investigated, we cannot place a great deal of significance on the fact that no identifiable petroleum derived hydrocarbons were detected. It is of significance, however, that organic material can be extracted from plants indigenous to the lake and that of the material extracted, some is identifiable as hydrocarbon in nature.

Summary

- Approximately 200 samples of lake water taken from Lake Maracaibo during a seven month period between September 1973 and March 1974 were analyzed for extractable organics using infrared spectrophotometry. More than 90% were found to contain less than 0.5 parts per million (ppm) extractable organic matter. The highest frequency of values over 0.5 ppm occurred in the area of the Straits of Maracaibo, an area of high population density, industrial activity, and tanker traffic.
- Detailed examination of the organic extracts of lakewater samples has confirmed the findings of others that less than 20% of the material extracted by carbon tetrachloride is hydrocarbon. The bulk of the extract was a high boiling, polar material.
- An investigation of the hydrocarbon contents of the blue-green algae *Anacystis cyanea* by solvent extraction and gas chromatography detected no hydrocarbons which would indicate the presence of petroleum. A few paraffin compounds were detected which had properties consistent with biogenic hydrocarbons previously reported to be present in these plants.

ANALYSIS OF COMMERCIALLY IMPORTANT FISH SPECIES FOR HYDROCARBONS

Introduction

The analysis of fish tissues for hydrocarbons was undertaken in order to investigate a number of factors related to the fate of oil in Lake Maracaibo. Hydrocarbons have been demonstrated to enter into biological tissue when fish are exposed to oil/water mixtures (Vaughan, 1973). Thus, in view of the oil production in Lake Maracaibo, one might expect a significant exposure of the indigenous biotic species to petroleum hydrocarbons and a resulting direct accumulation in the tissues.

Another possible mechanism for the introduction of petroleum hydrocarbons into tissue is via transfer from other organisms, both plant and animal, through food chains. For instance, the existence of some naturally occurring hydrocarbons in the tissues of some carnivore fish is postulated to be a consequence of food chain transfer of plant hydrocarbon through plankton feeders (Blumer and Thomas, 1965). Food chain mechanisms present the possibility of accumulation of specific types of compounds in tissue far in excess of what would be expected from a consideration of the surrounding background concentrations.

Many of the major commercial species of fish from Lake Maracaibo, represent positions near the top of the food chain; therefore petroleum hydrocarbons accumulated through food chain transfer would be most likely to be present in these species. To avoid confusion as to whether hydrocarbon was actually present in the tissue (as opposed to hydrocarbon on the surface or in the gut), the analyses were limited to samples of dissected muscle tissue. Since the muscle tissue of commercial fish species represents a significant portion of the diet of the local population of the Maracaibo area, the analytical results take on even more significance.

Sample Selection and Analytical Methods

The fish samples from Lake Maracaibo which were analyzed for hydrocarbons represent a range of six species from as many locations as collection success would allow. Twelve curvina samples from seven areas representing the geographical extremes of the lake have been analyzed, robalo and lisa from the north, northwest, and the Bay of Tablazo have also been examined. Bocachico and manamana are only found in the south because of low salinity preference and were analyzed for a comparison with species that frequent the oil producing areas. On the other hand, the white shrimp prefer higher salinity present in the northern portions of the lake.

Fish from outside Lake Maracaibo were analyzed in order to evaluate the hydrocarbon distribution in fish not directly exposed to petroleum production. These consisted of: spotted sea trout from Galveston Beach, Texas; perch from Grays Harbor, Washington; trout from the Merida trout hatchery in Venezuela; and bocachico from a freshwater pond located near Lake Maracaibo and known to be free from the influence of oil.

It was anticipated at the outset that the hydrocarbon levels present in Lake Maracaibo environmental samples would be considerably lower than in samples subjected to lethal concentrations of petroleum hydrocarbons. Therefore, rather elaborate methodology was developed during the course of this program which permitted the detection and quantitation of n-alkanes and methylnaphthalenes at levels of about 7 and 4 parts per billion, respectively. These analyses represent an extension of previously reported work (Blaylock et al., 1973). Recovery validation studies are discussed in Appendix B.

A detailed discussion of the procedures used for the analysis of samples for total saturates, total aromatics, n-alkanes, and methylnaphthalenes is given in Appendix A. During the course of the program, two different extracting solvents were employed. Initially, carbon tetrachloride was used to extract the hydrocarbons from the digested tissue. This solvent had the advantages of being completely free from traces of hydrocarbons and of permitting the use of infrared measurements to determine the effectiveness of the cleanup procedures. However, carbon tetrachloride did not provide a sharp separation between saturates and aromatics compounds. In addition, our validation studies showed that only about 20% of added polynuclear aromatic hydrocarbon could be recovered from tissue with this solvent. Thus, for the latter phase of the work, n-hexane was used as the extracting solvent. Validation experiments with methylnaphthalenes and polynuclear aromatics demonstrated that this solvent gives superior results in terms of aromatics recoveries. However, this solvent was occasionally found to be contaminated with n-alkanes at levels of about 0.1 part per billion. This was sufficient to interfere with the analysis for n-alkanes at levels requiring maximum sensitivity. Thus, levels of n-alkanes were reported only when concentrations were at least five times in excess of those found in a corresponding procedural blank.

Detection and quantitation of hydrocarbons was performed with a gas chromatograph (GC), using a flame ionization detector. Shown in Figure 27 is a gas chromatogram showing response of n-alkanes at the maximum instrument sensitivity used, which provided approximately 50% scale inflection with 8.7 x 10^{-3} ug of C_{19} n-alkane. Figure 28 illustrates the retention time and peak conformation of a group of pure methyl substituted naphthalene compounds which were compared to the fish tissue extracts for qualitative identification.

A procedural blank was included with each group of four tissue samples to determine solvent background and procedural contamination (Figure 29). This background was subtracted from the appropriate fish extract samples prior to calculating concentrations of total hydrocarbons. A gas chromatogram displaying the contamination detected from a 600-fold concentration of the CCl_4 used in the procedure is shown in Figure 30. The major peaks which occur in the concentrated CCl_4 do not occur in the procedural blank, apparently being selectively lost during the extraction or chromatographic steps.

Characteristics of Gas Chromatograms of Tissue Extract Fractions

Gas chromatograms of the saturate extract fractions of curvina tissues from Stations 2 and 29 are shown in Figures 31 and 32, respectively. Station 2 is located in the northeast section and Station 29 is in the extreme south end of the lake (see map showing station locations: Figure 5 and on inside cover). Two peaks in the chromatogram of the curvina from Station 29 occurred close to the retention time of C_{17} and C_{18} n-alkanes (approximately 23.5 and 25.5 minutes, respectively). This sample was spiked with pure n-alkanes and the resulting chromatogram is illustrated in Figure 33. The retention times of the two peaks in the sample are consistent with n-heptadecane and n-octadecane.

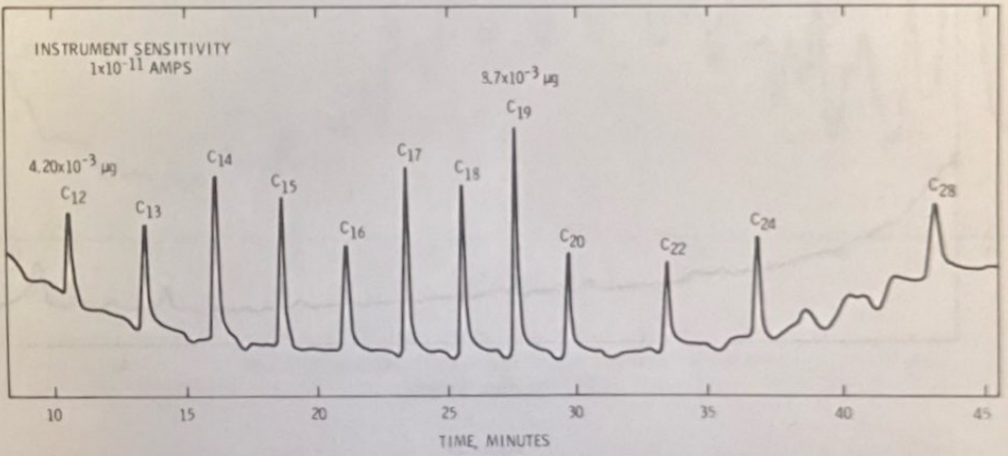


Figure 27
Gas chromatogram of normal paraffins in CCl_4 solvent

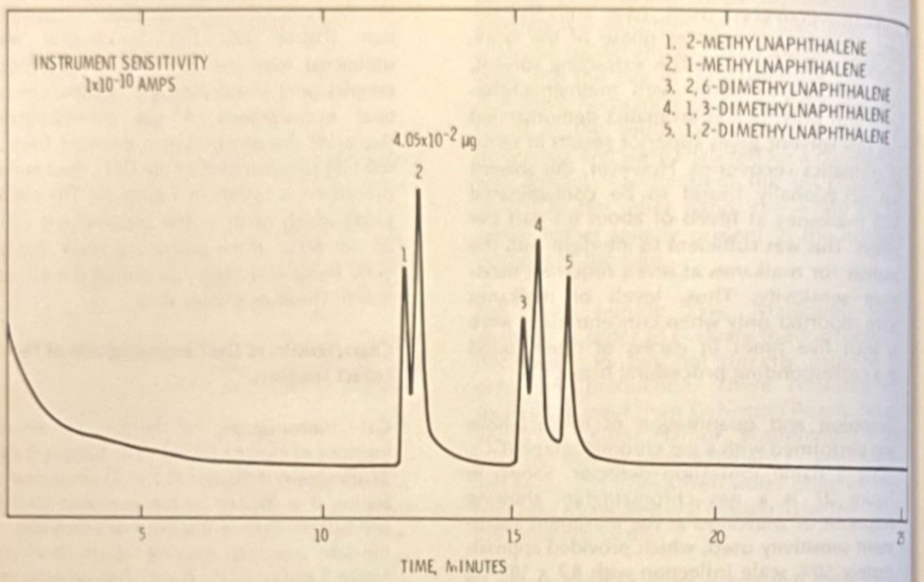


Figure 28

Gas chromatogram of methylnaphthalenes in CCl_4 solvent

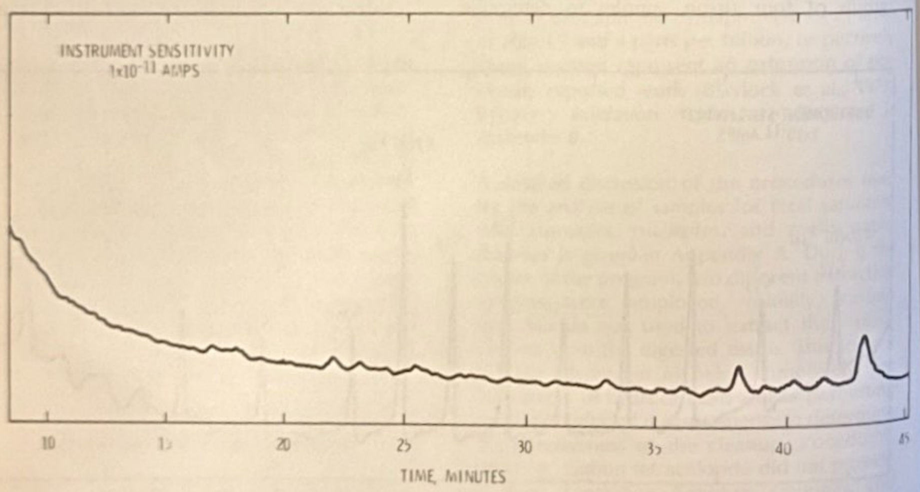


Figure 29

Gas chromatogram of the saturate fraction from a procedural blank

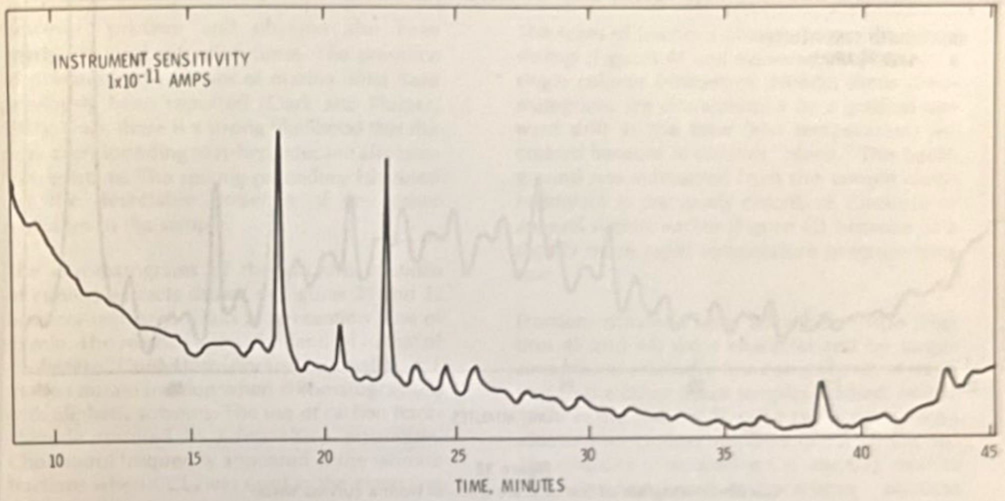


Figure 30

Gas chromatogram of Burdick and Jackson CCl_4, 600-fold concentration

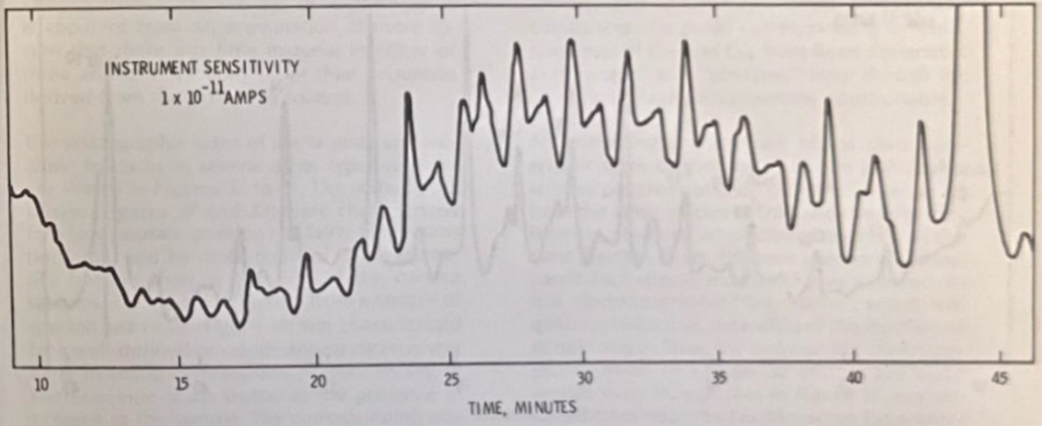


Figure 31

Gas chromatogram of the saturate fraction from a curvina tissue extract T338, Station 2

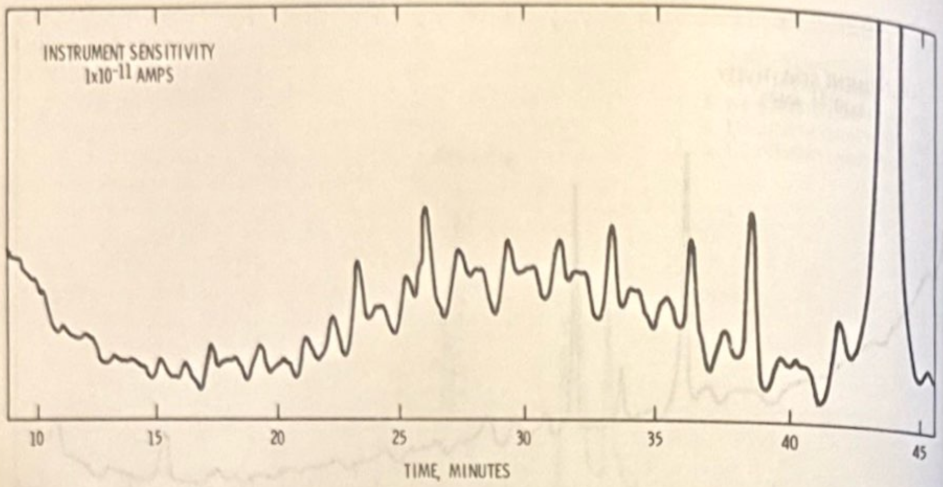


Figure 32
Gas chromatogram of the saturate fraction from a curvina tissue extract T356(B), Station 29

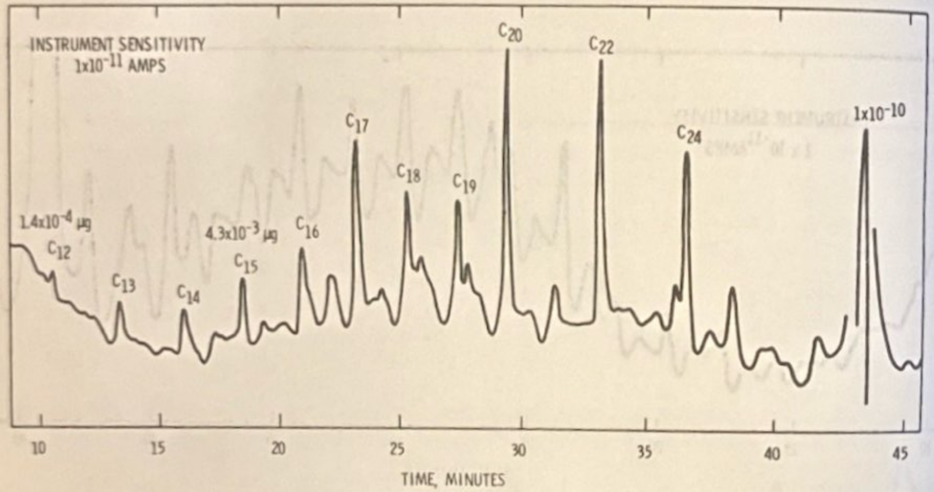


Figure 33
Gas chromatogram of the saturate fraction from a curvina tissue extract T356(B), Station 29, spiked with n-alkanes

However, pristane and phytane also have nearly identical retention times. The presence of pristane in the tissues of marine biota have previously been reported (Clark and Blumer, 1967). Thus, there is a strong likelihood that the peak corresponding to n-heptadecane also contains pristane. The spiking procedure has ruled out the detectable presence of any other n-alkanes in the sample.

The chromatograms of the saturate fractions of curvina extracts shown in Figures 31 and 32 both contain large peaks at a retention time of 43 min. The retention time is identical to that of cholesterol. Cholesterol does not normally occur in the saturate fraction when chromatographed with aliphatic solvents. The use of carbon tetrachloride resulted in a less sharp separation. Cholesterol frequently appeared in the saturate fractions when CCL_4 was used as the extracting solvent.

Gas chromatograms relating to the analysis of the aromatic fractions of the curvina samples are shown in Figures 34-36. Figure 34 shows the chromatogram of a procedural blank, and Figures 35 and 36 correspond to curvina tissue samples from Stations 2 and 29 respectively. It is apparent from an examination of these results that there was little material in either of these aromatic fractions other than impurities derived from the extracting solvent.

Chromatographic scans of the saturate and aromatic fractions of several other types of tissue are shown in Figures 37 to 44. The scans of the robalo (Figures 37 and 38) were characterized by a low saturate envelope at fairly long retention time, and by more material in the aromatics fraction than in the case of the curvina samples. The saturate fraction from a sample of spotted sea trout (Figure 39) was characterized by a well-defined envelope appearing relatively early in the chromatogram. A spike on top of the envelope is attributed to the presence of pristane in this sample. The corresponding aromatics fraction (Figure 40) was characterized by having most of the material appearing at longer retention times, indicative of higher boiling material.

The scans of fractions obtained from the white shrimp (Figures 41 and 42) were obtained on a single column instrument. Hence, these chromatograms are characterized by a gradual upward drift as the time (and temperature) increased because of column "bleed." This background was subtracted from the sample chromatogram as previously described. Cholesterol appears slightly earlier (Figure 42) because of a slightly more rapid temperature programming rate.

Fractions obtained from bocachico tissue (Figures 43 and 44) were characterized by larger amounts of relatively few components in contrast to the other tissue samples studied. Major peaks in the saturate fraction correspond to n-alkanes with carbon numbers of 15, 17 and 18. The difficulty of separating C_{17} anc C_{18} alkanes from the isoprenoid hydrocarbons pristane and phytane, on the column used for these studies makes the assignment of these peaks difficult. Whenever these peaks occurred in the saturate samples studied, they were not accompanied by a series of n-alkane peaks of comparable height, which have been described as a characteristic of petroleum contamination (Blumer and Sass, 1972). Hence, in these circumstances, the peaks corresponding to retention times of C_{17} and C_{18} have been designated as "pristane" and "phytane," even though the identity of these peaks remains questionable.

An interesting characteristic of gas chromatographic scans of the tissue samples is the similarity of patterns obtained on samples extracted from the same species of fish, and the large differences observed when chromatograms of the same fraction from different species are compared. Each species examined gave a characteristic chromatographic "fingerprint", which was quite reproducible, regardless of the location of sample origin. Thus, the two curvina chromatograms shown in Figures 32 and 33 are quite similar, even though that in Figure 32 was obtained from near the Las Morochas Laboratory and the other was taken from near the Catatumbo River. The differences in scans obtained among different species are likely a result of differences in diet and metabolism.

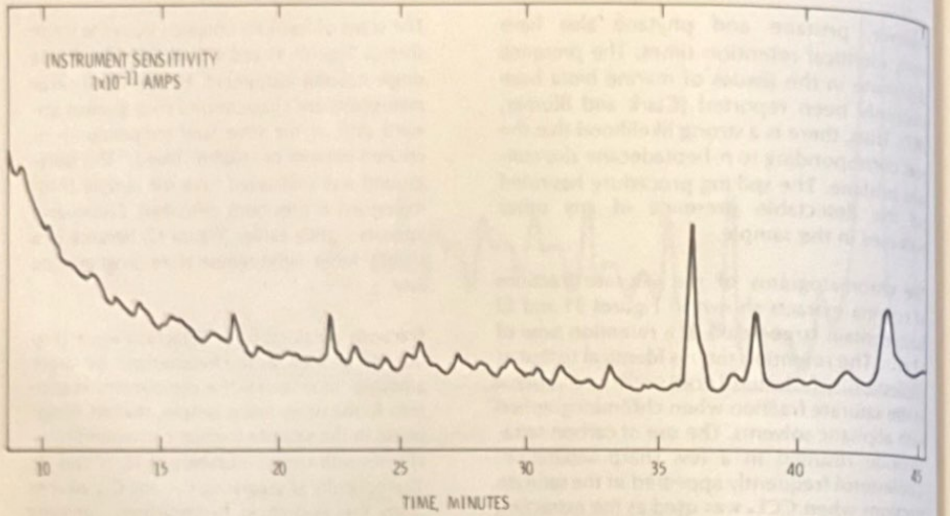


Figure 34
Gas chromatogram of the aromatic fraction from a procedural blank

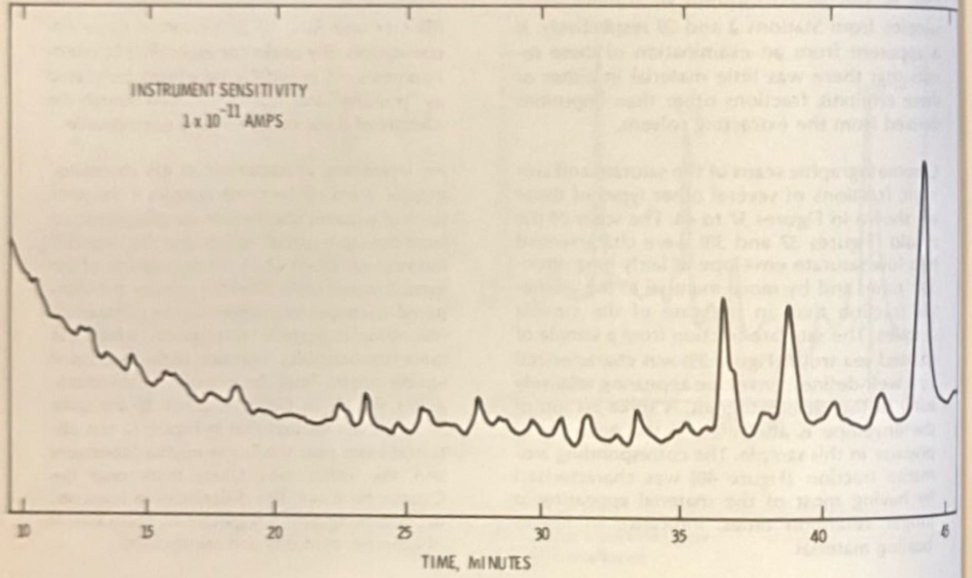


Figure 35
Gas chromatogram of the aromatic fraction from
a curvina tissue extract T338, Station 2

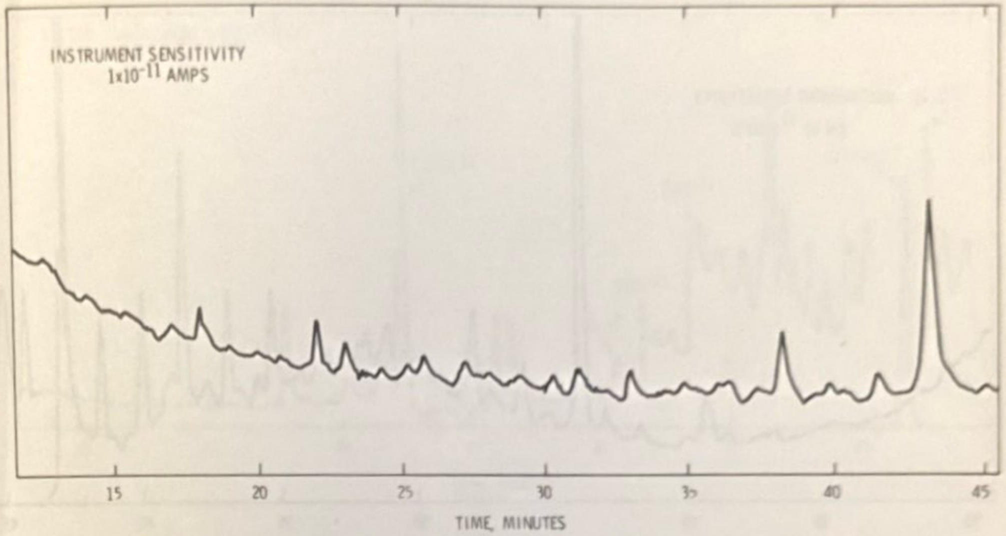


Figure 36
Gas chromatogram of the aromatic fraction from
a curvina tissue extract T356B, Station 29

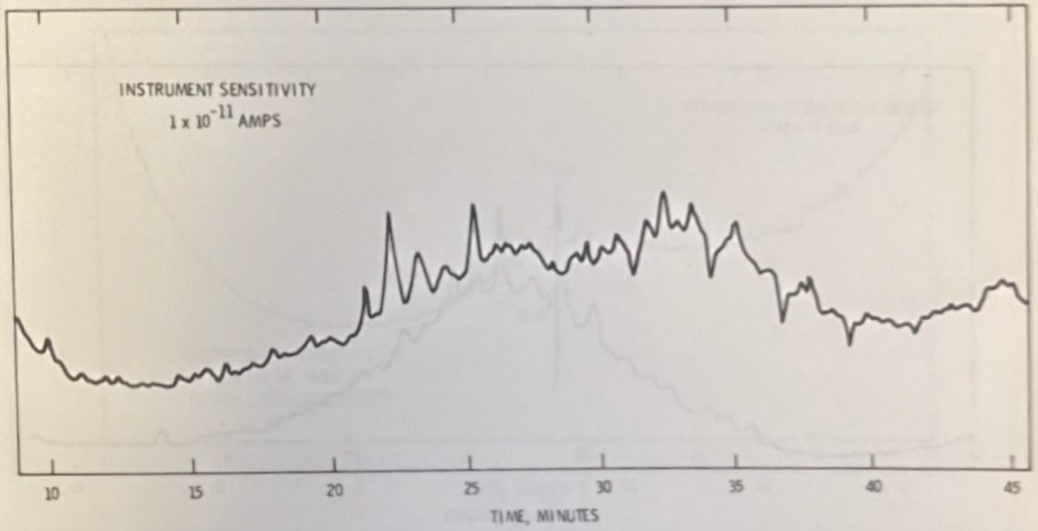


Figure 37
Gas chromatogram of the saturate fraction from
a robalo T346, Station 33

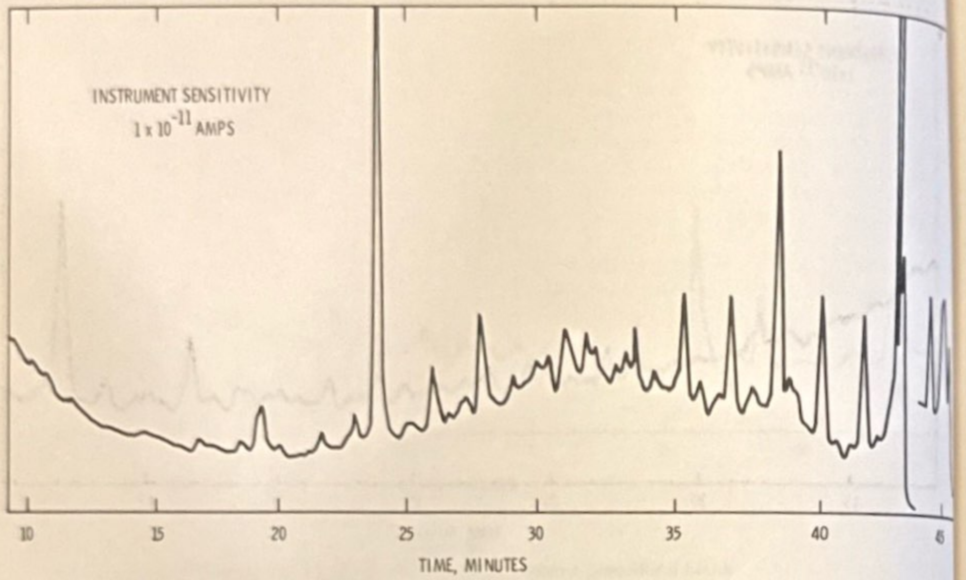


Figure 38
Gas chromatogram of the aromatic fraction from a robalo T346, Station 33

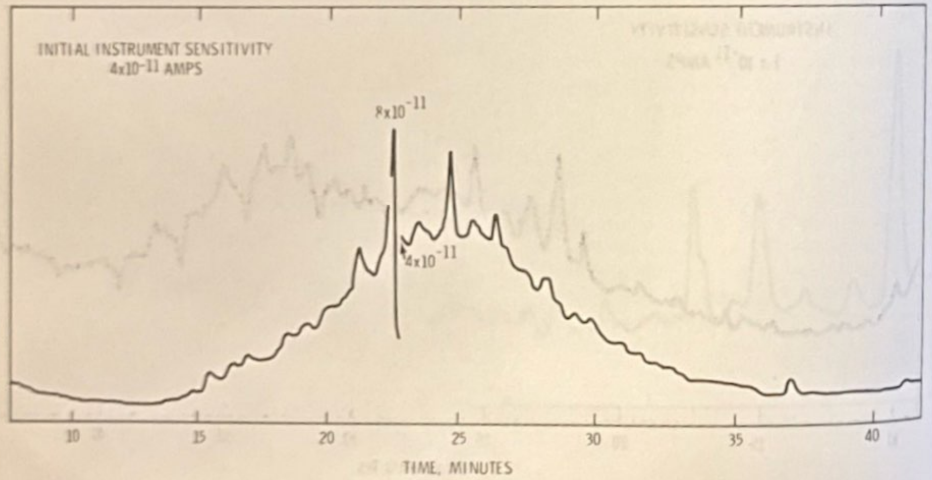


Figure 39
Gas chromatogram of the saturate fraction from a spotted sea trout (Galveston Beach 5-28-73)

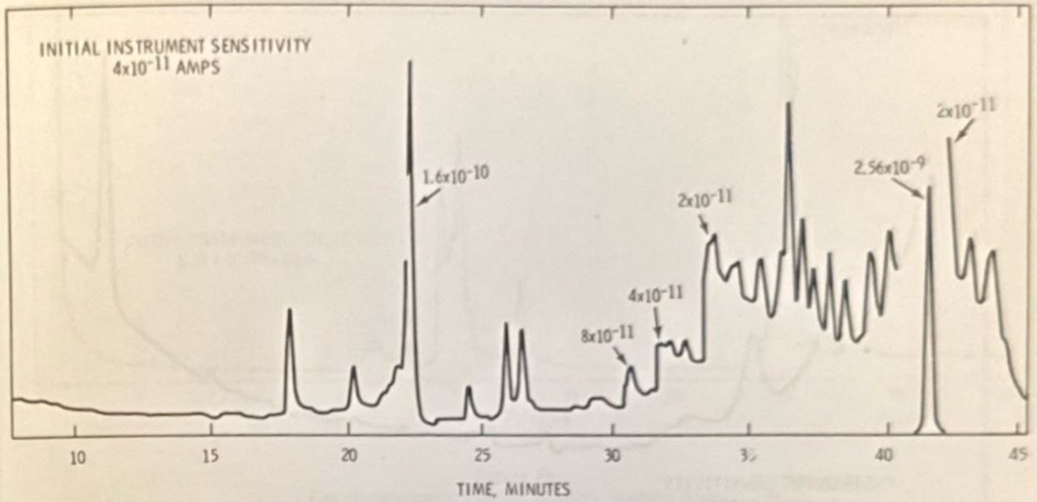


Figure 40
Gas chromatogram of the aromatic fraction from a spotted sea trout (Galveston Beach 5-28-73)

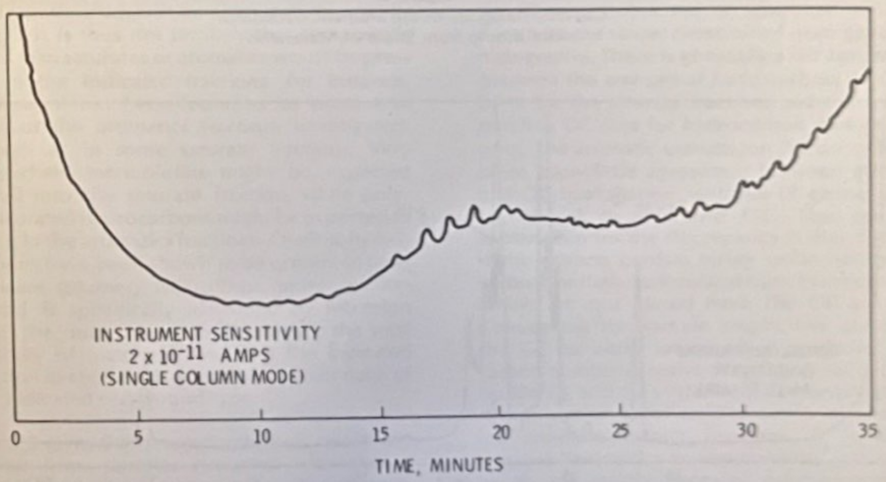


Figure 41
Gas chromatogram of the saturate fraction of white shrimp from Straits of Maracaibo

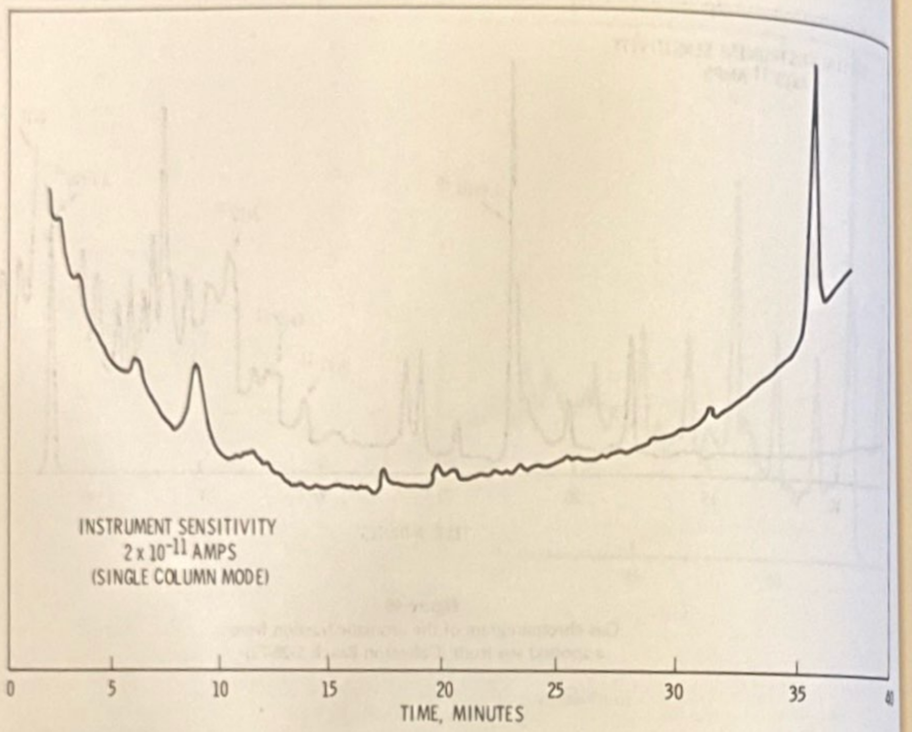


Figure 42
Gas chromatogram of the aromatic fraction of white shrimp from Straits of Maracaibo

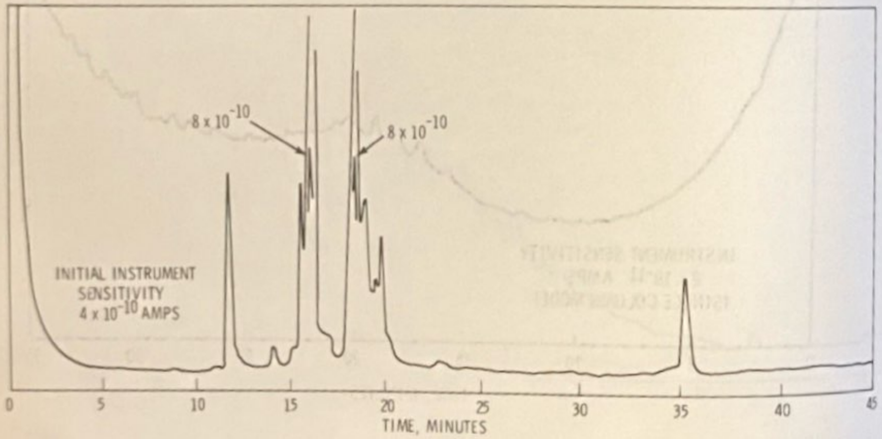


Figure 43
Gas chromatogram of the saturate fraction from a bocachico taken from a freshwater pond

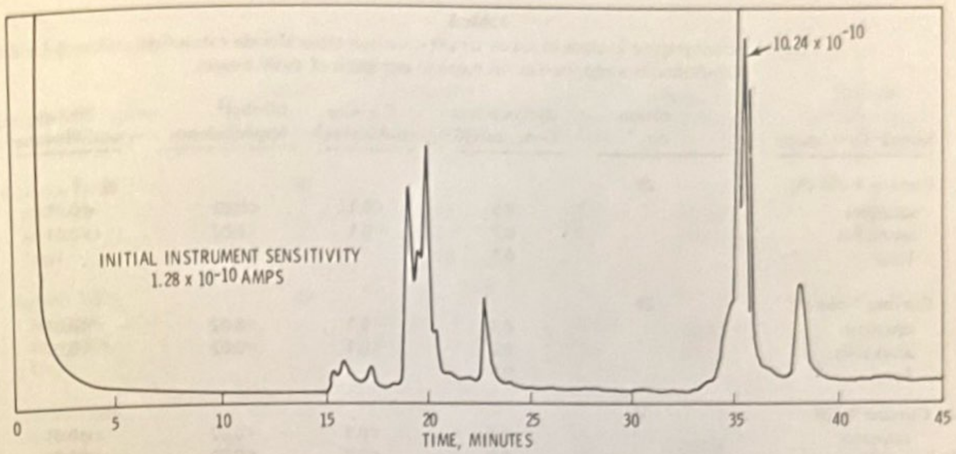


Figure 44
Gas chromatogram of the aromatic fraction from a bocachico taken from a freshwater pond

Quantitative Results

It is important in interpreting the data to recognize that the designation of tissue sample fractions as "saturates" and "aromatics" is based solely on their adsorption characteristics on silica gel. It is thus not unlikely that compounds other than saturates or aromatics would be present in the indicated fractions. For instance, cholesterol has been found to be present in most of the aromatics fractions investigated, as well as in some saturate fractions. Very long chain monoolefins might be expected to fall into the saturate fraction, while polyunsaturated hydrocarbons might be expected to occur in the aromatics fractions. Olefinic hydrocarbons have been shown to be present in biotic tissue (Blumer, 1967). Thus, unless a compound is specifically identified by retention time, the quantitative results refer to the total quantity of material present in the indicated fraction even though not all of the material is of the indicated compound type.

Table 5 gives the detailed analytical results obtained from samples extracted with carbon tetrachloride. Table 6 gives the results from samples extracted with hexane. The map in Figure 45 gives a summary of the data in terms of species, location of catch, and total quantity of hydrocarbons detected.

In addition to quantitation using gas chromatography, the hydrocarbon concentrations of tissue samples extracted with carbon tetrachloride were also measured using infrared methodology (see Appendix A). Table 7 gives a comparison of the IR values obtained on tissue extract fractions with the values determined from gas chromatography. There is generally a fair agreement between the amount of hydrocarbon reported by IR for the saturate fractions and the corresponding GC data for hydrocarbon plus cholesterol. The aromatic extracts, on the other hand, often show little agreement between infrared and GC quantitation, with the IR giving much higher values than the GC. The simplest explanation for the discrepancy is that the aromatic extracts contain either polar nonhydrocarbons or high molecular weight hydrocarbons which are not eluted from the GC column. Considering the sample origin, the ability of the GC to elute saturate hydrocarbons with carbon numbers greater than C_{30} (Triacontane, bp 304°C), and the evidence of carbonyl content (IR absorption band at 1715 cm^{-1}) in many of the aromatic extract fractions, it is likely that the "aromatics concentrations," reported by IR, are mostly traces of nonhydrocarbon lipid-derived material which the extraction and purification procedure has not removed. In addition, polyolefinic material, either present in the sample through biogenesis or through

Table 5

Gas chromatographic analysis of tissue samples: carbon tetrachloride extraction
(Concentrations reported as micrograms per gram of moist tissue)

Sample Description	Station No.[1]	Hydrocarbon Conc. by GC	C_{12}-C_{28} n-Alkanes[2]	Methyl[2] Naphthalenes	Pristane and Phytane[3]
Curvina T-354 (A)	29				
saturates		0.0	<0.1	<0.02	<0.01
aromatics		0.7	<0.1	<0.02	<0.01
Total		0.7			
Curvina T-354 (B)	29				
saturates		0.1	<0.1	<0.02	<0.01
aromatics		0.0	<0.1	<0.02	<0.01
Total		0.1			
Curvina T-308	13				
saturates		0.9	<0.1	<0.02	<0.01
aromatics		0.0	<0.1	<0.02	<0.01
Total		0.9			
Curvina T-365	34				
saturates		0.4	<0.1	<0.02	<0.01
aromatics		0.8	<0.1	<0.02	<0.01
Total		1.2			
Curvina T-335 (A)	2				
saturates		1.8	<0.1	<0.02	<0.01
aromatics		0.0	<0.1	<0.02	<0.01
Total		1.8			
Curvina T-335 (B)	2				
saturates		0.7	<0.1	<0.02	<0.01
aromatics		0.0	<0.1	<0.02	<0.01
Total		0.7			
Curvina T-342	17				
saturates[4]		-	-	-	-
aromatics		0.0	<0.1	<0.02	<0.01
Total		0.0			
Curvina T-331	3				
saturates		1.4	<0.1	<0.02	<0.01
aromatics		1.4	<0.1	<0.02	<0.01
Total		2.8			
Curvina T-356 (A)	29				
saturates		0.0	<0.1	<0.02	<0.01
aromatics		0.1	<0.1	<0.02	<0.01
Total		0.1			
Curvina T-356 (B)	29				
saturates		0.8	<0.1	<0.02	<0.01
aromatics		0.0	<0.1	<0.02	<0.01
Total		0.8			
Curvina T-338	2				
saturates		1.5	<0.1	<0.02	<0.01
aromatics		0.1	<0.1	<0.02	<0.01
Total		1.6			

Table 5 (continued)

Sample Description	Station No.[1]	Hydrocarbon Conc. by GC	C_{12}-C_{28} n-Alkanes[2]	Methyl[2] Naphthalenes	Pristane and Phytane[3]
Curvina T-339	18				
saturates		0.1	<0.1	<0.02	<0.01
aromatics		0.1	<0.1	<0.02	<0.01
Total		0.2			
Robalo T-327	14				
saturates		2.4	<0.1	<0.02	<0.01
aromatics		1.2	<0.1	<0.02	<0.01
Total		3.6			
Robalo T-346	33				
saturates		0.3	<0.1	<0.02	<0.01
aromatics		0.6	<0.1	<0.02	<0.01
Total		0.9			
Spotted Sea Trout (A)	Galveston Beach, Texas				
saturates		0.7	<0.1	<0.02	0.04
aromatics		1.0	<0.1	<0.02	<0.01
Total		1.7			0.04
Spotted Sea Trout (B)	Galveston Beach, Texas				
saturates		0.7	<0.1	<0.02	0.05
aromatics		1.7	<0.1	<0.02	<0.01
Total		2.4			0.05
Trout (A)	Merida hatchery, Venz.				
saturates		0.7	<0.1	<0.02	0.01
aromatics		0.4	<0.1	<0.02	0.12
Total		1.1			0.13
Trout (B)	Merida hatchery, Venz.				
saturates		2.5	<0.1	<0.02	0.26
aromatics		0.9	<0.1	<0.02	0.41
Total		3.4			0.67
Perch (A)	Grays Harbor, Wash.				
saturates		0.4	<0.1	<0.02	0.20
aromatics		0.4	<0.1	<0.02	0.11
Total		0.8			0.31
Perch (B)	Grays Harbor, Wash.				
saturates		0.4	<0.1	<0.02	0.19
aromatics		0.7	<0.1	<0.02	0.05
Total		1.1			0.24

[1] Map showing locations of stations, Figure 5

[2] The minimum detectable level for individual n-alkanes by gas chromatography is estimated at 0.007 μg/g based on a 50-gram tissue sample. Minimum level for individual methyl naphthalenes is 0.004 μg/g. Minimum detectable values of total n-paraffins (0.1 μg/g) and total methyl naphthlenes (0.02 μg/g) are estimated for considerations of baseline variation and instrument sensitivity. Recovery studies are discussed in the Appendix.

[3] Pristane and Phytane are difficult to separate from n-C_{17} and n-C_{18}, thus identification is tentative.

[4] Saturate fraction was contaminated with brownish forerun material. Rechromatography of this material on silica gel failed to remove the material.

Table 6

Gas chromatographic analysis of tissue samples: hexane extraction
(Concentrations reported as micrograms per gram of moist tissue)

Sample Description	Station No.[1]	Hydrocarbon Conc.	C_{12}-C_{28} N-Alkanes[2]	Methyl[2] Naphthalenes	Pristane and Phytane[3]
Bocachico T-367	31-32				
saturates		16.2	0.15	<0.02	7.06
aromatics		22.2	<0.1	<0.02	0.12
Total		38.4	0.15		7.18
Manamana T-369	31-32				
saturates		9.2	0.91	<0.02	7.34
aromatics		2.1	<0.1	<0.02	0.03
Total		11.3	0.91		7.37
Manamana T-370	31-32				
saturates		2.8	0.18	<0.02	1.54
aromatics		3.3	<0.1	<0.02	<0.01
Total		6.1	0.18		1.55
Robalo T-34	Bay of Tablazo				
saturates		0.6	<0.1	<0.02	0.16
aromatics		0.2	<0.1	<0.02	<0.01
Total		0.8			0.16
Robalo T-320	16				
saturates		0.4	<0.1	<0.02	0.12
aromatics		0.7	<0.1	<0.02	0.01
Total		1.1			0.12
Lisa T-23	2				
saturates		2.0	0.18	<0.02	1.37
aromatics		0.8	<0.1	<0.02	0.02
Total		2.8	0.18		1.39
Lisa T-87	16				
saturates		2.1	0.13	<0.02	1.50
aromatics		3.2	0.1	<0.02	0.43
Total		5.3	0.13		1.93
Bocachico (A)	Freshwater pond[4]				
saturates		9.1	0.85	<0.02	2.3
aromatics		3.4	-	<0.02	-
Total		12.5	0.85		2.3
Bocachico (B)	Freshwater pond[4]				
saturates		6.2	0.73	<0.02	3.1
aromatics		4.9	-	<0.02	-
Total		11.1	0.73		3.1
Shrimp (A) (6 individuals)	Straits of Maracaibo				
saturates		5.7	<0.1	<0.02	<0.01
aromatics		.3	<0.1	<0.02	<0.01
Total		6.0			
Shrimp (B) (7 individuals)	Straits of Maracaibo				
saturates		2.8	<0.1	<0.02	<0.01
aromatics		0.1	<0.1	<0.02	<0.01
Total		2.9			
Shrimp (C) (5 individuals)	Straits of Maracaibo				
saturates		3.0	<0.1	<0.02	<0.01
aromatics		0.2	<0.1	<0.02	<0.01
Total		3.2			
Shrimp Viscera (from 253 grams of shelled shrimp)	Straits of Maracaibo				
saturates		<0.1	<0.1	<0.02	<0.01
aromatics		<0.1	<0.1	<0.02	<0.01

[1] Map showing locations of stations, Figure 5.

[2] The minimum detectable level for individual n-alkanes by gas chromatography is estimated at 0.007 μg/g based on a 50-gram tissue sample. Minimum level for individual methyl naphthalenes is 0.004 μg/g. Minimum detectable values of total n-paraffins (0.1 μg/g) and total methyl naphthalenes (0.02 μg/g) are estimated from considerations of base-line variation and instrument sensitivity. Recovery studies are discussed in the Appendix.

[3] Pristane and phytane are difficult to separate from n-C_{17} and n-C_{18}, thus identification is tentative.

[4] Sample obtained from a freshwater pond in a region west of Lake Maracaibo. The pond is known to have no petroleum contamination or contact with the waters of Lake Maracaibo.

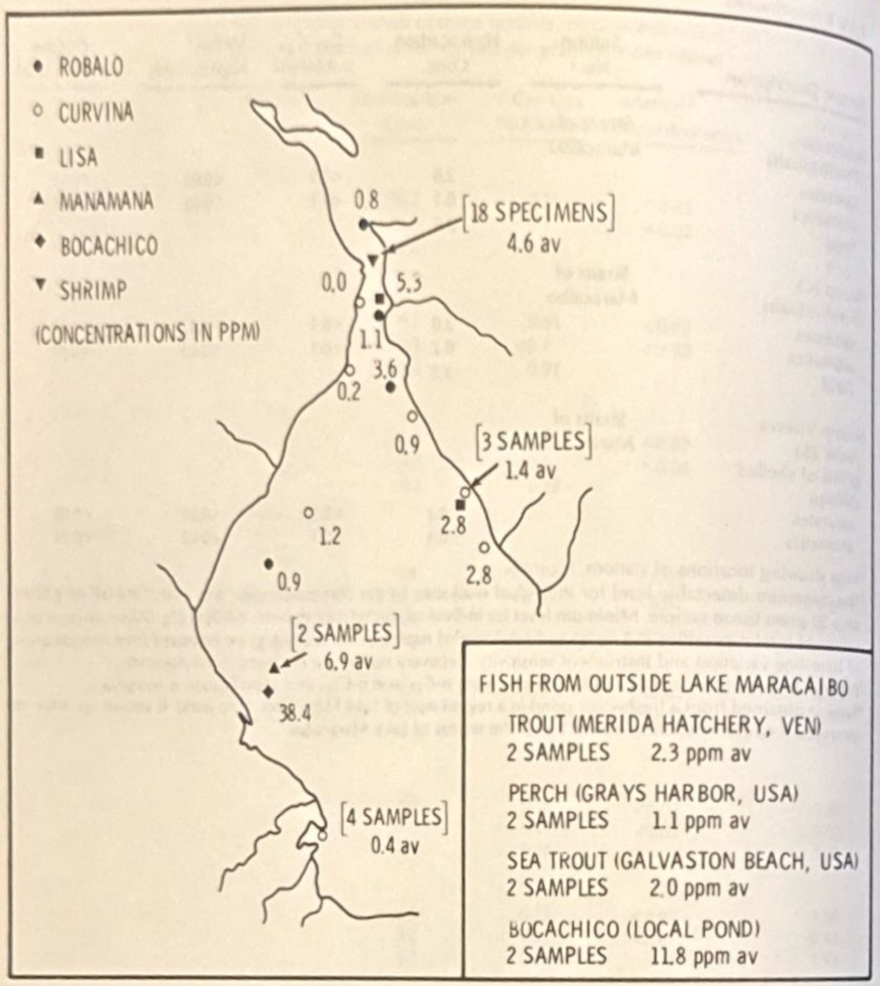


Figure 45
Analysis of tissue samples for total hydrocarbon (gas chromatography)

Table 7

Comparison of infrared absorption and gas chromatographic determination of hydrocarbon concentration in tissue samples.
(Concentrations reported as micrograms per gram of moist tissue)

Sample Description	Station No.	Hydrocarbon Conc. (IR)	Hydrocarbon Conc. (GC)	Sterol Conc. (GC)
Curvina	29			
saturates		0.3	0.0	0.0
aromatics		5.3	0.7	0.4
Curvina	29			
saturates		2.0	0.1	0.9
aromatics		2.1	0.0	0.0
Curvina	13			
saturates		3.0	0.9	0.4
aromatics		1.0	0.0	0.1
Curvina	34			
saturates		0.3	0.4	0.0
aromatics		3.3	0.8	0.3
Curvina	2			
saturates		1.8	1.8	0.7
aromatics		1.7	0.0	0.0
Curvina	2			
saturates		1.7	0.7	0.6
aromatics		2.8	0.0	0.0
Curvina	17			
saturates		-	-	-
aromatics		1.7	0.0	0.2
Curvina	3			
saturates		2.0	1.4	0.0
aromatics		6.9	1.4	3.6
Curvina	29			
saturates		0.0	0.0	0.0
aromatics		2.8	0.1	0.2
Curvina	29			
saturates		1.2	0.8	0.8
aromatics		1.6	0.0	0.0
Curvina	2			
saturates		2.5	1.5	1.6
aromatics		3.0	0.1	0.0
Curvina	18			
saturates		2.0	0.1	0.0
aromatics		2.4	0.1	0.3
Robalo	14			
saturates		2.7	2.4	0.0
aromatics		2.4	1.2	0.7

Table 7 (continued)

Sample Description	Station No.	Hydrocarbon Conc. (IR)	Hydrocarbon Conc. (GC)	Sterol Conc. (GC)
Robalo	33			
saturates		0.9	0.3	0.0
aromatics		2.0	0.6	1.0
Sea Trout	Galveston Beach, Texas			
saturates		2.7	0.7	0.0
aromatics		8.0	1.0	5.6
Sea Trout	Galveston Beach, Texas			
saturates		2.6	0.7	0.0
aromatics		11.9	1.7	10.5
Trout	Merida hatchery, Venz.			
saturates		0.3	0.7	0.0
aromatics		3.5	0.4	0.0
Trout	Merida hatchery, Venz.			
saturates		0.5	2.5	0.0
aromatics		7.7	0.9	0.0
Perch	Grays Harbor, Wash.			
saturates		0.8	0.4	0.0
aromatics		2.5	0.4	0.0
Perch	Grays Harbor, Wash.			
saturates		1.8	0.4	0.0
aromatics		2.8	0.7	0.0

alcohol dehydration on the silica gel column, would appear in the aromatics fraction.

The total hydrocarbon concentration in curvina tissue from seven different stations did not indicate any trend that would correlate with the oil producing areas. There were no detectable concentrations of n-alkanes or methylnaphthalenes in any of the curvina or robalo samples analyzed. Lisa obtained from Stations 2 and 16 contained 0.18 and 0.13 µg/g tissue of total n-alkanes. In addition, the total hydrocarbon content of the lisa samples was approximately 3 to 20 times higher than the total hydrocarbons found in the curvina. Samples of lisa from areas free from oil production could not be obtained.

The highest concentrations of hydrocarbons from fish tissue analyzed were found in the bocachico (38.4 µg/g) and manamana (11.3 µg/g), which are restricted to the south end of Lake Maracaibo because of a low salinity preference. Because of the very high value for total hydrocarbons found in the bocachico, two samples were obtained from a pond located to the west of the lake, south of Maracaibo City. This pond was fed from waters having no relation to those of Lake Maracaibo, and had no contact with petroleum. Again, the total amount of hydrocarbons found in these species of fish was quite high with respect to other species analyzed (11.1 and 12.5 µg/g). It is of interest to note that, although the total quantities of hydrocarbons in each of the samples from the pond were somewhat less than those found in the lake sample, the content of saturate hydrocarbons corresponding in retention time to n-pentadecane and phytane were higher for the pond samples than the lake sample.

The fish samples obtained from Puget Sound, Washington; Galveston Beach, Texas; and the Merida fish hatchery, Venezuela, did not contain detectable concentrations of n-alkanes nor methylnaphthalenes. The two trout contained larger concentrations of total hydrocarbons than the curvina and robalo.

Table 8 summarizes the quantities of individual alkane compounds found in tissue samples to date, including the naturally occurring pristane, and possibly phytane. Previous investigations conducted by Battelle-Northwest dealing with exposure of fish to crude oil and refined products have shown that tissue uptake of hydrocarbons from crude oils include both n-alkanes and methylnaphthalenes (Blaylock et. al., 1973). It is, therefore, significant that no detectable amounts of methylnaphthalenes have been found in any fish from Lake Maracaibo analyzed to date. The failure to detect methylnaphthalenes in those fish samples containing paraffins presents the likelihood that n-alkanes may arise in the lipid pool of these species as a result of normal metabolic processes rather than exposure to petroleum.

In addition to the gas chromatographic studies of hydrocarbon in environmental tissue samples, a study was conducted to evaluate the possibility of using mass spectrometry as a tool to identify polynuclear aromatic hydrocarbons in the tissue extracts. The data obtained from this investigation is given in Appendix C. The results were disappointing in that limits of detectability for individual compounds were found to be in the vicinity of 0.25 µg. Considering that recoveries of polynuclear aromatic hydrocarbon from tissue samples were found to range from 20 to 50%, a reasonable estimate for the minimum detectable amount of an individual polynuclear hydrocarbon compound would be on the order of 0.1 to 0.25 parts per million. This value is rather high when compared to other methods for detecting specific polynuclear hydrocarbons; hence, only seven samples of aromatics fractions extracted from tissue were submitted. These samples represented carbon tetrachloride extracts from robalo, lisa, perch, and Merida trout, and hexane extracts from manamana, bocachico, and shrimp. No molecular ions corresponding to molecular structures of polynuclear aromatic compounds were found in the mass spectra of any of the samples submitted.

Table 8
Individual alkanes found to be present in Lake Maracaibo fish tissue samples
(Concentrations expressed as μg/g moist tissue)

	Sta. 31 Bocachico	Sta. 31 Manamana	Sta. 31 Manamana	Sta. 2 Lisa	Sta. 16 Lisa	Bay of Tablazo Robalo	Sta. 16 Robalo	Meridan Trout (A)
C12								
C13								
C14								
C15	0.03	0.25	0.06	0.07	0.06			
C16	0.04	0.10	0.02	0.04	0.05			
C17*	7.10	7.31	1.53	1.36	1.91	0.16	0.12	0.13
C18*	0.08	0.06	0.02	0.03	0.02			
C19		0.50	0.07	0.04				
C20			0.02					
C22								
C24								
C26								
C28								
Total	7.25	8.22	1.72	1.54	2.04	0.16	0.12	0.13

	Meridan Trout (B)	Grays Harbor Perch (A)	Grays Harbor Perch (B)	Galveston Trout (A)	Galveston Trout (B)	Pond Bocachico (A)	Pond Bocachico (B)
C12							
C13							
C14							
C15						0.85	0.73
C16							
C17*	0.67	0.31	0.24	0.04	0.05	1.29	1.40
C18*						1.00	1.70
C19							
C20							
C22							
C24							
C26							
C28							
Total	0.67	0.31	0.24	0.04	0.05	3.14	3.83

*n-heptadecane and n-octadecane have retention times almost identical to pristane and phytane, respectively.

Summary

- Muscle tissue from six species of Lake Maracaibo macrofauna which are commercially important to the region have been analyzed for normal alkanes, methylnaphthalenes, and total hydrocarbons. For reference, four species of fish from waters outside of Lake Maracaibo have been analyzed for hydrocarbon content.

- Recovery studies have shown that individual n-alkanes and methylnaphthalenes can be detected at levels of 4 to 10 parts per billion. In spite of the sensitivity of the method, no methylnaphthalenes have been found in any tissue samples analyzed.

- Paraffin hydrocarbons ranging in carbon number from C_{15} to C_{20} have been detected in four species of fish from the lake and all of the species studied from outside the lake. The most abundant hydrocarbon corresponded to n-C_{17}, which is difficult to separate from the biogenic hydrocarbon, pristane. The greatest quantities of paraffins were recovered from bocachico and manamana, which prefer the lower salinity of the southernmost reaches of Lake Maracaibo. No paraffins have been found in curvina tissue.

- The analytical data suggests that the hydrocarbon contents of the fish tissue examined is more a function of species and diet than of geographical location in the lake.

CHARACTERIZATION OF THE ORGANIC CONSTITUENTS OF MARACAIBO SEDIMENTS

Introduction

Analytical studies of sediments have the potential for providing a wealth of information relating to the overall ecological characterization of Lake Maracaibo. In contrast to samples of water and of biota, the sediments can be considered to be reflective of average local environmental conditions over a finite period of time. Thus, an appreciation of the impacts of local environment on organic content of sediments, coupled with some knowledge of rate of deposition and movement, can give valuable clues to the relative impacts of petroleum production, industrial and domestic effluents and natural debris on the lake ecosystem.

Two types of sediment sampling programs were undertaken. In the initial screening phase, the top 5 cm of piston core samples from 20 stations were analyzed to determine the extent of variability in composition, and to investigate trends relating the character of the organic fractions to geographic locations. Duplicate cores from a few locations were analyzed to estimate the variability in sampling. In addition to the core samples, larger dredge samples were taken from 15 stations. Fractionation of the organic constituents of the dredge samples was performed in triplicate. The replicate analyses provided sufficient organic material for determination of molecular weights and elemental composition and, in addition, yielded data relating to the precision of the analytical methodology. Figure 46 shows the locations of the core samples analyzed, and Figure 46A shows that of the sediment dredge samples.

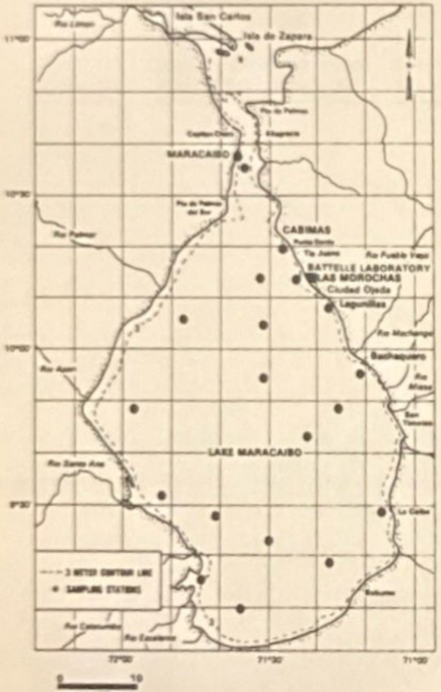


Figure 46
Locations of sediment core samples analyzed

Figure 46A
Locations of sediment dredge samples analyzed

Analysis of Sediments for Extractable Organic Content

Both types of samples were frozen at the site of collection and maintained in a frozen state until they were freeze-dried prior to analysis. The total organic extract (benzene-methanol) was obtained, and was further divided by solvent treatments into asphaltene-free extract and benzene-soluble asphaltenes. The asphaltene-free material was further separated by liquid chromatography into saturates, aromatics, and polar (NSO)* material. Methodological details, validation studies, and estimates of precision are included in Appendices A and D. The gravimetric data obtained on the fractions from core samples are presented in Table 9. Corresponding data for the dredge samples is given in Table 10, although, in this case, each determination represents the average of three replicates. Tables 11 and 12 give the total organic and carbonate carbon, as compared to the weight of the total organic extracts, for core and dredge samples.

Figure 47 shows how the quantity of organic material extracted from the sediment core samples varies with geographical location in the lake. Inspection of this figure reveals a general trend toward higher organic content in the northern sampling areas. This observation is consistent with what might be expected from

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*"NSO" denotes the presence of nitrogen-, sulfur-, and oxygen-containing polar compounds.

Table 9
Analyses of extractable organic matter in Lake Maracaibo sediment core samples

Station Number[1]	pH	Total Organic Extract (%)[2]	Asphaltene-Free Extract (μg/g)[2]	Composition of Asphaltene-Free Extract			Benzene-Soluble Asphaltenes (μg/g)[2]
				% Saturates	% Aromatics	% NSO	
17	8.3	2.70	3,955	16.8	53.1	26.1	1326
6	---	3.20	7,530	24.1	30.7	29.6	3472
13A	---	2.30	11,907	18.0	29.8	31.5	3968
13B	---	3.27	16,468	22.2	29.7	31.5	4169
12	8.3	1.30	6,356	20.8	31.3	31.3	1877
8A	8.3	1.60	9,099	21.8	31.7	28.9	2493
8B	7.0	---	2,041	16.1	30.0	29.6	1058
2	7.7	3.20	(13,838)[3]	(25.6)[3]	(42.0)[3]	(32.4)[3]	4523
34	7.1	0.95	1,381	18.4	30.7	30.1	1042
9	7.7	0.90	434	3.3	17.4	46.7	868
10	7.9	2.50	1,145	17.3	28.4	28.9	1558
4A	6.8	0.64	548	14.1	30.0	21.7	292
4B	---	0.02	84	5.1	7.7	12.8	56
24	8.2	0.74	738	20.0	23.3	45.6	926
32	8.3	0.50	633	15.5	38.2	34.8	394
25	8.0	1.50	<50	0.0	0.0	0.0	534
31	7.6	0.24	230	8.6	46.1	22.7	118
21A	7.7	1.50	<50	0.0	0.0	0.0	317
21B	7.6	2.50	1,536	0.0	17.9	0.0	638
27	---	---	2,234	10.5	34.3	16.7	1191
30A	--	2.69	1,585	3.4	16.1	28.2	1426
30B	8.2	4.16	6,112	12.9	45.9	26.4	3629
22A	---	1.10	429	16.0	0.0	34.7	503
22B	---	2.32	1,723	11.5	19.6	22.4	871
29	6.8	0.80	395	9.4	20.8	21.9	329
23A	6.9	---	220	20.5	11.4	38.6	405
23B	---	1.90	1,292	4.8	39.0	31.5	673

[1] Map of station locations; Figure 5.

[2] Based on freeze-dried sediment weight.

[3] Weight of asphaltene-free extract not determined; calculations are based on sum of saturates, aromatics, and NSO fractions.

Table 10

Analysis of extractable organic matter in Lake Maracaibo sediment[1] (dredge samples)

Station Number[2]	Total Organic Extract (%)[3]	Asphaltene (dredge) Free Extract (μg/g)[3]	Compositon of Asphaltene-Free Extract % Saturates	% Aromatics	% NSO	Benzene-Soluble Asphaltenes (μg/g)[3]
6	0.71	3,540	19.2	36.4	30.3	1,756
14	0.31	1,626	21.0	42.6	27.6	818
7	0.46	2,621	18.9	30.2	35.6	1,130
13	2.88	17,751	20.8	37.3	27.4	5,402
12	2.00	13,119	20.9	42.9	26.8	4,237
1	0.38	2,361	19.9	43.1	21.6	817
2	1.52	10,172	17.5	42.7	28.0	3,351
9	1.17	5,128	18.4	33.9	32.3	2,366
10	0.67	2,223	17.3	25.1	45.8	2,250
11	2.12	2,131	14.1	20.3	50.3	2,396
25	0.70	2,171	17.0	23.5	44.3	3,153
26	0.47	1,877	22.8	44.7	23.4	812
27	0.45	2,638	11.2	51.5	41.6	1,249
31	0.25	1,374	18.3	61.2	30.7	454
30	0.40	1,973	15.7	42.3	31.4	969

[1] Average of three replicate analysis.

[2] Map of station locations; Figure 5.

[3] Based on freeze-dried sediment weight.

Table 11

Total organic carbon and carbonate carbon of Lake Maracaibo sediment (core samples)

Station Number	Total Organic Extract (%)	Total Organic Carbon (%)	Carbonate Carbon (%)	Sediment pH
17	2.70	2.23	0.31	8.3
6	3.20	4.18	0.25	---
13A	2.30	3.42	0.29	---
13B	3.27	---	---	---
12	1.30	2.02	<0.05	8.3
8A	1.60	2.50	<0.05	8.3
8B	---	1.87	0.19	7.0
2	3.20	4.00	0.58	7.7
34	0.95	6.20	0.10	7.1
9	0.90	2.63	<0.05	7.7
10	2.50	4.43	<0.05	7.9
4A	0.64	1.56	0.20	6.8
4B	0.02	0.81	0.21	---
24	0.74	4.95	0.42	8.2
32	0.50	0.88	<0.05	8.3
25	1.50	2.87	<0.05	8.0
31	0.24	4.98	<0.05	7.6
21A	1.50	3.29	0.18	7.7
21B	2.50	3.37	0.48	7.6
27	---	2.80	0.55	---
30A	2.69	---	---	---
30B	4.16	5.91	0.09	8.2
22A	1.10	2.63	<0.05	---
22B	2.32	---	---	---
29	0.80	1.61	0.05	6.8
23A	---	2.54	0.11	6.9
23B	1.90	2.72	0.05	---

Table 12

Total organic carbon and carbonate carbon of Lake Maracaibo sediment (dredge samples)

Station Number	Total Organic Extract (%)	Sediment Total Organic Carbon (%)	Sediment Carbonate Carbon (%)	Sediment pH
6	0.71	1.72	0.68	8.3
14	0.31	0.54	<0.05	7.6
7	0.31	1.30	0.36	8.1
13	2.88	3.27	0.39	7.5
12	2.00	2.12	0.16	8.1
1	0.38	0.58	0.24	7.9
2	1.52	1.56	0.15	7.0
9	1.17	4.94	<0.05	7.6
10	0.67	5.95	0.17	8.3
11	2.12	6.64	0.40	7.7
25	0.70	7.80	0.33	8.0
27	0.45	3.06	0.10	6.6
31	0.25	1.27	0.13	7.3
26	0.47	2.35	0.21	7.6
30	0.40	3.04	<0.05	7.3

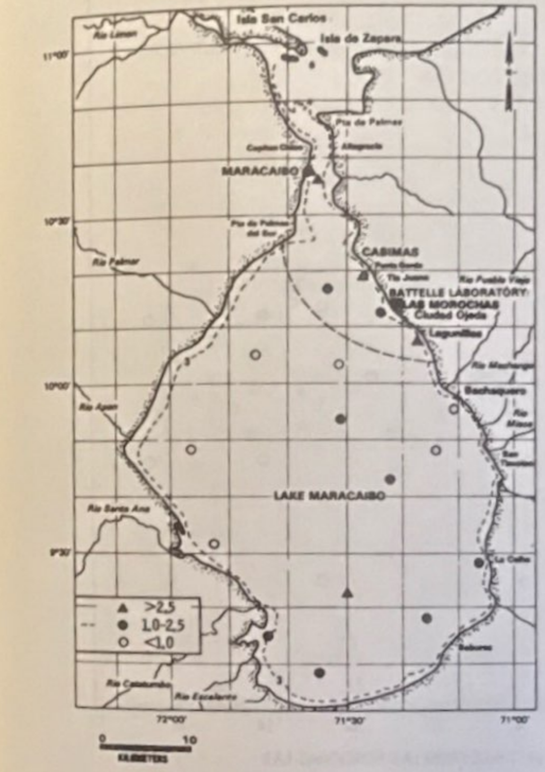

Figure 47
Percent extractable organic material in Maracaibo core sediment samples

a consideration of lake currents, river systems, prevailing winds, population density, petroleum production, and transportation activity. In the region of the northeast shore and in the Straits of Maracaibo, the winds and currents combine to add organic debris to the already heavy load imposed from sewage discharges and plankton. In order to confirm this preliminary observation, the gravimetric data for the core samples was subjected to a simple statistical test. In this analysis, a one-way analysis of variance (Sheffé, 1959), the means of two sets of data are compared in order to find if they are significantly different. Using this technique, it was found that the set of core samples taken at stations above the dashed line in Figure 47 were different from the rest of the samples with respect to total organic extract, benzene-soluble asphaltenes, and the percentage of saturates in the asphaltene-free extract. These differences were significant at the 0.01 level. When the data from sets of locations from the two sets shown in Figure 47 were statistically compared, the differences were far less significant.

Since this preliminary inspection of the sediment core gravimetric data indicated that the general area of intensive petroleum production was unique in sediment characteristics, a study was undertaken to determine how the data varied with the distance away from this zone. The gravimetric data from both the core and dredge samples were examined statistically

(Dixon and Massey, 1951) for correlation with the distance from the Battelle Laboratory at Las Morochas. This location, about midpoint between Cabimas in the north and Bachaquero in the south is centrally located in the oil-producing area.

Figures 48 and 49 show the concentrations of the asphaltene-free extract and the benzene-soluble extract plotted as a function of the relative distance of the samples from the Las Morochas Laboratory. Figure 50 shows a similar plot of the percentage of saturates in the asphaltene-free extract. These analytical variables correlate highly with distance at the 0.01 level of significance, with correlation coefficients of -0.558, -0.534, and -0.509. The general trend is a decrease in the quantities of these fractions as the distance from the oil-producing zone increases. The low values for the correlation coefficients are a quantitative confirmation of what is apparent from inspection of the plotted data; there is a great deal of scatter. That much of this scatter is due to errors associated with environmental sampling can be seen from comparing the values obtained for concentration of asphaltene-free extract between replicate cores (A and B) given in Table 9. For the seven pairs of duplicate cores analyzed, only one pair of values for asphaltene-free extract agree within a factor of three. Thus, it is not surprising to find that the correlations with distance, while highly significant, only account for about 30% of the observed variation.

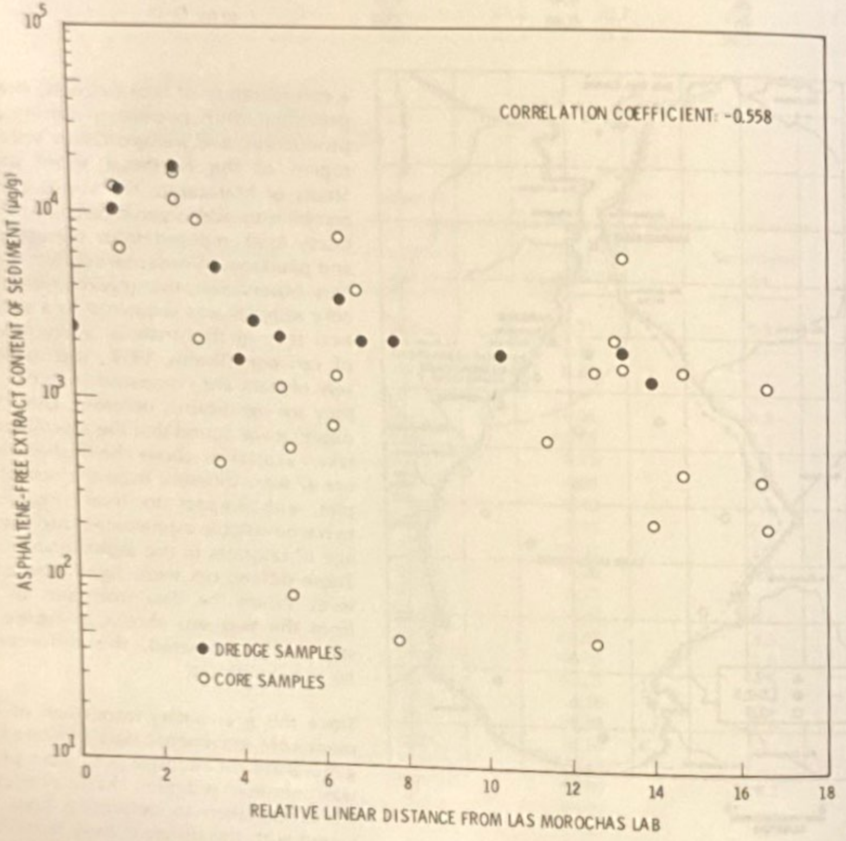

Figure 48
Aspaltene-free extract vs. distance from Las Morochas Lab

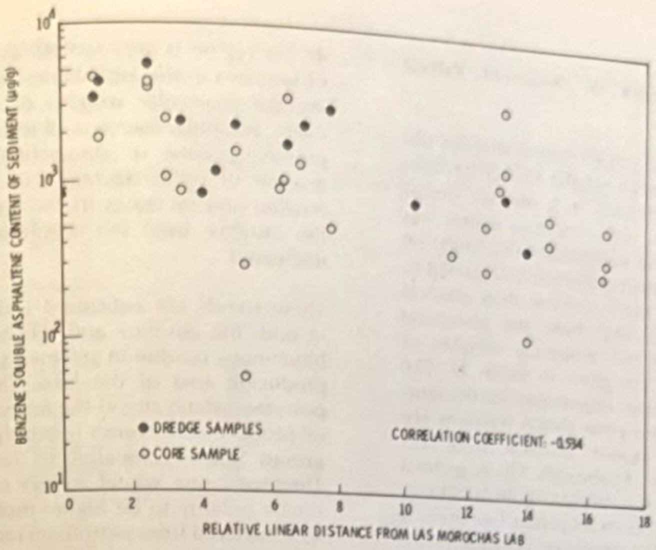

Figure 49
Benzene soluble asphaltene vs. distance from Las Morochas Lab

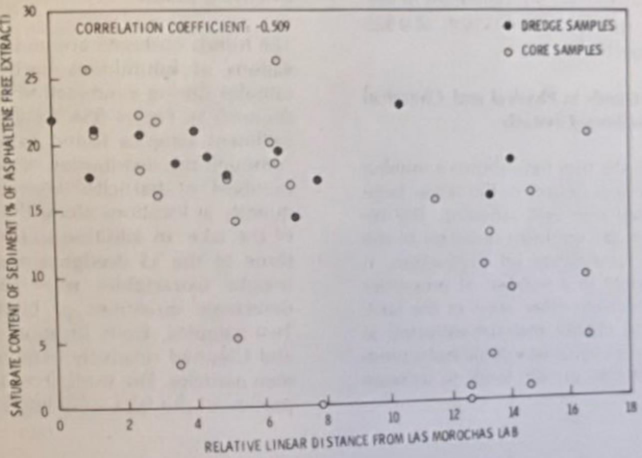

Figure 50
Percent saturates vs. distance from Las Morochas Lab

Chemical Properties of Sediment Extract Fractions

Molecular weights and elemental analyses obtained on the dredge sample extract fractions were treated statistically in a manner similar to the gravimetric data. The data scatter was not as great for this correlation. This might be expected if the general chemistry obtaining to any region were more uniform than physical variables. Data derived from the elemental composition and the molecular weights of dredge fractions are given in Table 13. The average mole weights determined for the saturate, aromatic, and polar (NSO) fractions are shown plotted against distance from Las Morochas (Figures 51 through 53). A general trend of decreasing average mole weight as distance increases from oil production areas is observed. While the correlation coefficients are greater for these data sets, only one set was correlated with distance at the 0.01 level of significance. Those for the saturate and NSO fractions were correlated at the 0.05 level. The lower level of significance is due in part to the smaller data set available, since only 15 dredge samples were analyzed. Figure 54 shows an increase in the number of atoms of sulfur per average molecule for the aromatics fractions as the distance from Las Morochas increases. Figure 55 shows the reverse trend for the NSO fraction. The correlations are significant at the 0.01 level with correlation coefficients of 0.925 and -0.827, respectively.

Discussion of Trends in Physical and Chemical Properties of Sediment Extracts

To summarize, the data have shown a number of significant trends in spite of the rather large errors associated with field sampling. The region located in the northeast quadrant of the lake, that of most active oil production, is distinctly different in a number of properties of its sediments from other areas of the lake. The quantity of organic material extracted as represented by the benzene soluble asphaltenes and asphaltene-free extract, tends to increase as this region is approached, as does percent of saturates in the asphaltene-free extract. The average molecular weights of saturate, aromatic, and polar fractions all increase as the oil producing zone is approached. The relative amount of sulfur decreases in the aromatics fraction and increases in the NSO fraction as the distance from the oil-producing zone is decreased.

These trends are consistent with an increase in both the absolute and relative amounts of bituminous residue in sediments from the oil-producing area of the lake. The maturation processes which attend the formation of crude oil produce compounds relatively free of polar groups when compared to recent detritus. Therefore, one would expect compounds of similar polarity to be higher molecular weight when derived from petroleum rather than from plant material.

A smaller number of sulfur atoms per molecule in aromatics fractions obtained from sediments near the oil producing zone can be attributed to a dilution of elemental sulfur, formed by sulfate reducing bacteria, with aromatic molecules from bituminous residue. A predominance of elemental sulfur in samples from the southern region was detected during the examination of fractions by mass spectrometric methods, discussed below.

The trends observed are consistent with observations of bituminous particles in sediment samples during examination for benthic fauna (Volume I). Figure 55A shows the locations of sediment samples found to contain bitumen. Although the distribution is widespread, large numbers of particles were found most frequently at locations along the northeast shore of the lake. In addition to these observations, three of the 15 dredge samples analyzed for organic extractables were found to contain detectable quantities of bituminous residue. Two samples, from locations near Lagunillas and Ulē, had relatively large numbers of bitumen particles. The third, from the southwestern portion of the lake, contained only traces.

Table 13

Summary of elemental analyses and molecular weight determinations of sediment fractions (dredge samples)

Station Number	Fraction	Average Molecular Weight	Hydrogen to Carbon Mole Ratio	Atoms Sulfur per Average Molecule	Atoms Nitrogen per Average Molecule	Ash (%)
6	Saturates	502	1.9			
	Aromatics	437	1.4	3.6	0.03	
	NSO	790	1.6	0.8	0.3	
14	Saturates	517	2.0			
	Aromatics	410	1.4	2.9	0.08	
	NSO	754	1.6	0.7	0.37	
7	Saturates	484	1.9			
	Aromatics	522	1.5	1.3	0.08	
	NSO	730	1.6	0.8	0.29	
13	Saturates	505	1.5			
	Aromatics	331	1.5	0.6	0.04	
	NSO	716	1.5	0.7	0.36	
12	Saturates	497	1.9			
	Aromatics	513	1.4	1.3	0.05	
	NSO	820	1.4	1.0	0.36	
1	Saturates	531	2.0			
	Aromatics	492	1.4	2.3	0.09	
	NSO	747	1.5	0.8	0.47	
2	Saturates	531	1.8			
	Aromatics	497	1.4	0.6	0.04	
	NSO	929	1.4	0.8	0.68	
9	Saturates	498	2.0			
	Aromatics	486	1.5	1.7	0.04	
	NSO	714	1.6	0.7	0.34	
10	Saturates	479	1.8			
	Aromatics	447	1.7	2.8	0.08	
	NSO	696	1.6	0.6	0.24	
11	Saturates	497	1.8			
	Aromatics	389	1.6	6.8	0.10	
	NSO	726	1.7	0.3	0.42	
25	Saturates	526	1.8			
	Aromatics	398	1.8	4.0	0.77	
	NSO	764	1.8	0.6	0.13	
26	Saturates	---	2.0			
	Aromatics	326	1.3	6.4	0.09	3.1
	NSO	779	1.8	0.5	1.59	9.3
31	Saturates	482	1.8			
	Aromatics	339	1.7	8.0	0.39	<0.1
	NSO	384	1.8	0.2	2.73	6.3
27	Saturates	---	1.8			
	Aromatics	327	1.3	8.7	0.10	1.0
	NSO	651	1.8	0.3	0.14	24.2
30	Saturates	438	1.8			
	Aromatics	326	1.3	7.6	0.24	<0.1
	NSO	691	1.6	0.5	0.13	

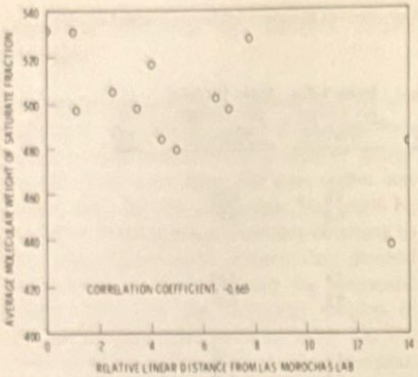

Figure 51
Molecular weight of saturate vs. distance from Las Morochas Lab

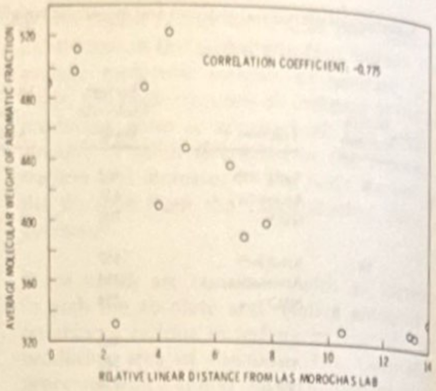

Figure 52
Molecular weight of aromatics vs. distance from Las Morochas Lab

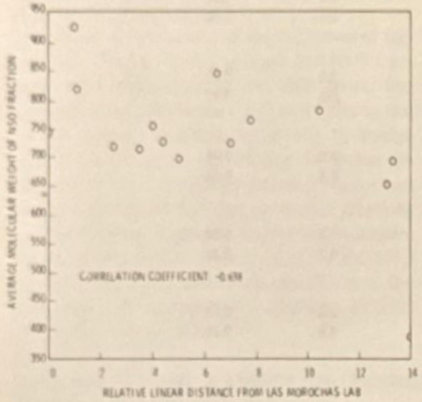

Figure 53
Molecular weight of polar (NSO) fraction vs. distance from Las Morochas Lab

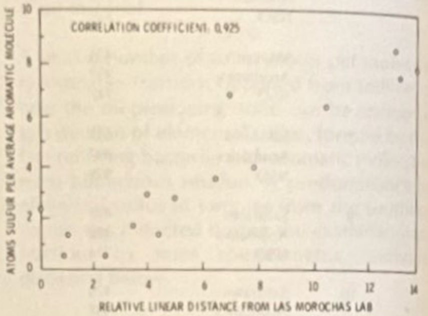

Figure 54
Atoms sulfur per aromatic molecule vs. distance from Las Morochas Lab

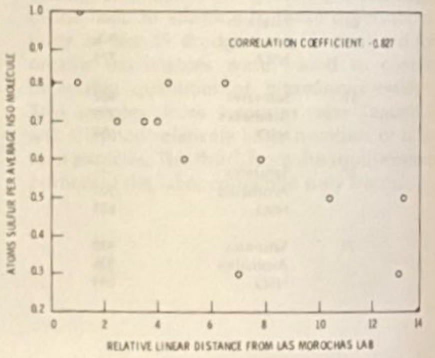

Figure 55
Atoms sulfur per polar (NSO) molecule vs. distance from Las Morochas Lab

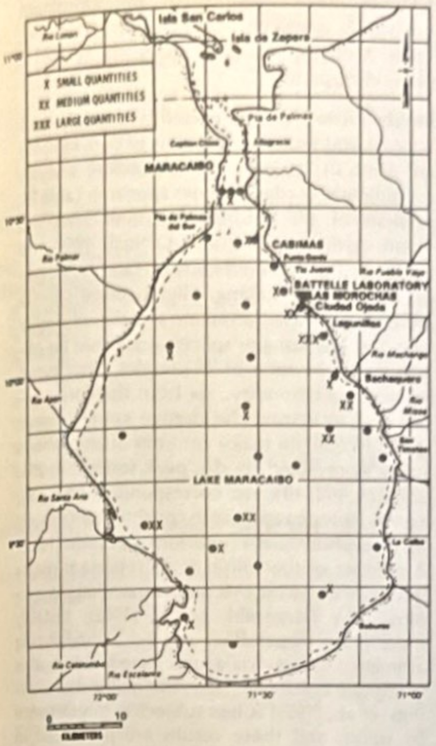

Figure 55A
Occurrence of bituminous particles in sediments

Gas Chromatography of Sediment Extract Fractions

Shown in Figure 56 are gas chromatograms typical of the saturate and aromatic fractions obtained from the dredge sediments. Gas chromatography has previously been employed successfully to detect the presence of petroleum hydrocarbons in sediments (Clark and Blumer, 1967). However, the method is limited to the lighter fractions of petroleum. There is little qualitative information to be gained from the gas chromatographic scans. No identifiable n-alkanes could be detected in any of the saturate fractions examined. Since these compounds predominate in the saturate fractions of crude oils, it is apparent that any organic material in the sediment samples arising from petroleum has been extensively altered. There was reasonable agreement between gravimetric and gas chromatographic determinations of the saturate and aromatic fractions investigated (Table 14), indicating that little nonvolatile material was present in these samples.

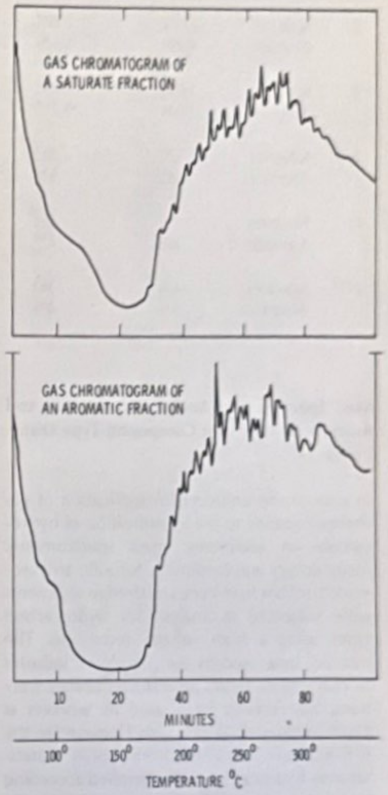

Figure 56
Gas chromatograms of sediment fractions

Table 14

Correspondence between gravimetric and gas chromatographic determinations of sediment fractions

Station Number		Gas Chromatography (µg/g)	Gravimetric (µg/g)
6	Saturates	568	681
	Aromatics	690	1,225
14	Saturates	453	393
	Aromatics	551	771
13	Saturates	3,394	3,797
	Aromatics	5,673	6,656
12	Saturates	3,138	2,827
	Aromatics	3,853	5,225
1	Saturates	559	534
	Aromatics	981	1,162
2	Saturates	2,025	2,282
	Aromatics	4,359	5,429
9	Saturates	1,059	995
	Aromatics	1,049	1,718
10	Saturates	520	362
	Aromatics	632	526
11	Saturates	339	292
	Aromatics	468	427
25	Saturates	444	361
	Aromatics	509	486

Mass Spectrometric Analysis of Saturate and Aromatic Fractions for Compound-Type Distribution

In view of the unsuccessful application of gas chromatography to the identification of hydrocarbons in sediments, mass spectrometric methodology was employed. Saturate and aromatic fractions from core and dredge sediments were subjected to analysis for hydrocarbon types using a high voltage technique. The method, long used by the petroleum industry to characterize heavy petroleum distillate fractions, has recently been used by workers at Exxon (Brown et al., 1973) to characterize the hydrocarbons extracted from ocean waters. Saturate hydrocarbons are identified according to number of naphthenic rings and aromatic hydrocarbons are identified according to the number and arrangement of aromatic nuclei.

Analyses were performed by Petroleum Analytical Research Corporation, Houston, Texas. A description of the procedures used is given in Appendix C.

Results from the compound-type analysis of saturate and aromatic fractions of core samples are given in Table 15. Corresponding analyses for sediment dredge samples appear in Table 16. Analysis of the saturate fractions using the Hood method (Hood and O'Neal, 1959) was performed with no difficulty on samples weighing as little as 1.8 mg. High voltage spectra were obtained for aromatic samples of similar size, but low voltage spectra could not be obtained. Of the total of 25 samples investigated by mass spectrometry, six from the core samples and four from the dredge samples were found to contain major amounts of amorphous sulfur as revealed by the peak series 258, 256, 226, 224, 192, 194, etc. corresponding to S_8, S_7, S_6, etc. Isotope ratios were consistent with those for amorphous sulfur reported by Beynon (1966). A number of the sulfur peaks represent major interferences with the matrix calculations employed by Fitzgerald et al., (1962); notably those for cycloparaffins and 1-, 2-, and 3-ring aromatics. Thus, data calculated using this technique is not reported. The method of Hastings et al., (1956) is less subject to interference by sulfur, and these results are presented in Tables 15 and 16. For comparison, an analysis of South Tia Juana Medium Crude residue used for sediment validation studies also appears in Tables 15 and 16.

The aromatics analyses for the sediment fractions appears to be consistent with that for crude oil residue. However, interfering elemental sulfur in aromatics fractions from sediment samples creates some doubt about the reliability of the percentages of compound types reported. Another possible source of error is in the matrix used for the calculation of the compound-type distributions. There are very low relative values for naphthalene types in most of the samples analyzed, including the South Tia Juana Medium residuum. Naphthalenes are usually a predominant compound type in most petroleums. The surprisingly high relative values for benzothiophenes and dibenzothiophenes in the South Tia Juana residue are also an indication that the relative values reported for the aromatic compound types may be somewhat in error. In spite of apparent difficulties, the data obtained can be considered to give at least a qualitative picture of the aromatic composition of the organic

Table 15

High voltage mass spectrometric analysis of sediment organic fractions: core samples

	Station Number										S. Tia Juana Medium Residuum
	2	6	8	10	13	17	22	24	27	32	
SATURATES, Conc. in sediment (µg/g)	3481	1815	1984	198	3656	664	198	345	235	98	
M.S. RING ANALYSIS (Hood Matrix)											
% Paraffins	14.6	16.5	13.0	23.1	15.1	16.1	35.2	27.1	30.8	17.6	37.2
% 1-Ring	28.3	27.4	25.8	41.6	28.2	34.1	35.1	27.2	27.8	27.1	20.4
% 2-Ring	36.5	30.6	26.9	19.0	29.0	29.1	9.3	19.8	22.1	28.4	17.0
% 3-Ring	17.1	22.4	19.0	14.2	19.7	13.2	18.0	13.3	15.2	19.0	11.9
% 4-Ring	3.1	2.1	12.5	0.0	7.1	4.8	1.6	8.6	3.4	5.9	9.6
% 5-Ring	0.2	0.0	2.7	0.0	0.0	0.0	0.0	1.9	0.0	0.1	3.1
% 6-Ring	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9
% Monoaromatics	0.0	1.0	0.1	1.7	1.0	2.9	0.7	2.2	0.8	2.0	<0.1
AROMATICS, Conc. in sediment (µg/g)	5811	2311	2884	325	4891	2100	338	401	766	242	
M.S. ANALYSIS (Hasting Matrix)											
% Benzenes	13.3	22.1	18.8	67.0[1]	16.4	[3]	17.9[2]	68.9[1]	[3]	[3]	17.9
% Indan	8.8	12.1	18.1	13.3	11.0		9.3	13.8			10.5
% Indenes	9.2	13.2	9.2	12.9	11.3		10.6	13.5			9.0
% Napththalenes	1.6	1.9	1.0	2.2	1.8		2.6	3.8			1.6
% Acenaphthenes	5.0	3.6	4.0	---	4.7		6.1	---			6.4
% Acenaphthalenes	9.3	8.7	7.5	---	10.3		28.2	---			13.6
% Phenanthrenes	7.7	3.7	4.7	---	4.0		19.4	---			8.0
% Pyrenes	16.3	7.5	11.5	---	10.5		---	---			5.4
% Chrysenes	7.6	2.3	2.5	---	3.7		---	---			1.5
% Benzothiophenes	8.4	14.1	7.4	4.6	12.7		3.2	---			13.1
% Dibenzothiophenes	6.9	7.4	11.2	---	9.1		2.7	---			12.9
% Naphthobenzothiophenes	5.8	3.3	4.0	---	4.5		---	---			0.2

[1] About 50% of total ion intensity due to sulfur.

[2] About 75% of total ion intensity due to sulfur.

[3] 99+% of total ion intensity due to sulfur.

Table 16

High voltage mass spectrometric analysis of sediment organic fractions: dredge samples

	Station Number							
	6	14	7	13	12[2]	1	2[2]	9
SATURATES, Conc. in sediment (μg/g)	680	341	495	3692	2741	470	1780	943
M.S. Ring Analysis (Hood Matrix)								
% Paraffins	11.4	15.9	13.9	11.8	12.0	10.3	7.5	18.7
% 1-Ring	16.7	16.8	22.2	20.4	13.7	19.3	15.5	17.8
% 2-Ring	22.4	22.0	24.7	27.6	20.2	24.1	22.4	20.6
% 3-Ring	20.3	21.2	19.3	23.7	22.3	20.2	23.0	16.3
% 4-Ring	17.4	17.2	13.5	13.1	19.5	16.7	20.3	15.6
% 5-Ring	8.8	5.3	4.8	3.4	8.9	7.3	8.7	8.0
% 6-Ring	3.1	1.6	1.6	n.d.	3.3	2.2	2.7	3.0
% Monoaromatics	n.d	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
AROMATICS, Conc. in sediment (μg/g)	1290	693	792	6621	5628	1018	4343	1738
M.S. Analysis (Hastings Matrix)								
% Benzenes	11.0	12.1	11.1	9.3	10.2	9.4	8.3	11.1
% Indan	6.6	7.9	10.2	7.1	7.3	6.5	6.4	7.6
% Indenes	7.6	1.5	10.4	7.5	7.3	7.4	8.0	7.4
% Naphthalenes	1.4	1.5	1.2	1.2	1.2	1.4	1.6	1.3
% Acenaphthenes	5.8	6.2	3.5	6.1	5.3	5.3	6.8	6.2
% Acenaphthalenes	10.9	11.5	8.8	11.1	9.2	8.9	13.8	11.5
% Phenanthrenes	7.3	8.7	5.3	7.9	6.4	13.0	7.7	10.8
% Pyrenes	11.2	13.4	13.7	12.6	14.1	0.9	7.0	12.7
% Chrysenes	7.2	8.3	1.3	7.8	6.9	15.8	10.7	7.1
% Benzothiophenes	10.2	9.1	12.7	9.3	9.9	8.3	6.9	8.2
% Dibenzothiophenes	12.5	11.2	13.2	12.7	13.7	7.9	12.0	9.5
% Naphthobenzothiophenes	8.3	8.6	8.7	7.5	8.6	15.2	11.0	6.7

	Station Number							S. Tia Juana Medium Residuum
	10	11	25	27[1]	31[1,2]	26[1]	30[1]	
SATURATES, Conc. in sediment (μg/g)	385	300	369	297	251	430	309	
M.S. Ring Analysis (Hood Matrix)								
% Paraffins	15.3	17.5	18.2	20.6	14.8	25.1	19.9	37.2
% 1-Ring	21.0	21.5	19.4	17.0	15.7	25.2	23.9	20.4
% 2-Ring	24.1	23.5	18.4	18.9	16.0	20.1	20.0	17.0
% 3-Ring	19.3	21.1	18.1	16.4	25.4	13.6	15.9	11.9
% 4-Ring	13.8	12.8	16.1	14.9	13.3	8.5	13.0	9.6
% 5-Ring	5.2	3.6	7.1	6.5	8.8	4.6	4.9	3.1
% 6-Ring	1.4	n.d.	2.7	2.4	4.4	1.3	0.4	0.9
% Monoaromatics	n.d.	n.d.	n.d.	3.3	1.6	1.7	2.0	<0.1
AROMATICS, Conc. in sediment (μg/g	558	432	510	1359	840	843	834	
M.S. Analysis (Hastings Matrix)								
% Benzenes	13.6	34.3	14.5	17.2[1]	15.9[1]	14.0[1]	26.2[1]	17.9
% Indan	8.0	5.2	8.2	6.1	8.1	7.5	20.9	10.5
% Indenes	9.3	6.2	9.0	6.2	9.1	11.8	7.6	9.0
% Naphthalenes	2.1	0.4	1.6	30.0	5.1	6.1	10.2	1.6
% Acenaphthenes	8.8	3.2	6.5	4.1	13.4	8.0	8.6	6.4
% Acenaphthalenes	17.8	7.2	12.9	11.0	16.0	18.9	8.5	13.6
% Phenanthrenes	1.2	15.8	12.7	3.3	0.9	3.2	4.2	8.0
% Pyrenes	7.7	9.3	7.3	5.8	14.8	10.3	3.0	5.4
% Chrysenes	7.9	3.1	5.0	2.0	0.3	0.4	5.2	1.5
% Benzothiophenes	5.2	0.3	8.9	4.8	5.8	10.2	2.8	13.1
% Dibenzothiophenes	13.2	10.6	11.4	6.3	5.1	8.9	1.4	12.9
% Naphthobenzothiophenes	5.1	4.5	2.1	3.3	5.4	0.7	1.4	0.2

[1] About 90% of total ion intensity due to sulfur. Sulfur peaks subtracted from spectrum to obtain analysis.

[2] Presence of bituminous particles detected in freeze-dried samples.

material in Maracaibo sediments, and also to suggest a close similarity in composition to the compounds found in petroleum bitumens.

Graphical presentation of the saturate compound type distributions determined for the sediment core samples is given in Figure 57, together with an indication of sample location. The corresponding dredge sample data is shown in Figure 58. The most abundant distribution pattern obtained for both core and dredge samples is that in which the 2-ring naphthenes predominate. Of the 10 core samples examined, 6 have a predominance of 2-ring compounds; for the dredge samples, 7 out of 15 have this pattern. A less frequently occurring pattern is that in which 1-ring compounds predominate; 2 core and 2 dredge samples have this compound type preference. Of interest is that only 3 of 25 samples had a clear predominance of paraffins (no rings), particularly in view of the saturate pattern determined for South Tia Juana Medium residue (see Table 15), in which 37.2% of the saturate hydrocarbons were found to be paraffinic compounds. None of the three dredge samples containing bituminous particles (Stations 2, 12 and 31) showed a predominance of paraffins.

It is likely that the relative deficiency of paraffinic hydrocarbons in the saturate fractions of most of the sediment samples examined is a result of selective microbial oxidation of these compound types, since saturate hydrocarbons are attacked by more microbial species, and more rapidly, than either naphthenic or aromatic compounds (Zo Bell, 1969). This being the case, a direct comparison of the saturate patterns of crude oil residues with those of the sediment organic extracts would provide little evidence for or against the presence of petroleum bitumen in the sediments. A more fruitful approach might be one in which the saturate patterns of sediments known to contain bitumen are compared with those from other sediments. Qualitatively, it would appear that over one-half of the samples have saturate patterns similar to the sample taken near Lagunillas (Station 2) or Ulē (Station 13); i.e., a predominance of 2- and 3-ring naphthenes.

An attempt to quantify the degree of similarity of saturate patterns has been attempted using the χ^2 test for goodness of fit (Dixon and Massey, 1951). For each sample, the differences in percent distribution of each saturate compound type from that at Station 2 and at Station 13 are treated statistically to estimate the probability that the samples are identical with either Station 2 or 13. The value obtained for the probability is interpreted to indicate the degree of correspondence or similarity between the two patterns. The calculated probabilities are given in Table 17. Figure 59 shows the sample locations of the 14 saturate fractions which have a reasonable chance (0.24 to 0.97) of being identical to either that from Station 2 or from Station 13. Of these, 13 were obtained from the production region of the lake. Only 4 out of the 17 samples from the production zone did not have saturate patterns similar to Station 2 or 13.

This type of approach is rather specific in that it attempts to identify the type of bitumen in the sample as well as the presence of bitumen. Thus, the comparison technique rejects the possibility that dredge samples from Station 2 and Station 13 are the same (Table 19), even though both were found to contain significant quantities of bituminous particles. A more general approach might be to build a data base by analyzing bituminous particles taken from a number of locations in the lake, and estimating the amount of variation to be expected. Comparison of unknown sediment saturate patterns against this reference base may enable a determination of the degree of bitumen content in the sample.

The results obtained from the distance correlation studies, and from the mass spectrometric analyses of saturate and aromatic fractions of sediment organic extracts, indicate that bituminous material is present in the sediments of most regions in the lake. The results further suggest that on the average, there is more bitumen present in sediments from the oil producing areas than in those from other areas. This trend is not particularly surprising in view of the long history of intensive oil production in Lake Maracaibo. In addition to residue deriving from oil discharges, bituminous residues in sediments arise from the numerous natural seeps that are present in almost every region of the lake. In view of the rapid rate of sedimentation in the lake system, and the generally anoxic conditions of the lake bottom, the sediments probably represent a relatively permanent repository for the insoluble nonvolatile portions of petroleum discharged into lake waters.

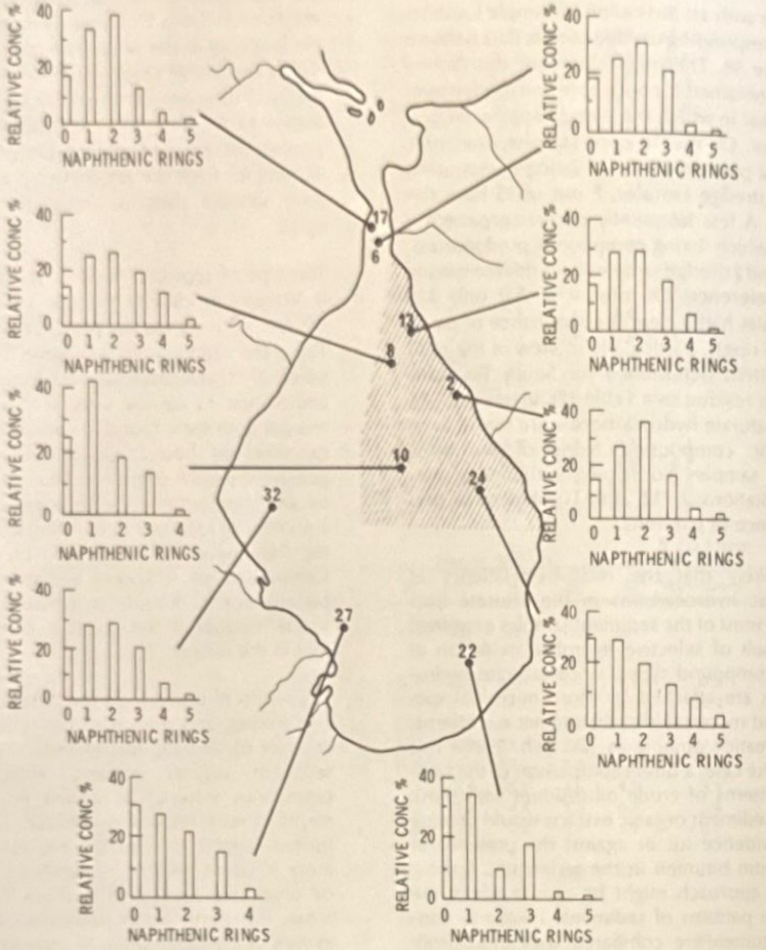

Figure 57
Saturate compound-type distribution in Lake Maracaibo sediment core samples
(Numbers on map refer to station numbers)

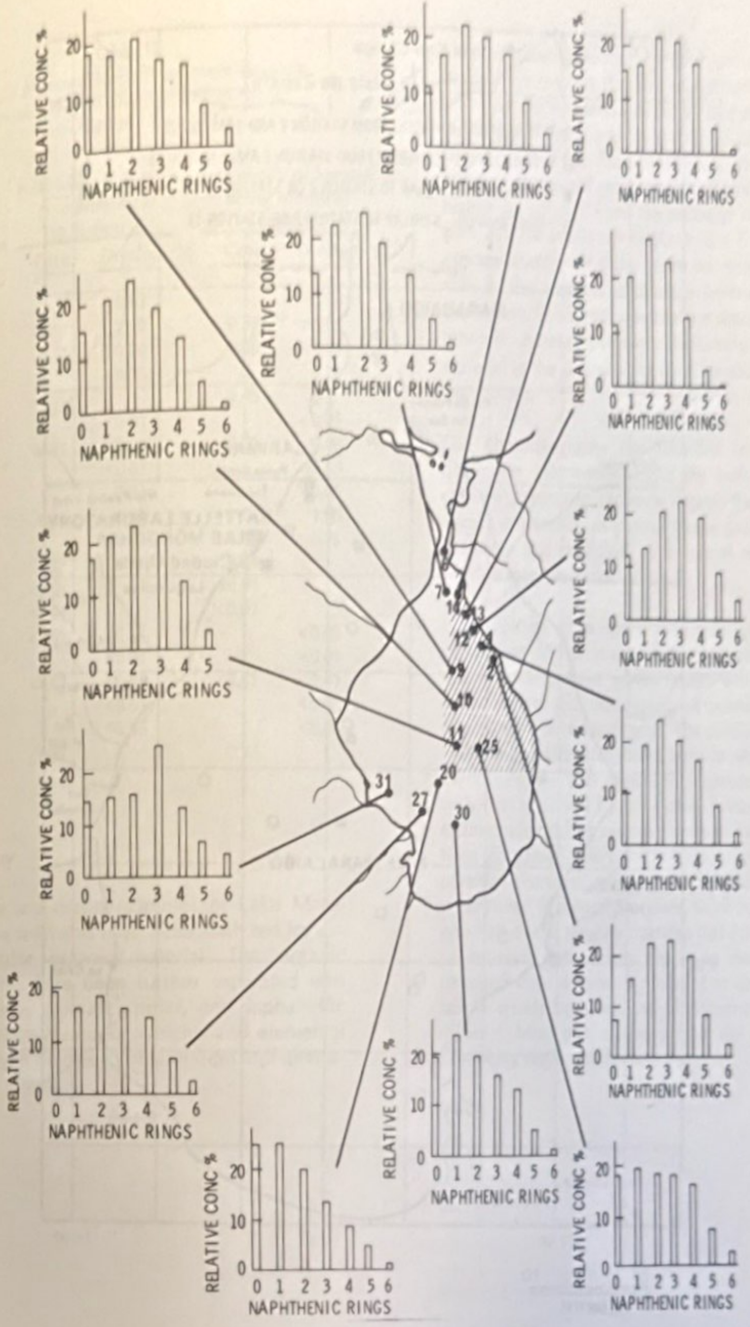

Figure 58
Saturate compound-type distribution in Lake Maracaibo sediment dredge samples
(Numbers on map refer to station numbers)

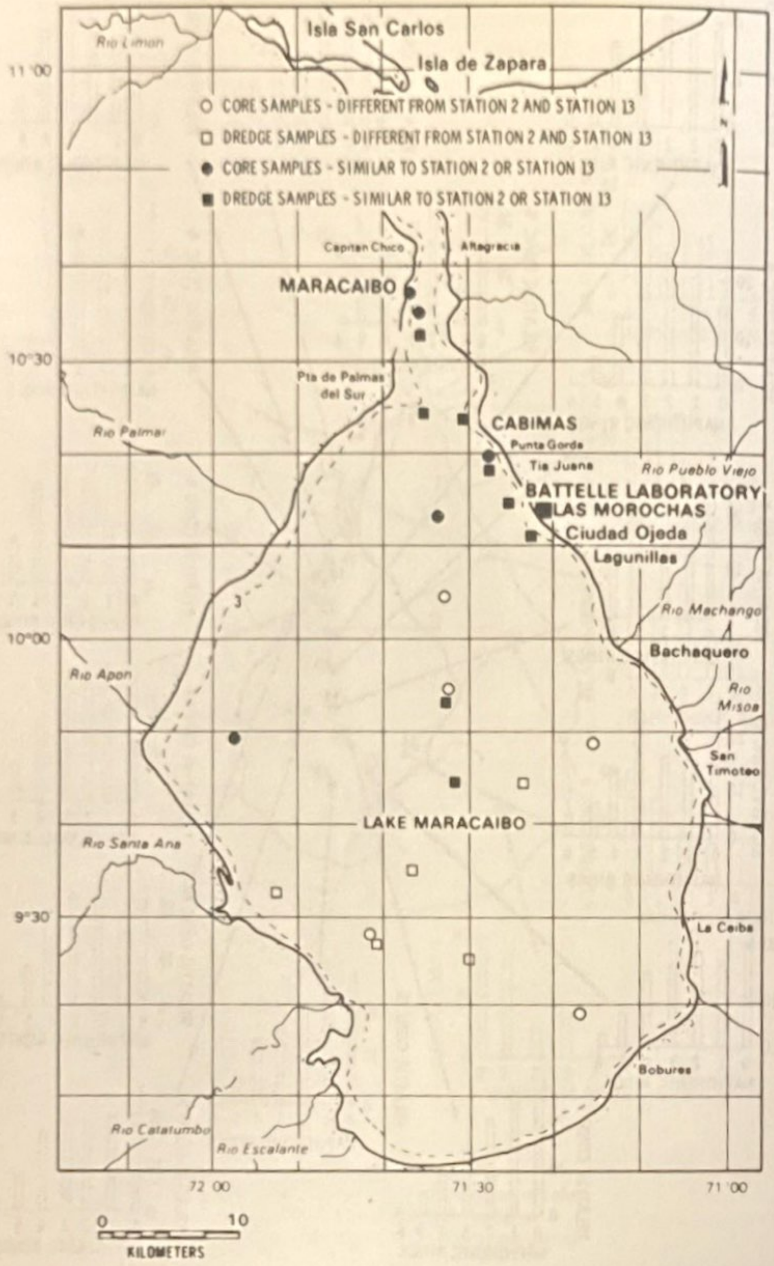

Figure 59
Summary of comparison of sediment saturate hydrocarbon distribution with those from Stations 2 and 13

Table 17
Results of comparison of sediment saturate compound-type distributions to those from Stations 2 and 13

Station No.	Probability of Sample Being Identical to Station 2		Probability of Sample Being Identical to Station 13	
	Core	Dredge	Core	Dredge
1		0.63		0.15
2	1.00	1.00	0.36	<0.01
6	0.47	0.70	0.88	<0.05
7		<0.05		0.73
8	<0.01		0.45	
9		<0.01		<0.01
10	<0.01	<0.05	<0.01	0.55
11		<0.01		0.60
12		0.53		<0.05
13	0.24	<0.05	1.00	1.00
14		<0.05		0.15
17	0.37		0.24	
22	<0.01		<0.01	
24	<0.01		<0.01	
25	<0.01	<0.01		<0.05
26		<0.01		<0.01
27	<0.01	<0.01	<0.01	<0.01
30		<0.01		<0.05
31		<0.05		<0.05
32	0.37		0.97	

Summary

- Core and dredge samples of Lake Maracaibo sediments have been analyzed for extractable organic material. The organic extracts have been further separated into saturate, aromatic, polar, and asphaltenic fractions. Molecular weights and elemental analyses have been obtained on asphaltene-free extracts.

- There is statistical support for the hypothesis that the organic fractions of sediments from the northeastern area of the lake are both qualitatively and quantitatively different from those from other areas. The quantity of material extracted is significantly higher in samples from the northeast region, as is the average molecular weight. The average number of sulfur atoms per molecule in the aromatic fractions is lower in this region. The trends observed are consistent with increasing amounts of bituminous material in the sediments of the oil producing regions.

- Gas chromatography has provided little qualitative information about the hydrocarbons present in sediment organic fractions. An absence of normal alkane peaks indicates that microbial utilization of any residue may have taken place.

- High voltage mass spectrometric analysis of saturate and aromatic fractions of sediment extracts have given results which are consistent with the presence of bituminous material in the sediments. The distributions of aromatic compound types in sediment samples are reasonably consistent with that observed for a weathered Maracaibo crude oil. The sediment saturate fractions contained lesser relative amounts of paraffins than the reference crude, which is attributed to natural processes. More than one-half of the saturate fractions had compound-type distributions similar to those obtained from sediments observed to contain relatively large amounts of bituminous material. Most of these were from the oil producing region of the lake.

STABLE ISOTOPE RATIOS OF CARBON

Introduction

Stable isotope ratios of carbon have provided information on many natural geochemical processes. These variations have also demonstrated that man's activities can be determined where the natural stable isotope ratios have been shifted by significant additions or removals of material with different but characteristic stable isotope ratios.

In the field of isotope geochemistry the data is expressed in terms of δ (Del), the per mil difference between the isotope ratio of the sample and a standard material. $\delta^{13}C$ is defined as

$$\delta^{13}C = \frac{(^{13}C/^{12}C)\text{ sample} - (^{13}C/^{12}C)\text{ standard} \times 1000}{^{13}C/^{12}C\text{ standard}}$$

In these studies the reference standard is Solenhafen Limestone from the National Bureau of Standards (NBS 20). Based on this definition, stable isotope ratio variations are expressed as small positive or negative numbers. A negative δ means the sample is enriched in the light isotope relative to the standard, while a positive δ means the sample is depleted in the light isotope. Analysis by mass spectrometer has been carried out by Teledyne Isotopes Inc. under sub-contract.

As a result of the equilibrium isotope effect, atmospheric CO_2 is about 7/mil enriched in ^{12}C relative to the bicarbonate of sea water. The small amount of CO_2 dissolved in sea water and the large amount in fresh water will remain at -7/mil so long as it is in isotopic equilibrium with atmospheric CO_2.

Petroleum and natural gas have the most negative $\delta^{13}C$ values of any of the major geochemical reservoirs of carbon. It has been calculated, using this method, that about 50% of the carbon atoms in the Houston Ship Canal are derived from petrochemical pollution (Calder and Parker, 1968). Many oils have values of $\delta^{13}C$ of between -25 and -33. The oil supplied to the laboratory at Lake Maracaibo had a $\delta^{13}C$ value of -26.6.

Since Lake Maracaibo has been subjected to oil seeps, leaks and discharges, and untreated sewage discharges for many years, it was of interest to examine the use of stable isotope ratios of carbon as indicators of changes in the natural system. The studies were initiated to determine, if possible, the routes by which petroleum derived carbon is available to the biological system, and the degree of incorporation. However the method provides no information about the chemical structure of organic pollution or its toxic properties.

To date, published studies have confined themselves to determination of pollution by measurement of the $\delta^{13}C$ of inorganic carbon, dissolved and particulate organic carbon in water. The objective of this study was to explore the possibilities of applying the technique to samples which had been subjected to oil contamination for many decades, the hypothesis being that incorporation into the ecosystem of carbon derived from petroleum may be able to be determined by a shift in the $\delta^{13}C$ values of biological material.

Biological Samples

For a preliminary assessment, a variety of biological samples were selected from a wide variety of collection sites. These included the lake itself, Gulf of Venezuela and the eastern side of the Paraguaná peninsula. The majority of samples were of muscle tissue of fish, shrimp and crabs, though some samples of viscera and gut contents were also examined. Biotic samples were dried, weighed, and submitted for analysis of CO_2 by mass spectrometry and reported as $\delta^{13}C$ compared to the National Bureau of Standards reference (NBS 20). Values are given in Table 18.

It is apparent that there is a range of $\delta^{13}C$ values in fish muscle from -15.3 to -24.6. Those fish samples from uncontaminated areas tend to have values from -15.3 to -17, whereas those samples taken from within the lake range from -17 to -24.6, the higher values from the eastern side of the lake off Lagunillas. The $\delta^{13}C$ values of visceral tissue from selected species were inconsistent but in the same range.

Species differences may well account for most of the variation. Eight samples of the fish, curvina, were sampled and showed a range from -17 to -19.5. Parker (1964) found that individual fish of the same species which were collected at the same time and place have an isotopic composition within ± 1/mil. Published values for shrimp from a shallow marine estuary in Texas were in the range $\delta^{13}C$ of -11 to -14. For fish the range was -7.7 to -17.0.

Table 18

Stable isotope ratios of carbon in Maracaibo biological samples

Sample	Sampling Area	Tissue $\delta^{13}C$	Viscera $\delta^{13}C$
Phytoplankton	Terminales Maracaibo	-14.3	
Lisa	Guajira Peninsula	muscle -15.3	
Freshwater Catfish	La Ceiba	muscle -15.3	
Blue Crabs	Bridge - La Rita	gill -15.4	*-13.8
Blue Crabs	L-80 off Lagunillas	whole -16.7	
Corocora	Manzanillas, East of Paraguaná Peninsula	muscle -15.8	
Curvina	Barranquitas	muscle -17.0	-18.2
Carpeta	Barranquitas	muscle -17.2	
Shrimp	Bridge - La Rita	muscle -17.3	
Shrimp	Bridge - La Rita	muscle -17.4	-13.2
Shrimp	Off La Represa	muscle -17.7	
Moharra	Off La Represa	muscle -18.0	
Shrimp	Bridge - La Rita	muscle -18.0	
P. tropicus	Bridge - La Rita	muscle -18.3	-17.0
Curvina	L-80 off Lagunillas	muscle -18.7	-.18.3
Curvina	Off La Ceiba	muscle -18.8	-19.2
Curvina	Off La Ceiba	muscle -18.8	-20.1
Curvina	Off La Represa	muscle -19.0	
Curvina	Bridge - La Rita	muscle -19.3	-16.8
Curvina	Bridge - La Rita	muscle -19.5	
Curvina	Bridge - La Rita	muscle -19.5	-16.5
Bagre	L-80 off Lagunillas	muscle -19.7	-18.1
Jack	Off La Represa	muscle -19.9	
P. tropicus	Bridge - La Rita	muscle -20.6	-21.6
Robalo	L-51 off Lagunillas	muscle -21.4	-25.3
Robalo	L-51 off Lagunillas	muscle -24.6	-23.0

*entire remainder of crab used

When normal and pollutant carbon are mixed, the $\delta^{13}C$ of the mixture is given by

$$\delta m = \frac{Cn\delta n + Cp\delta p}{Cn + Cp}$$

where C = concentration of carbon mg/ℓ, δ = $\delta^{13}C$, n refers to non-pollutant carbon, p refers to pollutant carbon, and m refers to the mixture (Calder and Parker, 1968). The ratio of the amount of pollutant carbon to normal carbon in the mixture is given by

$$\frac{Cp}{Cn} = \frac{\delta n - \delta m}{\delta m - \delta p}.$$

Substituting the $\delta^{13}C$ values for the extreme curvina samples (-17 and -19.5), and the determined $\delta^{13}C$ value for oil of -26.6, gives a result indicating that 26% of the carbon in the curvina having the lowest value (-19.5) is derived from petroleum. This result assumes that the one having the highest value (-17) contains no carbon derived from petroleum. It should be kept in mind, however that the range of values for curvina is very nearly that of the reported variation within species. Thus, the data obtained must be considered inconclusive with respect to the contribution of petroleum to the carbon content of Lake Maracaibo biota.

Sediment Organic Extracts

In addition to biotic samples, the stable carbon isotope ratios of the organic extracts from sediment samples were determined. Six samples were selected for these exploratory studies;

three from locations likely to contain bitumen in the sediment, and three from locations far from the oil producing region. The results are presented in Table 19, together with the values obtained for South Tia Juana Medium Crude, a weathered sample of the same crude oil, and for a bituminous particle removed from the sediment sample obtained at Station 2. The values of the isotope ratios of Maracaibo sediment extracts are extremely low compared to values obtained in a true marine environment. Values of $\delta^{13}C$ for marine plants and biota generally fall in a range between -4 and -15 (Calder and Parker, 1970). However, the lipid fractions of land and freshwater plants can have much lower values (Silverman, 1967). It is therefore likely that the low values for the sediment extracts reflect the presence of detritus from freshwater and land plants.

The "percent bitumen carbon" values given in Table 19 are based on the assumption that the lowest value of $\delta^{13}C$ found for the sediment extracts (-41.6) is representative of the value expected for a sample containing no bituminous residue. A value of -27.1 is taken as that representing 100% bitumen, since this value was reported for the bitumen particle recovered from Station 2. Calculation of percent bitumen carbon is based on the equation previously given. Of the three sediment extracts calculated to have 49% or more bitumen carbon, it is perhaps significant to note that two (Station 2 and 31) were observed to contain bituminous particles, and the third (Station 6) was from sediments from the vicinity of the ship channel in the Straits of Maracaibo.

The assumptions used for the calculations, as in the case of the tissue studies, are of necessity over-simplified; much more data is needed concerning the $\delta^{13}C$ values of other types of material discharged into the lake before such calculations can be accepted as true indicators of the presence or absence of bituminous residues. The trends observed in this exploratory study are, however, very encouraging and suggest that further studies along these lines may be profitable.

Table 19

Stable isotope ratios of carbon in Maracaibo sediment extracts[1]

Station Number	$\delta^{13}C$	Calculated Percent Bitumen Carbon[2]
2	-33.7	54
6	-30.0	80
14	-38.8	19
26	-41.6	0
27	-38.8	19
31	-34.5	49
Tia Juana Medium Crude Oil	-26.6	—
Weathered South Tia Juana Medium	-27.9	—
Bitumen particle from Station 2	-27.1	—

[1] Benzene-methanol extraction

[2] Assuming Station 26 sample contains no bitumen

Summary

- The $\delta^{13}C$ values determined for samples of Maracaibo fish muscle tissue ranged from -15.3 to -24.6. Those samples from areas of petroleum production, and of domestic and industrial wastewaters, have values ranging from -17.0 to -24.6. Those samples from other regions had a range of -15.3 to -17.0. It is difficult to assess the impact of petroleum hydrocabons on the values reported however, since those within any one species were reasonably close together.
- The $\delta^{13}C$ values reported for Lake Maracaibo sediment organic extracts were all below -30.0, suggesting the presence of lipids from freshwater and land plants. Values obtained on two samples which contained noticeable quantities of bitumen were much higher than the lowest value, -41.6, obtained for a sediment extract from the southern portions of the lake.

EFFECTS OF OIL: EXPERIMENTAL STUDIES OF TOXICITY OF CRUDE OIL TO LAKE MARACAIBO BIOTA

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EFFECTS OF OIL: EXPERIMENTAL STUDIES OF TOXICITY OF CRUDE OIL TO LAKE MARACAIBO BIOTA

INTRODUCTION

Bioassays are the most effective method of assessing potential toxicity to aquatic organisms. With most bioassays the chemistry of material entering a water system is well understood, although changes in chemistry after passing into the water may be obscure. However, petroleum is ill-defined at the outset; the isolation and identification is made complex in at least three ways. First, the term "petroleum" is applied to a wide variety of "natural" or crude oils and a host of refined products. Many of the same individual components commonly occur in the unpolluted system. Second, even after specification of a given entity as the "petroleum" of definition, one is concerned with a complex mixture of organic components with wide variety in properties of volatility, water solubility, and toxicity. Third, the chemical composition of many of the compounds may change after entering water. Conditions of turbulence and temperature may radically alter the dynamics of "oil-water" mixtures, and partitioning among oil, water, and gas phases may differ greatly depending on the hydrocarbon types present in crude oils.

There are two types of bioassays to determine acute toxicity in general use, 1) the flow-through bioassay in which the test solution is continually renewed and 2) the static bioassay in which the test solution is not changed during the test period. For each type of test, acute toxicity is described in terms of the median tolerance limit (TLm), which is the concentration of the test substance at which 50% of the test organisms survive for a specified period of time. TLm is reported for 24, 48, 72, or 96 hours. The objective in this study was to determine the concentration of crude oil which would kill half of the test population during a 24-hour test period. The concentration values for crude oil toxicity are based on the amount of oil added to the water rather than the amount of oil actually present in the water column. These values are expressed in parts of oil per million parts of water (ppm). The types of crude oils used in the experimental toxicity studies were all from the Lake Maracaibo field. Since the test oils used varied widely in both physical and chemical properties, they are listed in Table 20 together with pertinent data.

Table 20
Crude oils used in toxicity testing experiments

Crude Oil	API Gravity	Dehydration Chemical Added	Remarks
South Tia Juana Medium Crude Oil	24°	No	Supplied in bulk to a holding tank at the Las Morochas Lab. An oil/water emulsion layer was present, and this layer was used in some of the preliminary experiments.
Tia Juana Medium Crude Oil	25°-26°	Yes	This and the following oils listed were obtained at production flow stations.
Tia Juana Low Pour Crude Oil	24°	Yes	
Tia Juana Heavy Crude Oil	18°	Yes	
Tia Juana Light	31°	Yes	

CONTINUOUS FLOW METHODOLOGY

Acute Toxicity Studies

A flow-through bioassay system, similar in concept to the system at Battelle's Marine Laboratory, Sequim, Washington, (Vaughan, 1973) was constructed at the Las Morochas Laboratory. The system consisted of a constant level water head tank fitted with a manifold with several water delivery tubes. Each tube delivered diluent water to the individual bioassay tanks. The oil delivery system consisted of a large glass tank with bent glass delivery tubes to meter constant amounts of oil to the individual bioassay aquaria. The water and oil were combined in a large-mouthed funnel prior to discharge into the bioassay aquaria. Water and oil flow were adjusted to maintain a displacement time of 1 hour in the bioassay aquaria.

This system did not function satisfactorily with Lake Maracaibo waters due to the high productivity and heavy growth of sessile organisms within the water system. Because of extreme variability in the continuous flow bioassay data, the results of the acute toxicity tests are inconclusive.

Studies of Artificial Oil Spills in a Flow-Through System

When considering the toxic effects of oil discharged in Lake Maracaibo, one must keep in mind the following points which characterize a typical "spill." The release of oil occurs over a period of hours so that for each spill the organisms are exposed to a single "slug" of oil which is subjected to continuous degradation by microbial activity, loss of light ends by volatilization, and movement into the water of the soluble fractions.

Both continuous flow and static methodologies were employed to simulate these conditions. Three continuous flow, simulated spill bioassays were conducted. An 8-foot trough was divided across the midpoint by a partition that extended below the water surface to a depth of two inches. Water inflow and outflow located at opposite ends of the tank; a constant water exchange of 3,000 mls a minute was maintained. The tank was stocked with 15 lisa *(Mugil curema)* and 15 white shrimp *(Penaeus schmitti)* for a two-day acclimation period before introducing the oil.

Oil was introduced on the inflow side of the partition by gently pouring through a large-mouthed funnel. All of the oil was retained behind the partition. Undoubtedly, soluble fractions entered the water column and passed under the partition; however, no visual evidence of oil was noted past the partition. For the first experiment one gallon of oil was discharged into the tank which contained 90 gallons of water. Five gallons of oil were discharged into the tank during the second and third experiments. The fish and shrimp were observed for a period of four days. During this time, no additional oil was introduced into the test system.

No mortality was observed throughout the entire test period for the three experiments. The fish and shrimp could swim under the partition; however, no avoidance of the oil-containing half of the tank was noted. Actually, the fish appeared to favor the oil-containing area and were found most of the time under the floating oil. Zooplankton seemed to be concentrating within 5 cm of the edge of the floating oil. However, no measurements were taken to verify any difference in zooplankton density between the oil-containing and oil-free portions of the tank.

This design probably closely simulates the conditions following a single release of oil except that there was no agitation of the oil and water which naturally occurs through wave action. Agitation would most likely result in a faster rate of solubilization and potentially place more toxic compounds in the water, but would also release toxic compounds to the atmosphere through evaporation. Additionally, agitation could disperse small droplets of insoluble oil throughout the water column. These droplets, adhering to gill tissue and the swimmerets of shrimp, may cause mechanical damage that could result in or contribute to the death of the organism.

STATIC BIOASSAY METHODOLOGY

After careful consideration of the problems which were attendant in the use of the continuous flow bioassay system and after repeated attempts to correct these problems failed to reduce experimental variability, a new experimental approach was implemented. Formulation of the approach, which utilizes the static bioassay concept as the fundamental tool, was possible only after a period of trial and error. An understanding of the biology of Lake Maracaibo gained by the staff scientist on site played a key role in the design of a more realistic bioassay approach. Input from a concurrent Battelle-Northwest program on bioassay methodology (Bean et al., 1974) was utilized, when feasible, in the design of the bioassays at the Las Morochas Laboratory.

Using this system it was possible to determine the effects of weathering on the toxicity of the crude oil, the diminution of toxicity with time of oil subjected to artificial spill conditions, and to compare the toxicity of several crude oils.

An integral part of the experimental design for oil bioassay studies at the Las Morochas Laboratory was the measurement of hydrocarbon concentrations in the test media. Duplicate samples of bioassay water were extracted with hexane and with carbon tetrachloride and shipped to the Richland laboratories. Carbon tetrachloride extracts were analyzed for total oil using infrared spectroscopy (IR). The hexane extracts were analyzed for monocyclic aromatic compounds using gas chromatography. Details of methodology employed for these analyses are given in Appendix E.

Exploratory Phase

Six exploratory bioassays were conducted using South Tia Juana Crude Oil to determine the impact of agitation of oil with water. The desired amount of oil was mixed with 15 liters of lake water by inverting a glass carboy 20 times in rapid succession. The mixture was allowed to separate into two phases for 30 minutes. The water phase (hypophase) was siphoned into 1 gallon glass jars and the test organisms added. Ten animals were added to each of 3 liters of test medium. The desired concentration was arrived at by dilution of the hypophase (100%) with lake water to yield 75, 50, and 32%. Mortality in the test vessels and a control vessel was observed frequently over the 24-hour period.

Emulsified South Tia Juana Medium Crude (see Table 20) was not toxic to shrimp when 90 and 150 milliliters were used according to the above methodology. These volumes of oil correspond to an oil/water contact concentration of 6,000 to 10,000 ppm. When 2 liters of demulsified oil were used (133,000 ppm, Figure 60) or 3 liters (200,000 ppm, Figure 61), the results were essentially the same; it required approximately 96% of the concentration of the whole aqueous extract to produce mortality of 50% in 24 hours; i.e., the TLm_{24} value equaled 96%.

The crude oil emulsion was dehydrated by addition of a chemical demulsifier, and subsequently tested for toxicity according to the exploratory procedure above. The dehydrated oil was found to be more toxic than the crude oil emulsion. The 150 milliliters of dehydrated oil extracted into 15 liters of water produced toxicity sufficient to require a dilution to about 72% of the original strength in order to induce mortality in one-half of the test animals (Figure 62). When 2 liters of demulsified oil were tested, the value of the TLm_{24} was estimated to be 50% of the concentration of the original aqueous extract (Figure 63).

In a final exploratory experiment, one liter of emulsified oil was "weathered" for 8 hours in direct sunlight at lake temperature and extracted with 15 liters of lake water. The TLm_{24} value was determined to be about 90% of the extracted water phase (Figure 64).

The above experiments provided a basis for more detailed investigations into the TLm_{24} of South Tia Juana Medium Crude Oil, a comparison of TLm_{24} of several crude oils, the "weathering" of crude oil, and the diminution of toxicity with time of artificial oil spills.

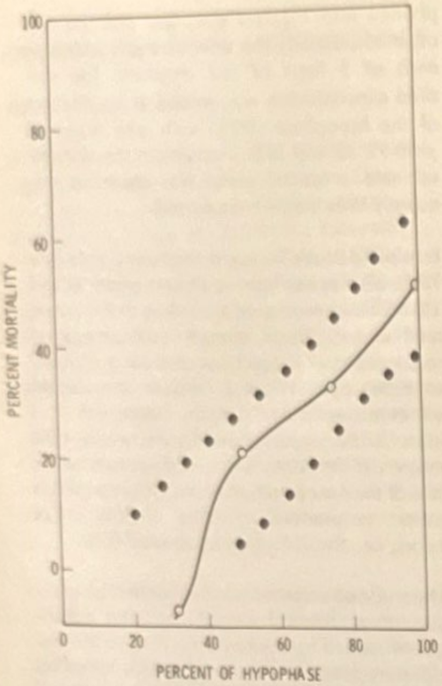

Figure 60
Relationship between the mortality of white shrimp and dilution of hypophase. Ratio of crude oil emulsion to water in extraction vessel 2:15 liters (133,000 ppm)

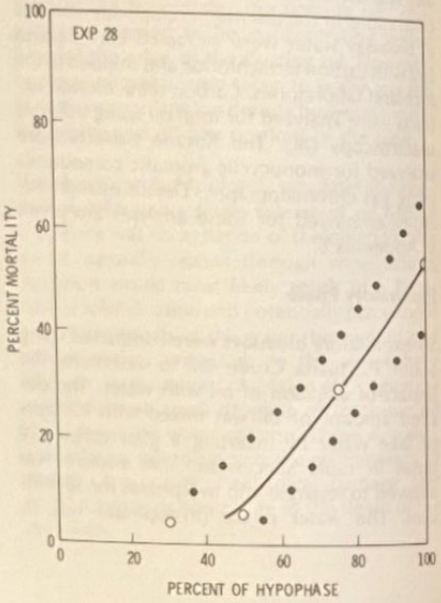

Figure 61
Relationship between the mortality of white shrimp and dilution of hypophase. Ratio of crude oil emulsion to water in the extraction vessel was 3:15 liters (200,000 ppm)

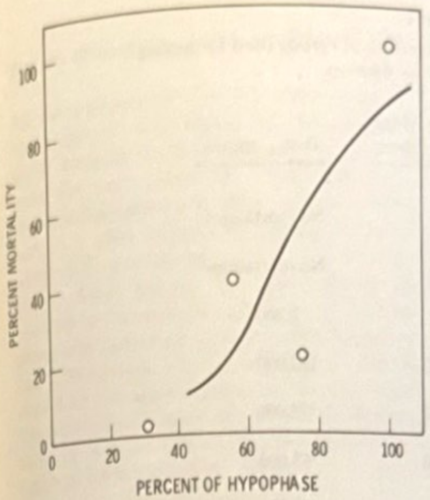

Figure 62
Relationship between the mortality of white shrimp and dilution of hypophase of water extraction of crude oil emulsion. Ratio of crude oil emulsion to water in extraction vessel was 0.15:15 liters (10,000 ppm)

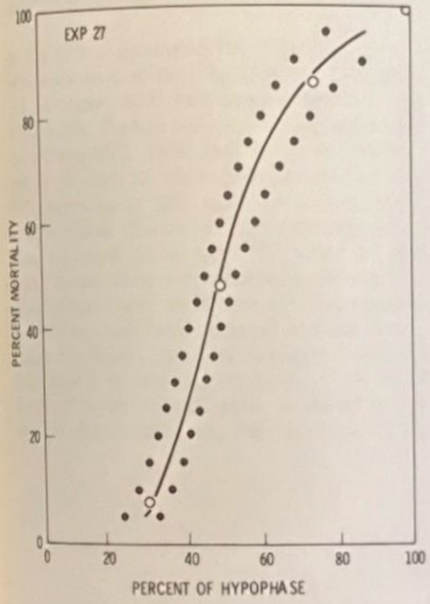

Figure 63
Relationship between the mortality of white shrimp and the dilution of the hypophase of a water extraction of dehydrated crude oil containing chemical demulsifier. Ratio of oil to water in extraction vessel 2:15 liters (133,000 ppm)

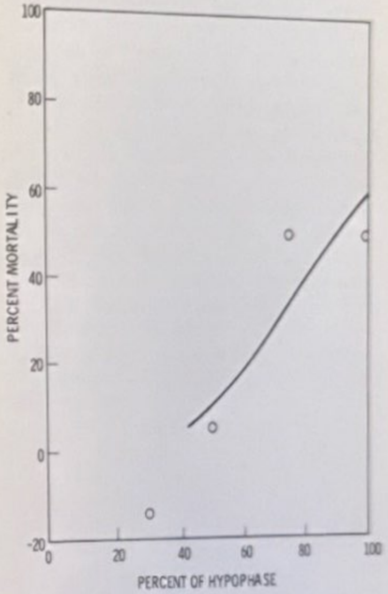

Figure 64
Relationship between the mortality and the dilution of the hypophase of a water extraction of crude oil emulsion which had been "weathered" for 8 hours in direct sunlight. Ratio of crude oil emulsion to water 1:15 liters (67,000 ppm)

Summary Table 1

Experimental results of exploratory phase: Inversion of carboy used for mixing. Concentration arrived at by dilution.

Organism	Oil Type	Concentration Range of Crude Oil (ppm)	TLm_{24} (ppm)
P. schmitti	STJM Emulsion	0-6,000	No mortalities
P. schmitti	STJM Emulsion	0-10,000	No mortalities
P. schmitti	STJM + C.D.[1]	0-10,000	7,200
P. schmitti	STJM Emulsion[2]	0-200,000	192,000
P. schmitti	STJM Emulsion	0-133,000	128,000
P. schmitti	STJM + C.D.	0-133,000	69,000
P. schmitti	STJM Emulsion	0-66,700	61,000

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[1] C.D. = Chemical Demulsifier

[2] "Weathered" for eight hours

Toxicity of South Tia Juana Medium Crude Oil

For these experiments a new mixing technique was employed (see Figure 65). All subsequent static bioassay experiments were performed using the same procedure. The water and oil, in desired proportions, were mixed for 10 minutes in a glass carboy containing 15 liters of water with a magnetic stirrer set at a fixed speed. After mixing, the two phases were allowed to separate for 30 minutes. The water phase was siphoned so as to avoid contamination with surface oil. The organisms were then added to the water phase in the test system. The experiments were carried out in five replicates of ten animals each, with the time of death of each individual noted.

The White Shrimp. The commercial value of the white shrimp, *Penaeus schmitti*, is greater than that of any other in Lake Maracaibo (Volume 1) and is easily kept in captivity for use in toxicity experimentation. It is also an important member of the food web leading to the production of commercial species of fish (Volume 1).

In the early experiments there appeared to be a sharp increase in toxicity between 1333 ppm, oil to water, and 2666 ppm (Experiment 43, Figure 66). Further refinement was attempted by adding 1667, 1333, 1000, 667, and 0 ppm of oil to 15 liters of water. This resulted in only 12% mortality at 1667 ppm and only scattered deaths below that value. The same experiment was repeated using ratios from 667 to 2667 ppm. Again, there was a breaking point at 1333 ppm (Experiment 49, Figure 67). The concentration intervals were reduced and the TLm_{24} value for South Tia Juana Medium Crude Oil was found to be 22.6 milliliters of oil in 15 liters of water or 1507 ppm, as tested in this system (Experiment 44, Figures 68 and 69). The 95% fiducial limits for the TLm_{24} are 17 to 25 milliliters or 1133 to 1666 ppm.

Lisa and Robalo. Similar tests were performed using the lisa, *Mugil curema*, and one of the species of robalo, *Centropomus ensiferus*. Tests on these animals were less extensive due to the lack of animals of suitable size.

The robalo appear to be somewhat more resistant to the effects brought about by the mixing of crude oil and water in which they live than lisa or shrimp. Experiment 50 with robalo (Figure 70) shows that the amount of oil is seven times that required to produce the same response in shrimp. The TLm_{24} value for robalo was calculated to be 10,667 ppm (Figure 71).

Experiment 65 (Figure 72) was performed on lisa using the standard procedure and a TLm_{24} was found to be 2400 (2325-2465) ppm (Figure 72A). Another experiment with the lisa differed slightly from the outlined procedure in that the different concentrations were produced through dilution of concentrated aqueous extract with lake water. The ratio of oil to water in Experiment 34 (Figure 73) was extremely high, (66,667 ppm). The sharp break which occurs between 50 and 75% would correspond roughly to between 33,333 and 50,000 ppm of oil to water. This is rather high compared to what one finds in nature. From this experiment it would appear that the lisa would not suffer in case of an oil spill in Lake Maracaibo. This value is quite different from that arrived at in Experiment 65, when mixing was accomplished using a magnetic stirrer, and no subsequent dilution. It is likely that the process of dilution caused substantial losses of volatile hydrocarbons from the water, with a corresponding rise in the TLm value reported.

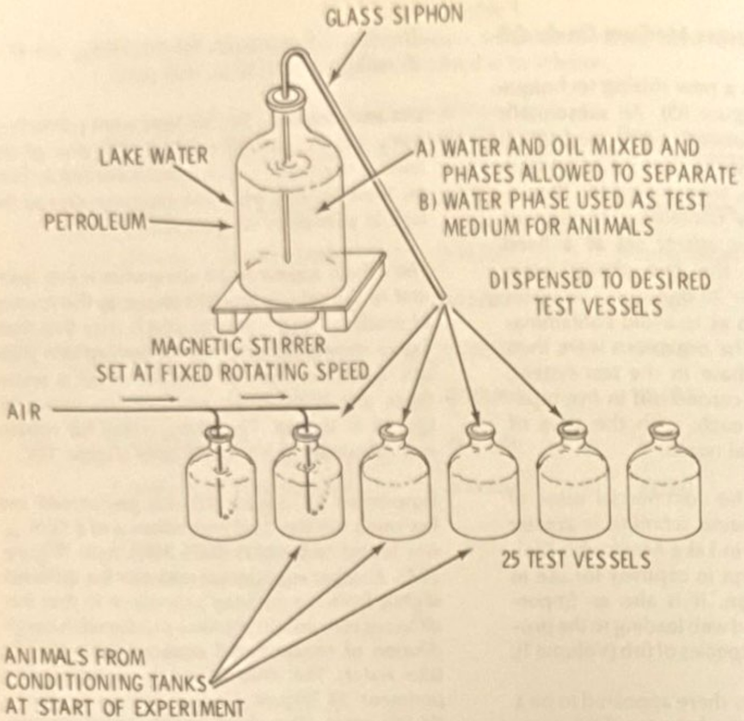

Figure 65.
Static bioassay system

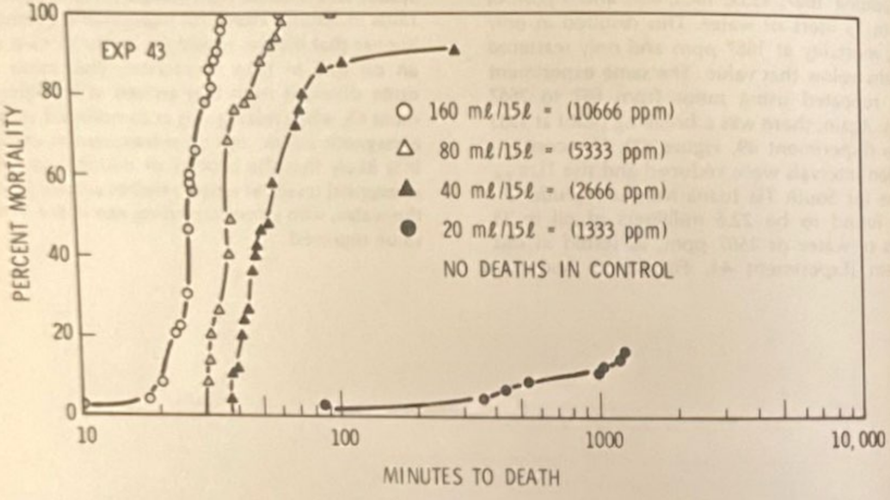

Figure 66
P. schmitti survival at varying concentrations of South Tia Juana Crude Oil.
Points represent death of individual test animals.

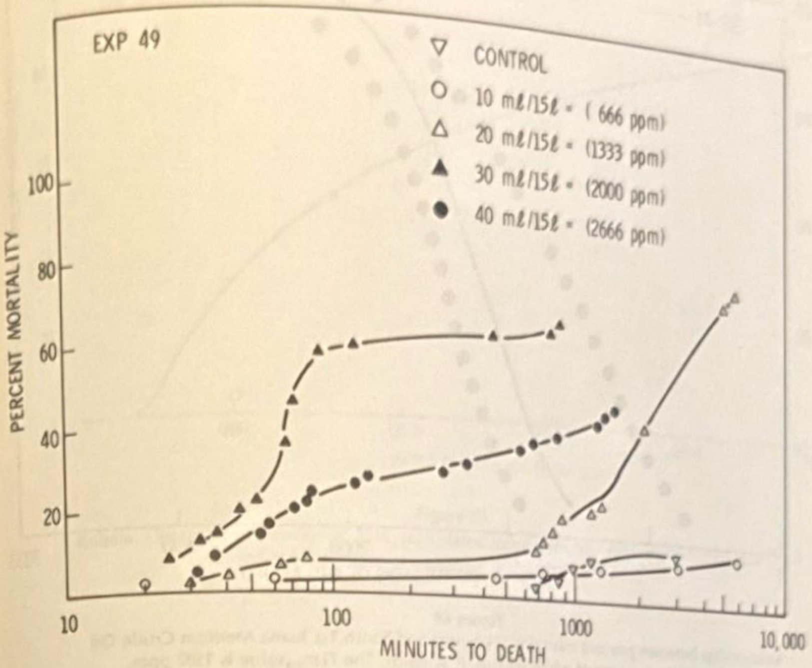

Figure 67
P. schmitti survival at varying concentrations of South Tia Juana Medium Crude Oil

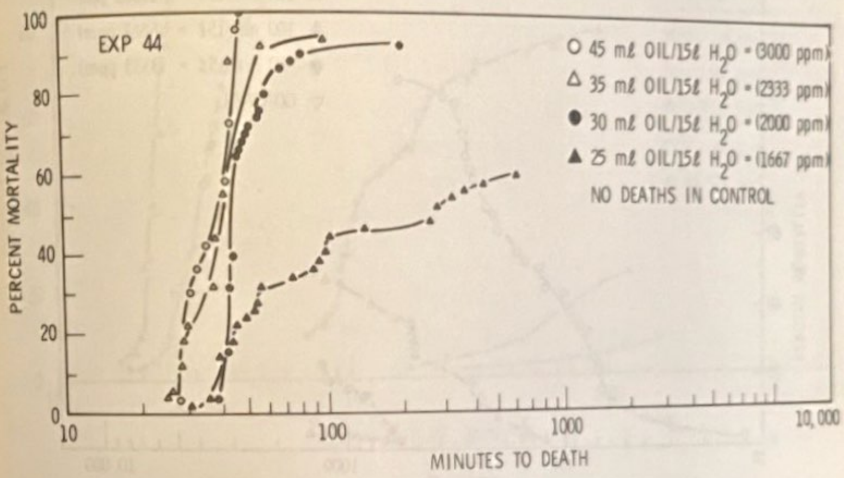

Figure 68
P. schmitti exposed to concentrations of South Tia Juana Medium Crude Oil of 0 to 3000 ppm

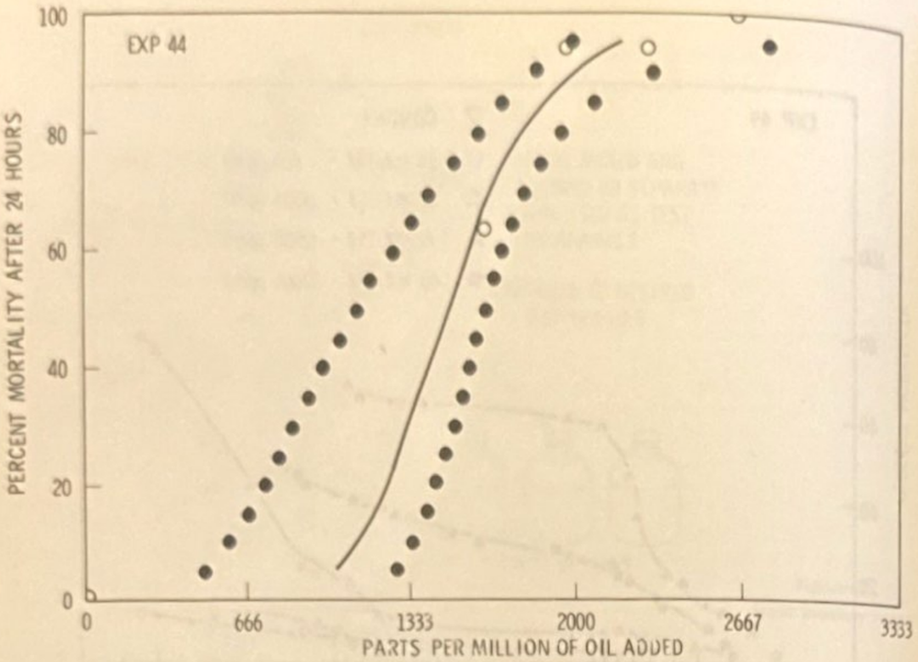

Figure 69
Relationship between percent mortality (24 hours) and South Tia Juana Medium Crude Oil concentrations in water of white shrimp, *P. schmitti*. The TLm_{24} value is 1507 ppm.

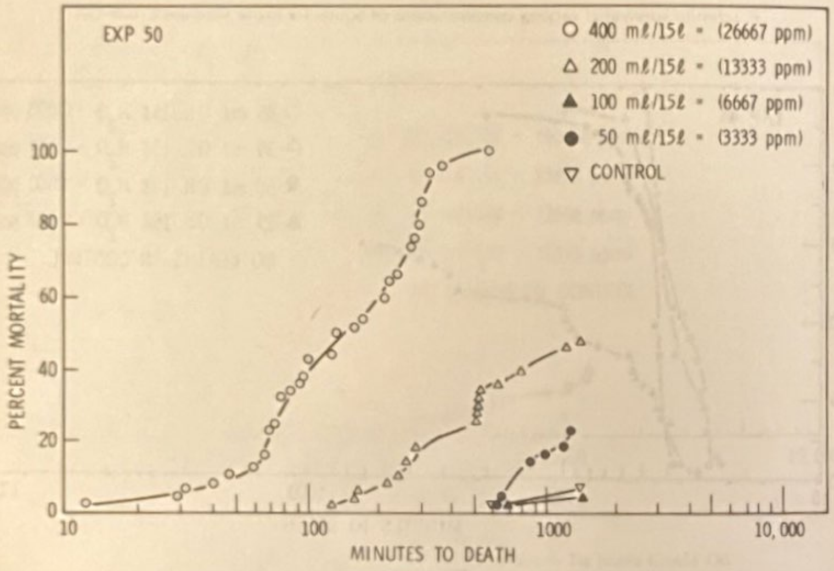

Figure 70
Response of robalo, *Centropomus ensiferus*, to varying combinations of South Tia Juana Medium Crude Oil and water

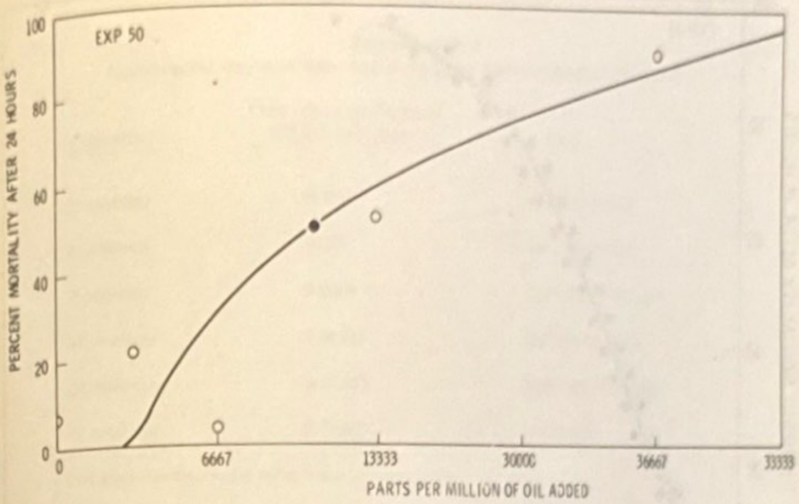

Figure 71
Robalo, *Centropomus ensiferus*. TLm_{24} calculated using nonlinear regression. TLm_{24} value reported is 10,667 ppm of South Tia Juana Medium Crude Oil.

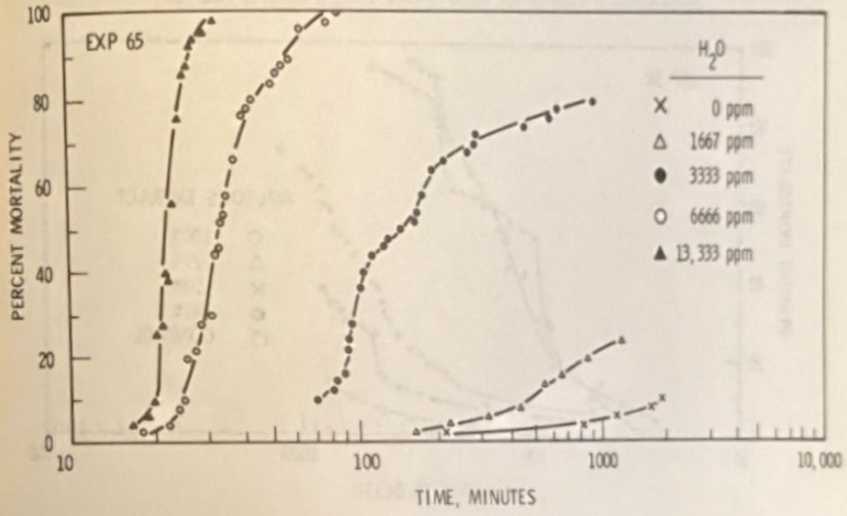

Figure 72
Mortality rates of lisa, *Mugil curema*, in concentrations of 0 to 13,333 ppm of South Tia Juana Medium Crude Oil

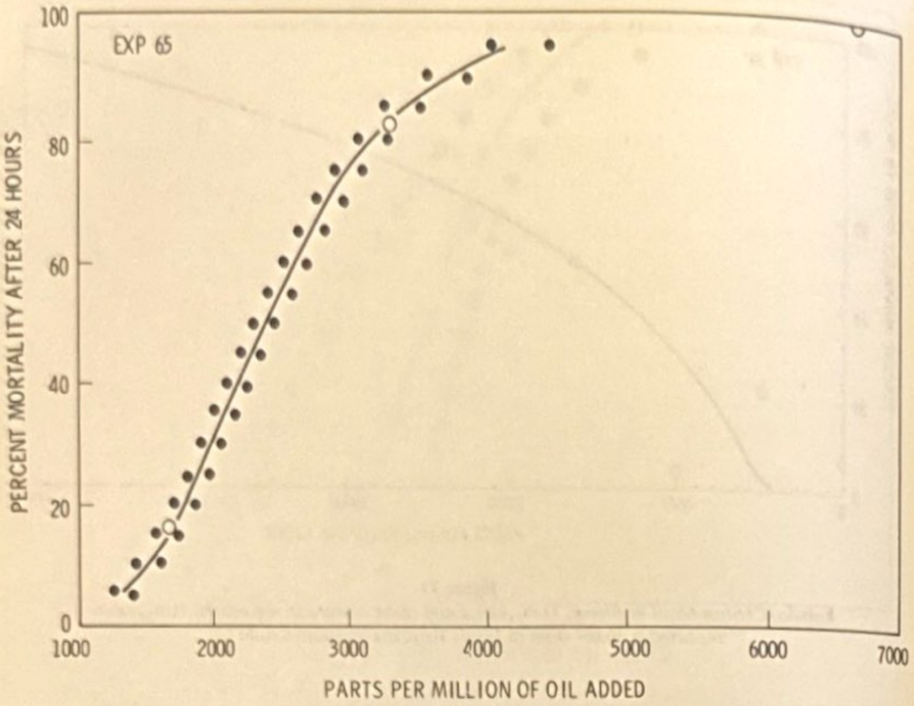

Figure 72A
Relationship between percent mortality (24 hours) and South Tia Juana Medium Crude Oil concentration in water for lisa, *Mugil curema*. TLm_{24} is equal to 2400 ppm.

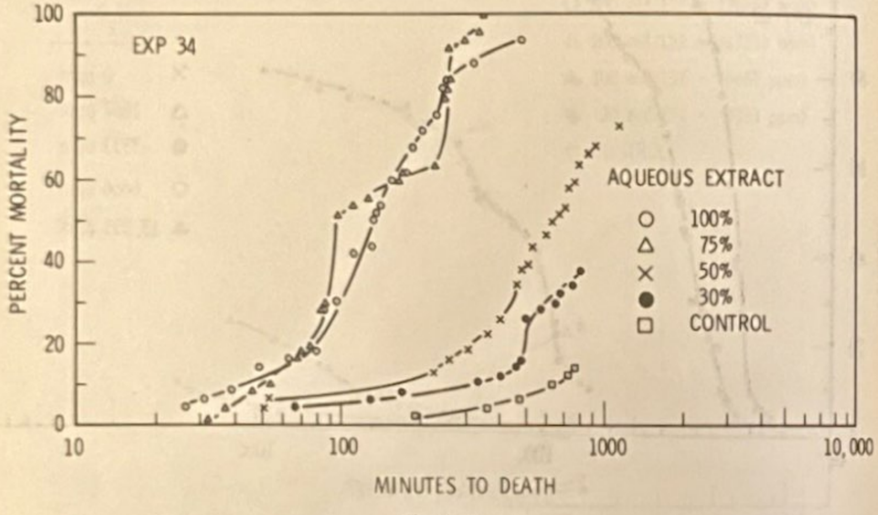

Figure 73
Mugil curema exposed to different concentrations of South Tia Juana Medium Crude. The water phase of the oil-water mixture was diluted with lake water to arrive at the varying concentrations. 100% is equal to 66,667 ppm of oil to water.

Summary Table 2
Experimental results of static tests using South Tia Juana Medium Crude Oil

Organism	Concentration Range of STJM Crude (ppm)	TLm_{24}
P. schmitti	0-10,667	Not determined
P. schmitti	0-2667	Not determined
P. schmitti	0-3000	1507 (1133-1166) ppm
M. curema[1]	0-66,667	Not determined
M. curema	0-13,330	2400 (2325-2465) ppm
C. ensiferus	0-26,670	10,670 ppm

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[1] Dilution method used to arrive at concentration.

Comparative Toxicity of Medium Low Pour, Light, and Heavy Tia Juana Crude Oils

A number of experiments were performed using crude oils, all of which had a chemical dehydration agent added. All experiments were performed using the white shrimp as test animals and employing the magnetic stirrer mixing technique. Experiment 51 (Figure 74) was conducted with a medium gravity oil with low pour point. The mortality results were equivalent to those with South Tia Juana Medium Crude Oil but the induction time for the response was reduced. Statistical analysis indicates that the TLm_{24} is 1600 ppm with 95% fiducial limits of 933 to 2670 ppm (Figure 75). Further experimentation with the low pour Tia Juana Medium gave a TLm_{24} value of 1783 ppm with 95% fiducial limits of from 1200 to 2650 ppm (Experiment 58, Figure 76). Experiment 52 (Figure 77) was performed using Tia Juana Medium Crude Oil. This crude oil was taken from the production line at Ulé Tank Farm. Once again there was a definite reduction in the induction time. Although the data was not amenable to probit analysis a TLm_{24} of 2160 ppm was calculated. Thus, it appears that South Tia Juana Medium Crude Oil, Tia Juana Low Pour Crude Oil and Tia Juana Medium Crude Oil have approximately the same TLm values.

Experiments with Tia Juana Heavy Crude Oil with demulsifier indicated that this oil was less toxic than all other crude oils tested (Experiment 56, Table 21).

Experiment 53 (Figure 78) used Tia Juana Light Crude Oil with dehydration chemical added in the production line. There appears to be no correlation between the toxic effects and the amount of crude oil added. However, analysis of light aromatics in the test water (Figure 79) showed that the concentration of the light aromatic fraction, rather than the oil-to-water ratio, is consistent with the mortality data. It would appear that properties of the light oil influenced its mixing efficiency.

To achieve more effective mixing 9 liters of lake water, rather than 15 liters, were used to extract the Tia Juana Light Crude Oil (Figure 80). Tia Juana Light Oil appears to be less toxic than all medium crude oils tested. Statistical analysis of the data yielded a TLm_{24} or 2800 ppm with 95% fiducial limits of 1650 to 4750 ppm.

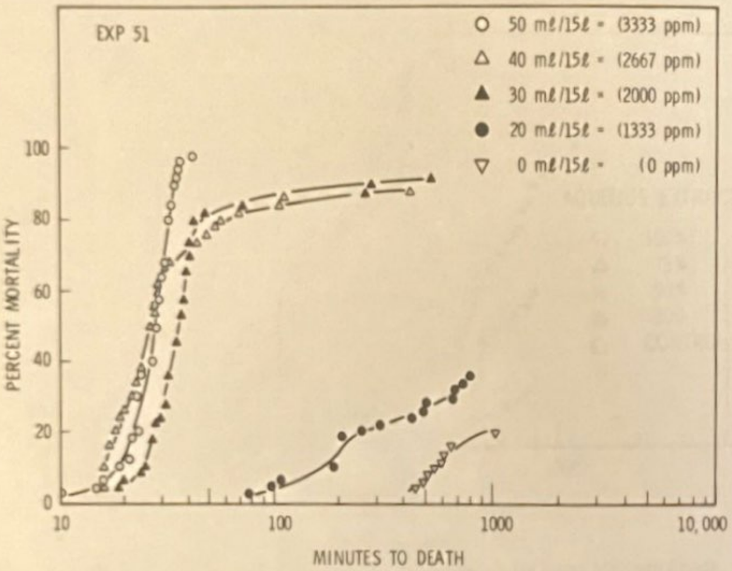

Figure 74

Shrimp, *P. schmitti*, exposed to TJ-102, a medium weight crude oil with a low pour point, and dehydration chemical added in production line. Oil sample was taken from production line.

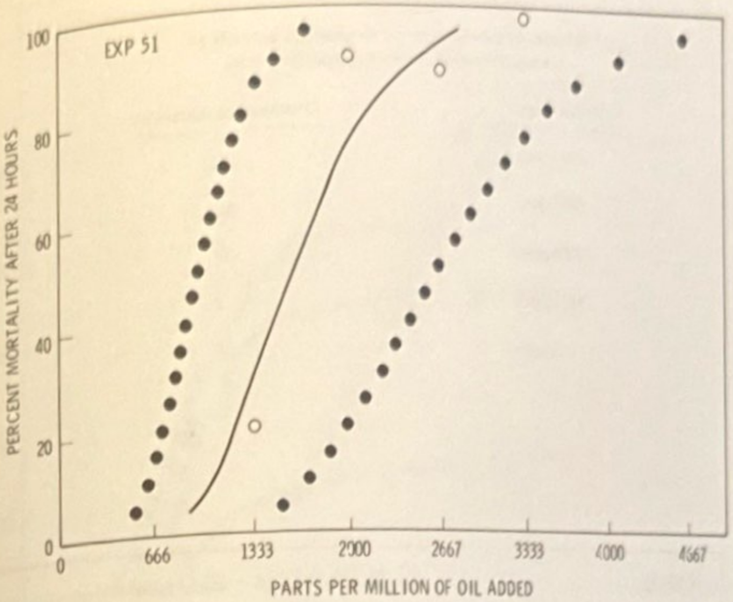

Figure 75

Relationship between percent mortality of white shrimp and oil concentration of TJ-102 crude oil with dehydration chemical.

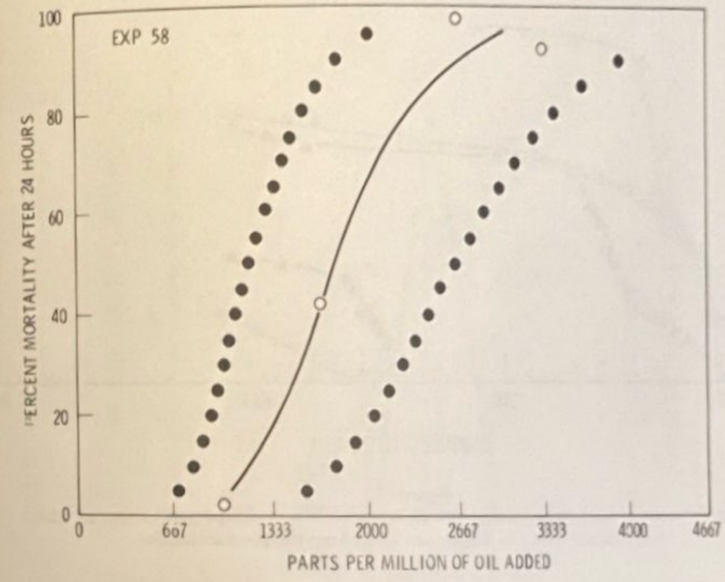

Figure 76

Relationship between percent mortality of white shrimp and the concentration of TJ-102 crude oil with dehydration chemical.

Table 21
Mortality of shrimp exposed to aqueous extracts of
Tia Juana Heavy Crude Oil (Experiment 56)

Oil/Water Ratio	Percentage Mortality
3333 ppm	20
2667 ppm	18
2000 ppm	24
1333 ppm	8
0 ppm	4

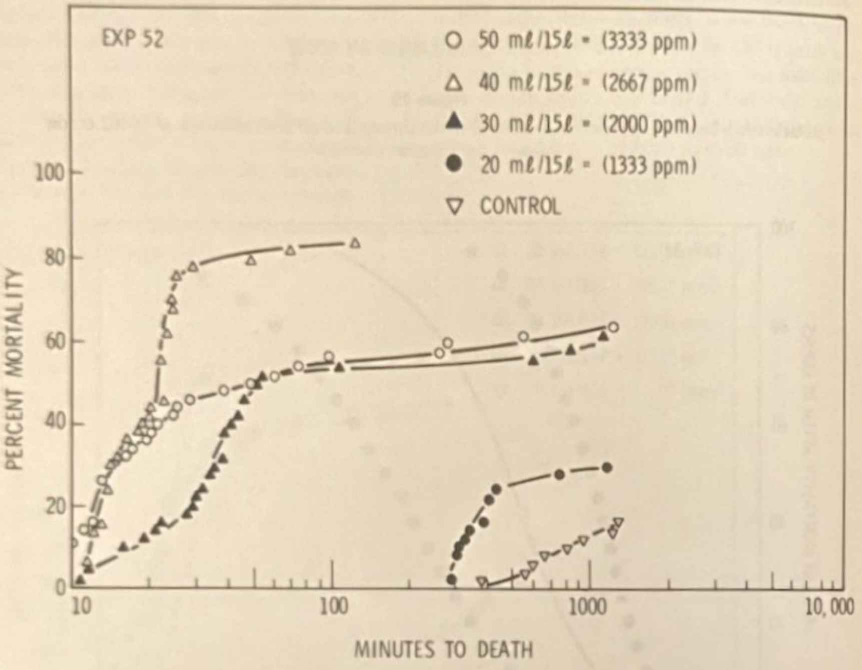

Figure 77
Shrimp, *P. schmitti*, exposed to Tia Juana Medium from Ulé with dehydration chemical added in production line. Oil sample was taken from the production line.

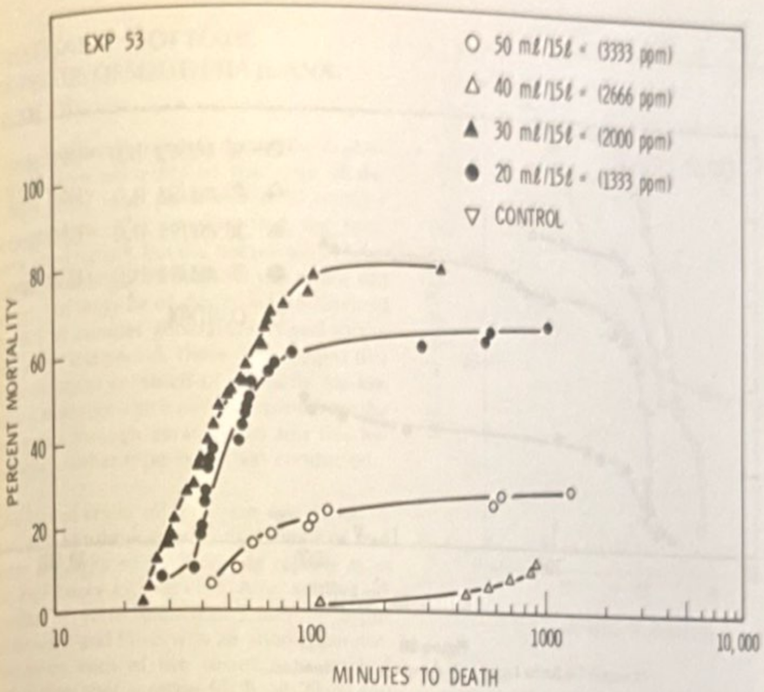

Figure 78
P. schmitti exposed to Tia Juana Light Crude Oil which has dehydration chemical added

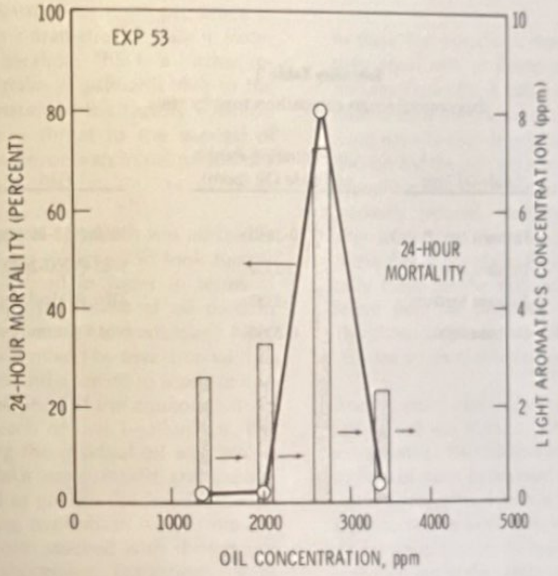

Figure 79
Relationship of light aromatic concentration
to mortality of Tia Juana Light Oil

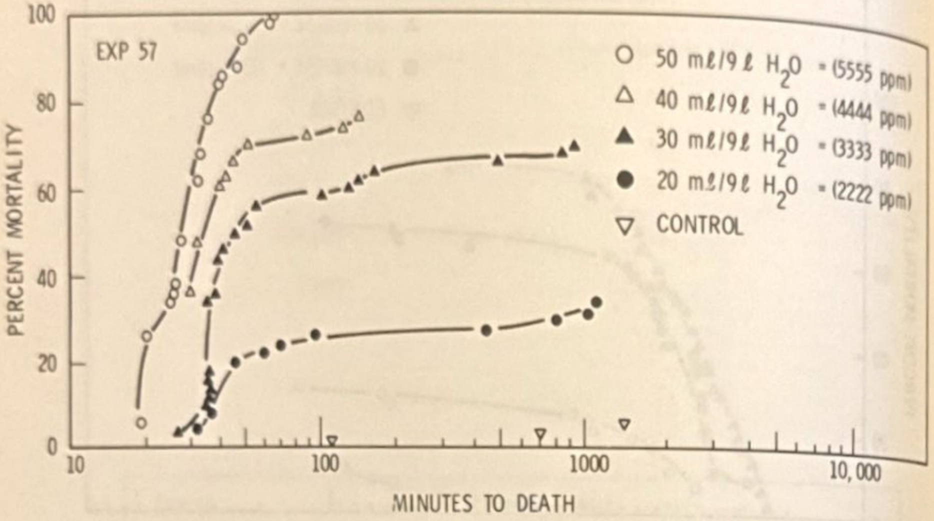

Figure 80
Tia Juana Light with dehydration chemical

Summary Table 3
Experimental results comparison toxicity tests

Organism	Crude Oil Type	Concentration Range of Crude Oil (ppm)	TLm
P. schmitti	Tia Juana Low Pour	0-3330	1600 (953-260) ppm
P. schmitti	Tia Juana Low Pour	0-3330	1783 (1200-2650) ppm
P. schmitti	Tia Juana Medium	0-3330	2160 (Calculated)
P. schmitti	Tia Juana Light	0-3330	Not Determined

INVESTIGATIONS OF TOXIC PROPERTIES OF SOUTH TIA JUANA CRUDE OIL

Mortality observations made during the exploratory experiments indicated that most of the shrimp deaths took place within 80 minutes after exposure. This suggests that the toxic agent acted rapidly, but did not remain in solution at full strength throughout the entire test period. The majority of shrimp which survived the first 80 minutes generally survived to the end of the test period. These data suggest that the toxic agent consisted of primarily the low boiling aromatics which were removed from the test media through aeration. To test this hypothesis, another experiment was conducted.

One liter of crude oil emulsion was added to each of five carboys containing 15 liters of lake water and agitated by inverting rapidly as in the exploratory experiments. After settling, 25 1-gallon jars were filled with 3 liters of hypophase water and fitted with aeration apparatus. Five series, each of five vessels, was stocked with shrimp after aeration for 0, 10, 20, 40 and 80 minutes. Mortality was observed frequently for 24 hours.

The results of this experiment are presented in Figure 81. There is a dramatic decrease in toxicity with time of aeration. This is a further indication that mortality is primarily due to the low boiling aromatics which rapidly volatilize and may not be a threat to the survival of aquatic organisms beyond an initial period of a few hours.

Another experimental approach was utilized in a series of bioassays designed to look further at the dynamics of oil in water in terms of toxicity to shrimp. The levels of oil used in the exploratory experiments were quite high. One liter of oil was mixed by inversion with 15 liters of lake water and allowed to separate into two phases. Three liters of the aqueous extract were placed in each of five 1-gallon jars. The carboy containing the residual oil was refilled with 15 liters of lake water, mixed, and the two phases separated as previously described. Gallon jars containing hypophase water from the first extraction were stocked with shrimp and aerated. Five successive extractions were carried out using the original oil sample. For each extraction, five replicate bioassays were performed on the hypophase water.

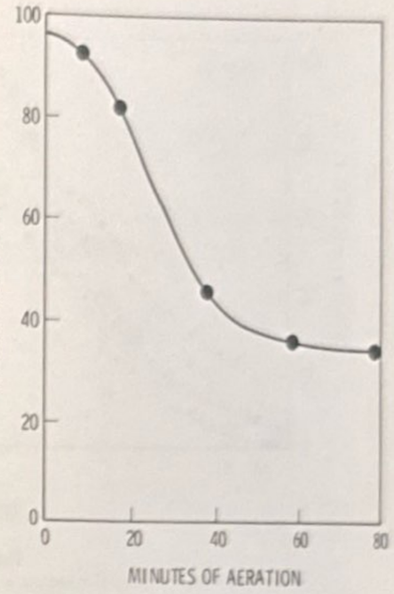

Figure 81
Minutes of aeration refer to the amount of time the test medium was aerated prior to the addition of white shrimp, *P. schmitti*

In these five extractions, mortality was essentially equal with all shrimp dying within 100 minutes (Figure 82). A sixth extraction was performed using the original oil sample; however, there was a 12-hour delay between the fifth extraction and the sixth extraction. Toxicity of the hypophase water after the sixth extraction was markedly reduced, mortality reaching 40% after 100 minutes (Figure 82). Visual inspection of the data indicates that the slope of the mortality curve for the sixth extraction was different from the preceding five extractions. This shows a different mortality rate and possibly the action of different toxic agents.

Another experiment similar in design to the preceding test was initiated, but with successive extractions of 150 milliliters emulsified oil with 15 liters of water. In this experiment the oil and water were agitated with a magnetic mixer. Eight successive extractions were carried out. After each extraction, the hypophase water was siphoned out of the carboy and 3 liters of this water were placed in each of five replicate 1-gallon jars. Each jar was stocked with ten shrimp and the test media aerated with com-

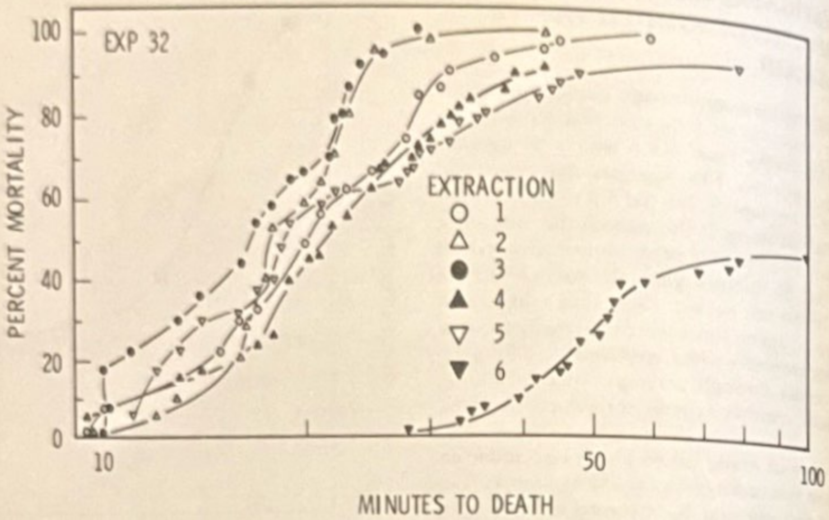

Figure 82
The effect of successive extractions of oil on mortality to white shrimp

pressed air. A ninth extraction of the original oil sample was made 12 hours after the previous eight successive extractions. The hypophase water from this extraction was also bioassayed using ten shrimp in each of five replicate gallon jars.

Nearly identical mortality rates were observed for the first eight extractions. Only two shrimp were killed as a result of exposure to hypophase water from the ninth extraction (Figure 83). Apparently there are sufficient toxic elements in 150 milliliters of oil to render 120 gallons of water lethal to shrimp. Additionally, this toxic constituent is lost with at least 12 hours weathering of the whole oil. As shown in earlier experiments, those toxic components extracted from whole oil are "weathered" out of aqueous solutions in 80 minutes and mortality is significantly reduced. The method used to agitate the oil/water mixture is important. Simple inversion of the carboy did not render 15 liters of hypophase water toxic, whereas mixing with a magnetic stirrer could render 120 gallons of hypophase water extremely toxic to shrimp.

Experiment 37 was conducted using lisa, *M. curema*. Four extractions of 150 milliliters of oil were made as above, and although the toxicant remained potent after four extractions, there is a substantial change in the form of the curve after the first extraction (Figure 84).

The data collected in all the bioassays suggested several additional experiments to further define the dynamics of oil in water as it relates to toxicity. One experiment was designed to determine the effects of weathering of whole oil on subsequent toxicity. A sample of oil was placed in a beaker and set on a magnetic stirrer and continuously agitated in direct sunlight. A 150 milliliter sample of oil was removed before "weathering" and subsequent 50 milliliter samples were taken after 60, 120, 240, and 300 minutes of "weathering." Each of the samples was placed in a carboy containing 15 liters of lake water and agitated with a magnetic mixer. After 30 minutes of rest the hypophase water was siphoned out of the carboy and placed in five replicate gallon jars, each containing 3 liters of test media.

The results of this series of bioassays are presented in Figure 85. A definite decrease in toxicity was apparent for the oil taken after 300 minutes of "weathering."

Experiment 38 (Figure 86) conducted on shrimp was also concerned with weathering in a one-phase system in which the petroleum was stirred in the sun and wind at constant temperature and 150 milliliter subsamples were taken from this oil at time intervals which increased in roughly logarithmic fashion. These subsamples were processed according to the method outlined above. The toxicity of these subsamples was tested individually. The important effect to be noted here is that the diminution of toxicity with increased weathering is more evident (Figure 86). The exposure of this amount of oil does not approximate a film in that the ratio of the surface to volume is very low.

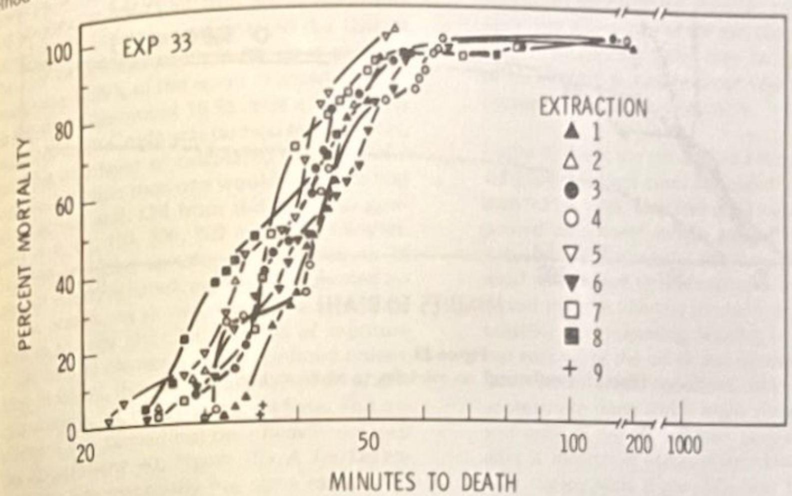

Figure 83
The effect of successive extractions of oil on mortality to white shrimp

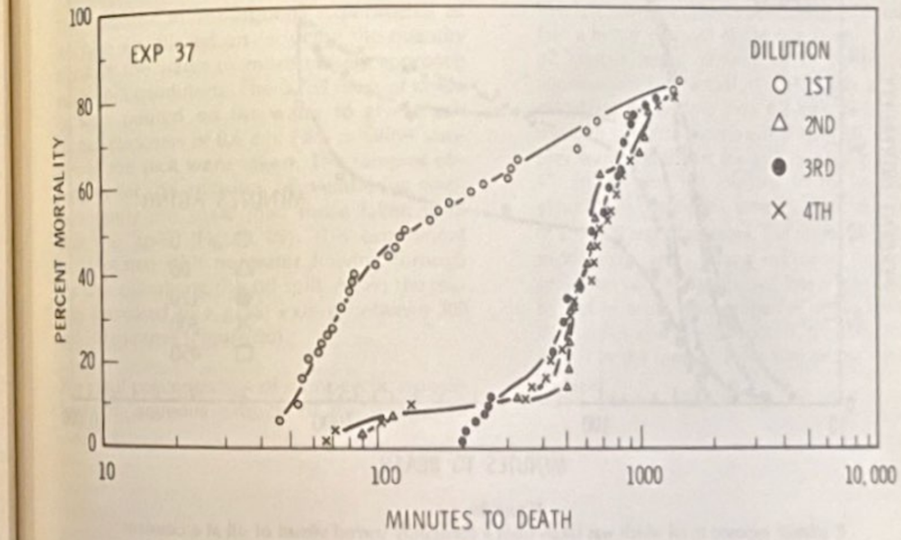

Figure 84
Lisa, *Mugil curema*, were exposed to successive dilutions of hypophase water from 10,000 ppm oil to water mixture

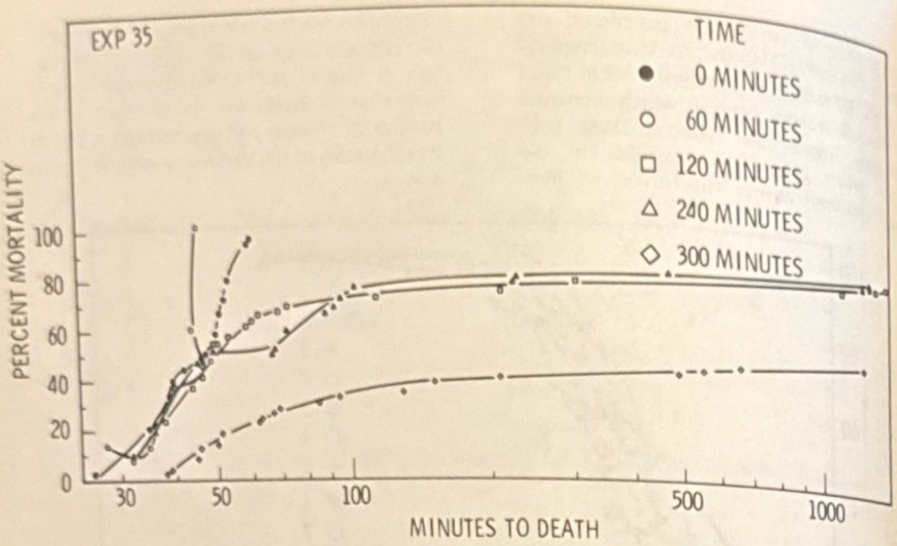

Figure 85
Effect of "weathering" on mortality to white shrimp

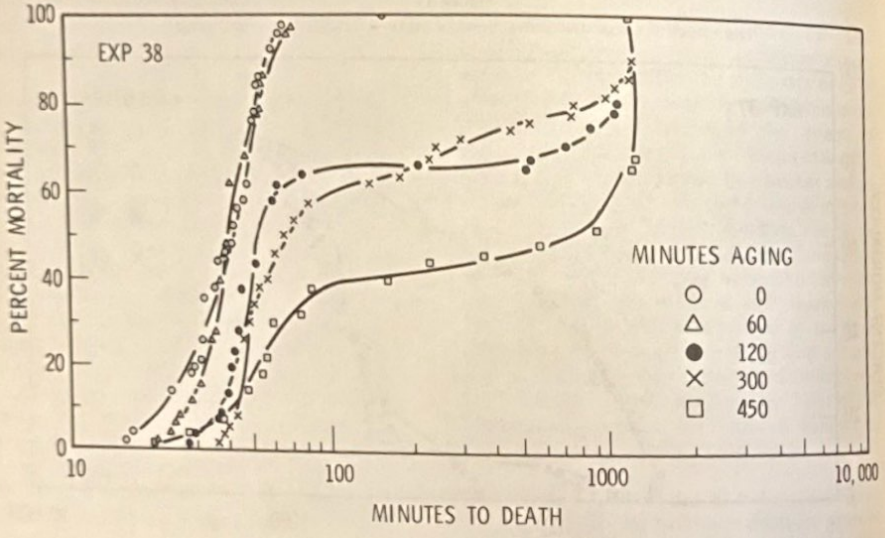

Figure 86
P. schmitti exposed to oil which was taken from a constantly stirred vessel of oil at a constant temperature of 29°C, in sunlight

EFFECTS OF WEATHERING ON OIL TOXICITY: ARTIFICIAL OIL SPILLS USING SOUTH TIA JUANA MEDIUM CRUDE OIL

A series of artificial spills were conducted in which oil was poured gently over the surface of water in a rectangular fiberglass tank, covering an area of 6262 cm². The water was maintained at 23 cm deep and entered the tank at the rate of 3 liters per minute, or a replacement time of 99% of the water in about 8 hours. In the first experiment 18.93 liters of South Tia Juana Medium Crude was poured on the water, resulting in a layer of calculated thickness of 3 cm, much thicker than one would expect to find in an actual spill. Oil from this slick was sampled at 0, 60, 120, 300, 500 and 1440 minutes. The 150 milliliter samples were added to 15 liters of water, agitated, separated, siphoned into test vessels. As shown, there is a significant drop in toxicity after 300 minutes of exposure to the natural elements in the confined system. After 24 hours, the toxic effect is, for all practical purposes, nil, on a short-term basis. This experiment was carried out on a heavily overcast day (Experiment 40, Figure 87). A further experiment was essentially the same except that the day was clear and the 24-hour determination was not made (Figure 88).

The emphasis in subsequent experiments of this type was placed on reducing the quantity of oil on the water to more closely approach actual spill conditions. The 3.785 liters of crude oil were poured on the water to give a calculated thickness of 0.6 cm. Fifty milliliter samples of the slick were taken. The samples obtained after 256 minutes of weathering were significantly less toxic than those taken soon after the "spill" (Figure 89). This experiment was repeated with no water flowing through the tank containing the oil spill. Again the toxicity decreased to a great extent between 300 and 500 minutes (Figure 90).

The initial concentration of monocyclic aromatics in the aqueous extracts of the weathered oil was found to diminish with the time of exposure of the oil film. Figure 90 shows how the mortality curves correspond to the aromatics concentrations in the extracts. Figure 91 shows the relationships among weathering time, mortality and light aromatics concentration in the test water. Although both the 2-hour and the 24-hour mortality curves correspond reasonably well with the aromatics concentrations, the divergence of the two curves at the longer weathering times may be indicative of an increase in vital response time with decreasing aromatics concentration.

Figure 92 shows the results from a film of South Tia Juana Medium crude calculated to be less than 0.2 cm thick. One liter of oil was carefully poured on a water surface area of 6273 cm². Actually, the thickness of this film varied with wind velocity and time of exposure. It was observed that the film was constantly stretching, breaking, and reforming, resulting in continuous renewal of the oil/air and oil/water interface. Under these conditions, which closely approximate those which might attend an actual spill of oil, 50 milliliters samples tested after 90 minutes of exposure were found to be only slightly toxic (Figure 92). Note that aromatics concentrations were also significantly reduced by the weathering.

The last two experiments were designed to obtain a better measure of the rate at which the oil toxicity might diminish under conditions approximating an actual oil spill. Again a film calculated to be less than 0.2 mm thick was used. In the first experiment (Figure 93), samples were withdrawn for toxicity testing at 0, 45, 60, 90 and 120 minutes. In the second experiment (Figure 94), samples were taken at 0, 5, 10, 20 and 40 minutes. The results of these experiments are a strong indication that oil spilled on Lake Maracaibo will lose at least one-half of its acute toxic properties within about 45 minutes after the spill occurs, providing the spill is in the form of a thin film on the water surface.

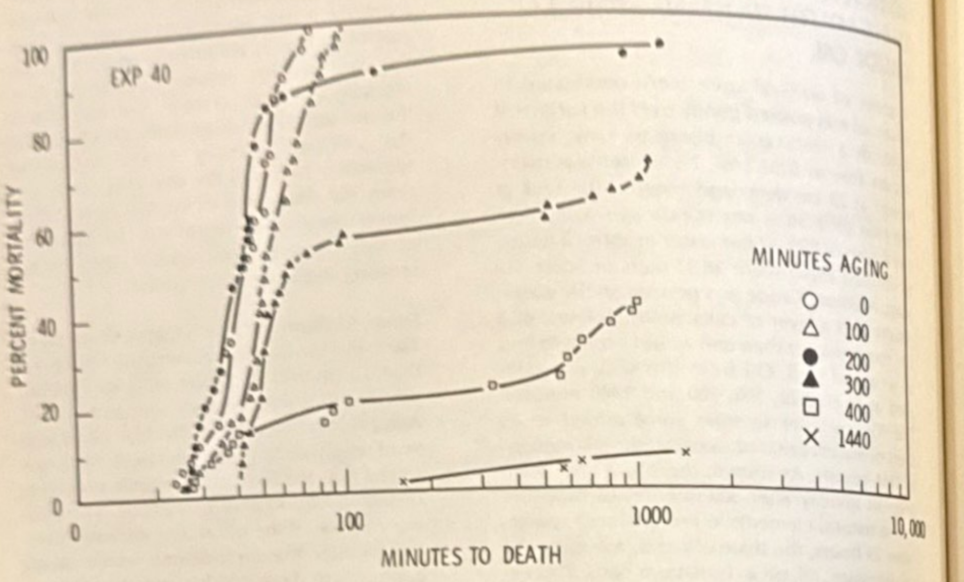

Figure 87

Toxicity of samples of South Tia Juana Medium Crude to *P. schmitti* taken from a surface film (calculated film thickness, 3 cm). Water flowing through oil exposure tank. Oil tested for toxicity at 3,333 ppm. Weather conditions, heavily overcast.

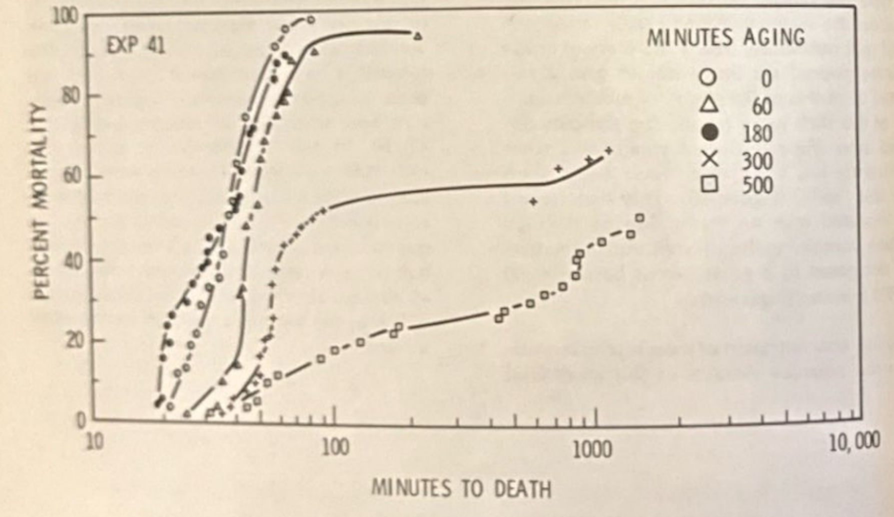

Figure 88

Replicate of experiment shown in Figure 87. Weather conditions, clear and sunny.

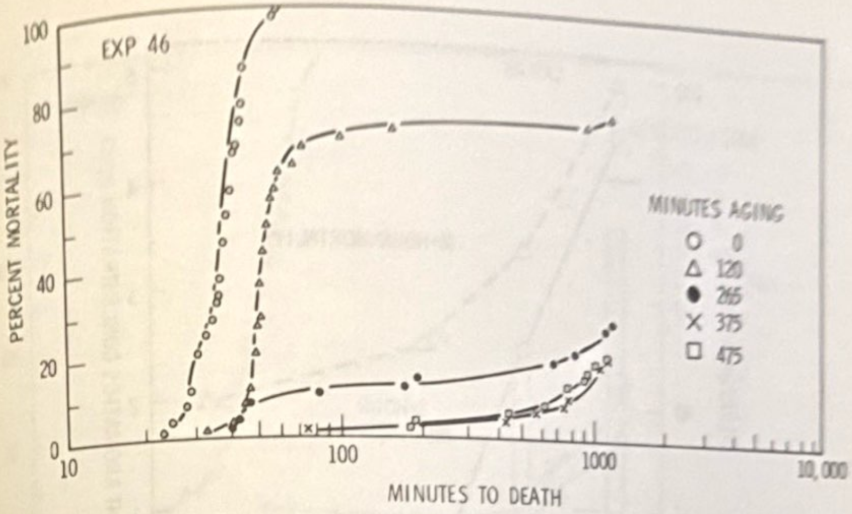

Figure 89

Toxicity of samples of South Tia Juana Medium Crude to *P. schmitti* taken from a surface film (calculated film thickness, 0.6 cm). Water flowing through oil exposure tank. Oil tested for toxicity at 3,333 ppm.

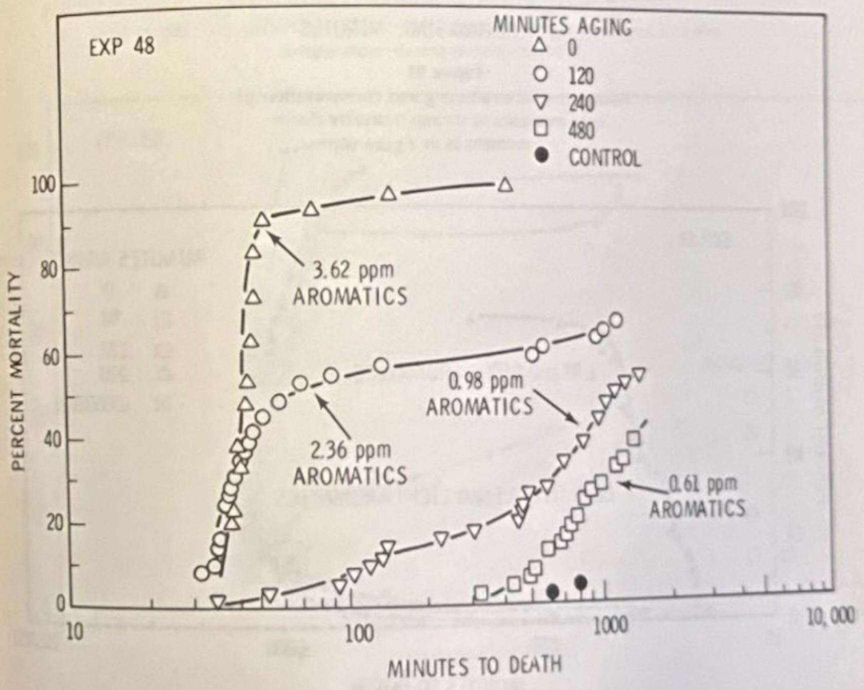

Figure 90

Replicate of experiment shown in Figure 89. No water flowing through oil exposure tanks. Data shown for aromatics refers to initial concentrations of monocyclic aromatics found in toxicity test water.

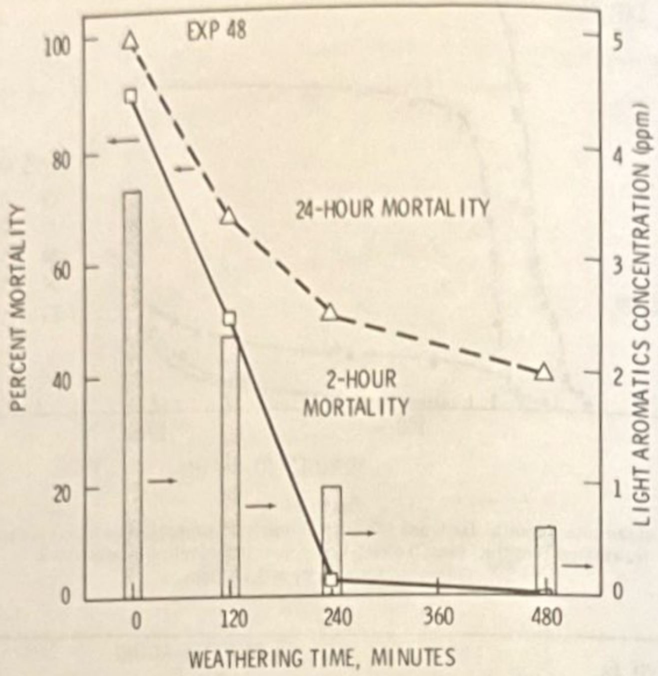

Figure 91

Relationship of weathering and concentration of light aromatics to shrimp mortality (Same experiment as in Figure 90)

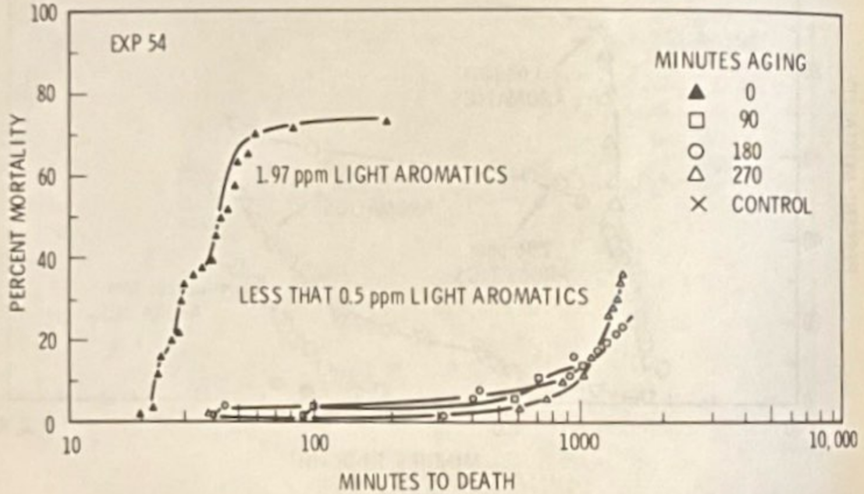

Figure 92

Toxicity of samples of South Tia Juana Medium Crude to *P. schmitti* taken from a surface film (calculated film thickness 0.16 cm). Oil tested for toxicity at 3,333 ppm.

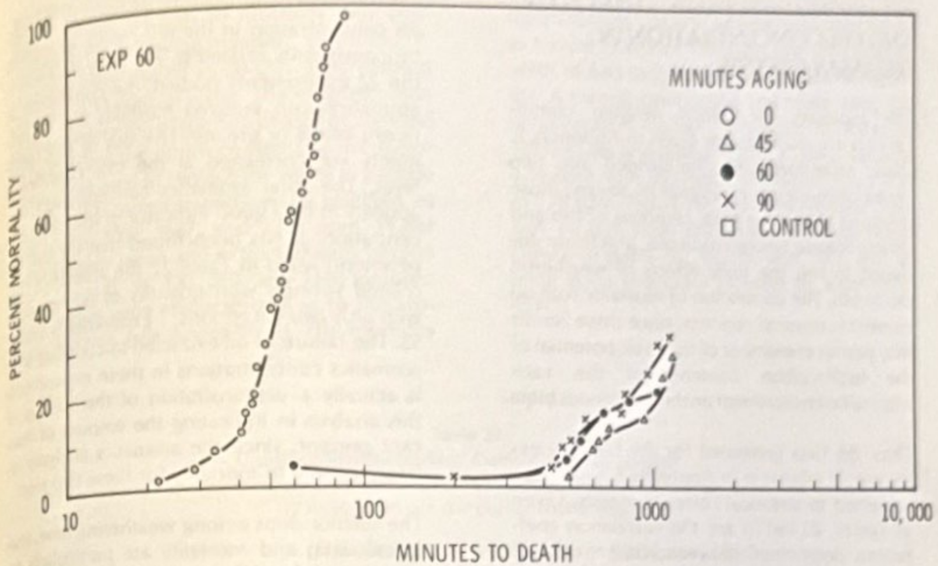

Figure 93

Toxicity of samples of South Tia Juana Medium Crude to *P. schmitti* taken from a surface film (calculated film thickness 0.17 cm). Oil tested for toxicity at 3,333 ppm. Ambient temperature during weathering 39°C.

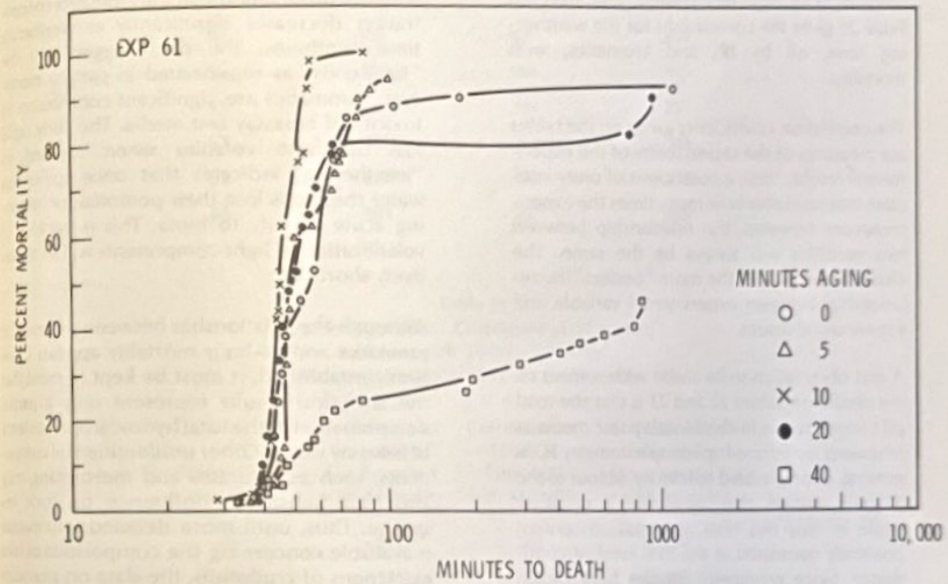

Figure 94

Oil weathering conditions identical to the experiment shown in Figure 93, except that ambient temperature was 33°C. Samples taken for toxicity testing over smaller time intervals. Oil tested for toxicity at 3,333 ppm.

RELATIONSHIP BETWEEN MORTALITY AND OIL CONCENTRATION IN BIOASSAY WATERS

The bioassays for which detailed chemical data are available are given in Appendix E. These experiments can be divided into two broad categories of experimental design; those designed to test the toxic response of fish and shrimp toward whole crude oils, and those designed to test the toxic effects of weathered crude oils. The correlation of mortality with oil content is of some concern, since these results may permit assessment of the toxic potential of the hydrocarbon contents of the Lake Maracaibo environment on the indigenous biota.

Thus, the data generated for the bioassay experiments tabulated in Appendix E have been subjected to statistical correlation tests. Given in Tables 22 and 23 are the correlation coefficients determined between acute toxicity of test medium and the physical/chemical parameters of the experiments. The data in Table 22 shows the correlation between the quantity of crude oil extracted, oil in test medium by IR analysis, and total monocyclic aromatics of test medium with mortality results. The data on Table 23 gives the correlations for the weathering time, oil by IR, and aromatics, with mortality.

The correlation coefficients given on the tables are measures of the repeatability of the experimental results; thus, a coefficient of unity indicates that no matter how many times the experiments are repeated, the relationship between two variables will always be the same. The closer to unity then, the more "perfect" the relationship between experimental variable and experimental results.

A first observation to be made with respect to the results on Tables 22 and 23 is that the total oil concentration in the bioassay test media as measured by infrared spectrophotometry is, in general, poorly related to toxicity. In four of the 11 tests studied, the correlation is negative, while in only two tests was total oil content positively correlated at the 95% level of confidence. Since gravimetric studies have shown that IR is a good indicator of oil concentration, we must conclude that the total oil content of lake water is poorly related to the 24-hour mortality induced.

On the other hand, the total monocyclic aromatics concentration in the test media appears to be consistently related to mortality. In each of the 22 experiments studied, the correlation of aromatics with induced mortality has a coefficient of 0.8 or greater. Five out of 22 experiments are correlated at the 95% confidence level. The total aromatics concentration also appears to be a good indicator of toxicant concentration. It has been found that for the experiments listed in Table 22, the aromatics correlated strongly with quantity of oil extracted with only two exceptions: Experiments 53 and 55. The failure of oil extracted to correlate with aromatics concentrations in these experiments is actually a demonstration of the success of this analysis in indicating the amount of toxicant present, since the aromatics analyses relate very well to mortality for these two tests.

The relationships among weathering time, analytical data, and mortality are particularly interesting (Table 23). In none of these tests was there even reasonably good correspondence between total oil concentration and toxicity. The correlation between total aromatics and mortality, however, was fairly good. When coupled with the observation that the aromatics concentration decreases significantly as weathering time continues, the data suggest that the "light ends" as represented in part by monocyclic aromatics are significant contributors to toxicity of bioassay test media. The fairly rapid loss of these volatiles when exposed to "weathering" indicates that once spilled on water these oils lose their potential for imparting acute toxicity to biota. This is because of volatilization of light components within a relatively short time.

Although the relationship between monocyclic aromatics and 24-hour mortality appears to be firmly established, it must be kept in mind that the analytical results represent only a partial determination of the total hydrocarbon contents of bioassay water. Other unidentified oil components, such as saturates and mercaptans, may also have substantial influence on toxic response. Thus, until more detailed information is available concerning the composition of lake extractions of crude oils, the data on aromatics are best considered as an indicator of total toxicant concentrations.

A further investigation of the relationship of the light aromatic fraction to mortality was conducted in the following manner: 25 shrimp (*P. schmitti*) were placed in 20-liter glass carboys containing 15 liters of water. The magnetic mixing and separation of the two phases proceeded as before but the entire contents of the hypophase were used to replace the water in a single carboy. Oil concentrations were selected at 1667, 1333, 1000, 1667, and 3333 ppm. Water samples were taken and extracted with hexane and CCl_4 at 0, 30, 120, 360, and 1440 minutes. The samples were characterized as to the aromatic fraction (see Appendix E). The mortality of the shrimp was studied with respect to total light aromatic fraction at time zero and found to have a TLm_{24} of 1.36 ppm with 95% fiducial limits of 0.65 to 2.9 ppm (Experiment 73, Figure 95). A second comparison was made using the concentration of light aromatics in water at 120 minutes and found to give a TLm_{24} value of 0.54 ppm with 95% fiducial limits of from 0.38 to 0.78 ppm (Figure 96). The light aromatic fraction escapes from the water in a logarithmic fashion (Figures 97, 98, 99, and 100), and at some point will cease to exert an acute toxic effect.

Table 22
Crude oil toxicity studies: Correlation of oil content variables with 24-hour mortality
(Values given are correlation coefficients)

Experiment Number	Correlation of Quantity of Oil Extracted with Mortality	Correlation of Total Oil Conc. (IR) with Mortality	Correlation of the Conc. of Total Monoaromatics (GC) with Mortality
44	.963	.920	.805
49	.816	.356	.823
50	.956	-.80	.810
53	.156	.963	.945
55	.387	-.394	.897
57	.990	.364	.968
62	.927	.723	.913
63	.884	-.217	.869

Table 23
Weathering studies: Correlation of oil content variables with 24-hour mortality
(Values given are correlation coefficients)

Experiment Number	Correlation of Quantity of Oil Extracted with Mortality	Correlation of Total Oil Conc. (IR) with Mortality	Correlation of the Conc. of Total Monoaromatics (GC) with Mortality
46	-.915	.008	.804
48	-.961	.143	.953
54	-.450	-.607	.868

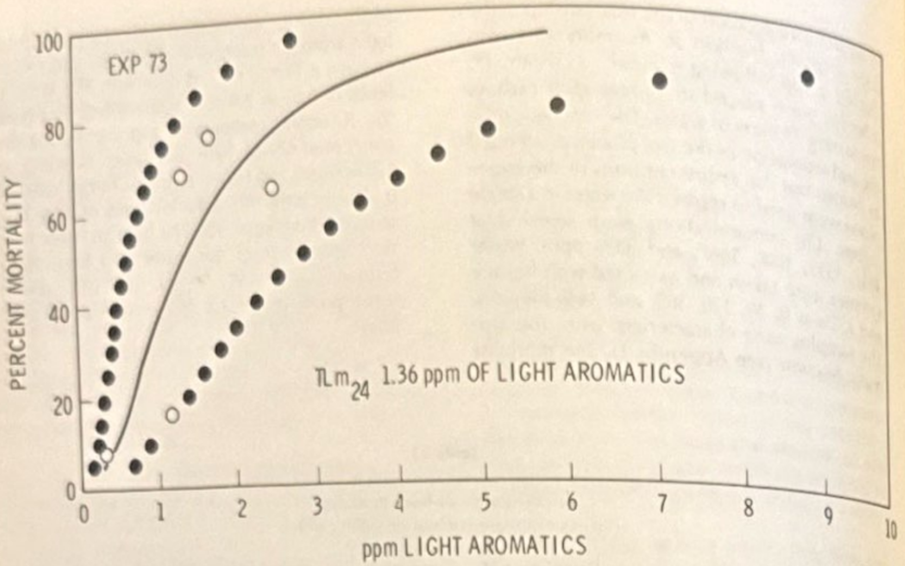

Figure 95

P. schmitti exposed to South Tia Juana Medium Crude Oil. The percent mortality is compared to the light aromatic concentration at the initiation of the experiment.

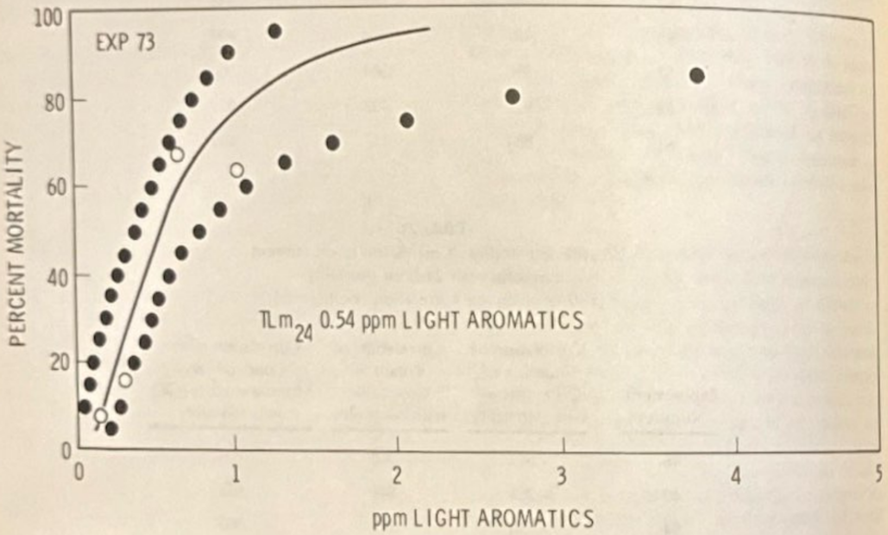

Figure 96

P. schmitti exposed to South Tia Juana Medium Crude Oil. The percent mortality is compared to the light aromatic concentration after two hours exposure.

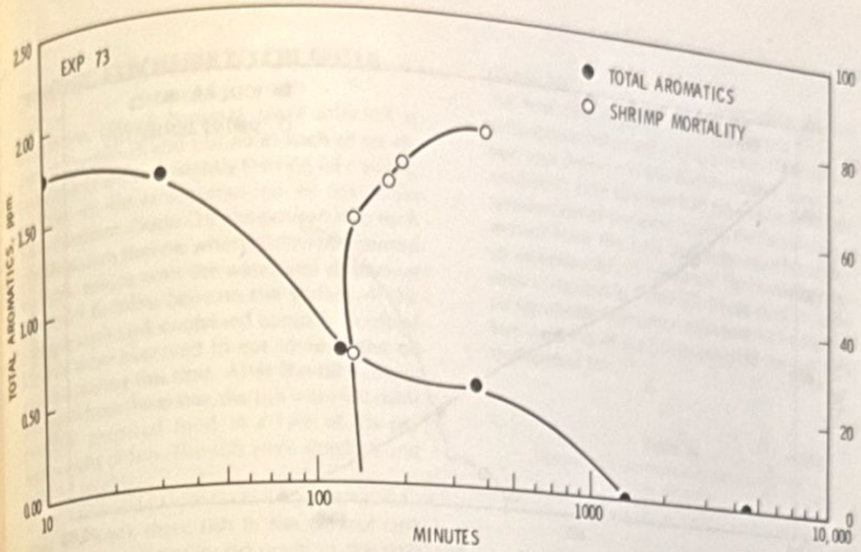

Figure 97

Mortality of *P. schmitti* and total aromatics in the test medium as a function of time. Concentration of oil added to water of 3,333 ppm.

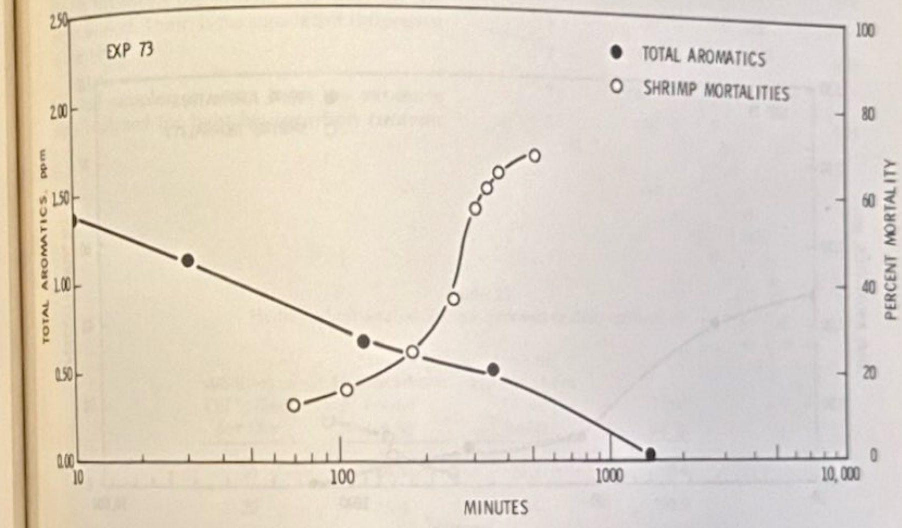

Figure 98

Mortality of *P. schmitti* and concentration of total aromatics as a function of time. Concentration of oil added to water of 2,667 ppm.

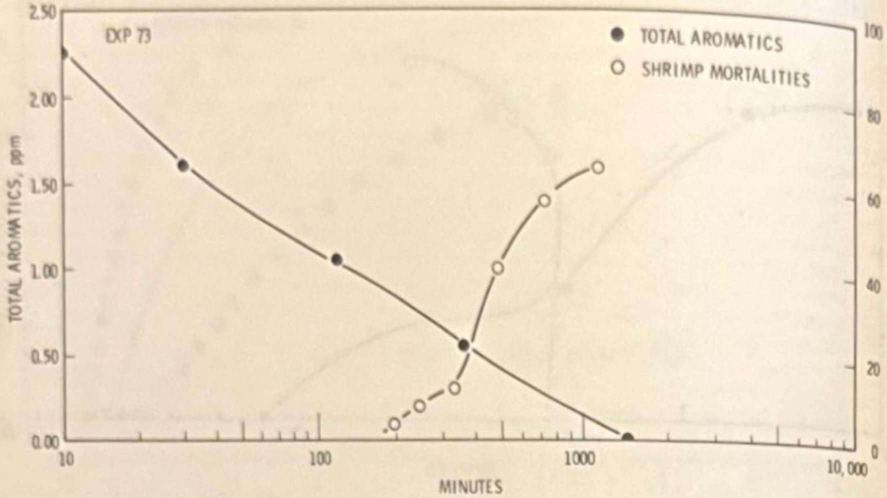

Figure 99
Mortality of *P. schmitti* and concentration of total aromatics in test medium as a function of time. Concentration of oil added to water of 2,000 ppm.

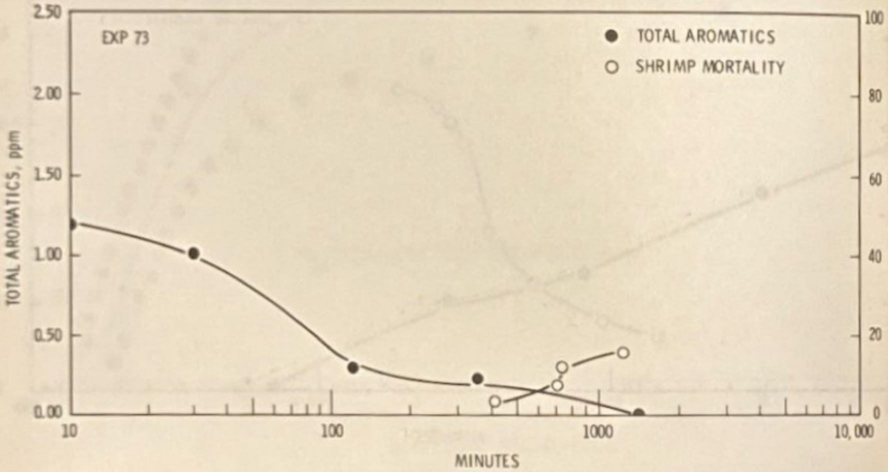

Figure 100
Mortality of *P. schmitti* and concentration of total aromatics in test medium as a function of time. Concentration of oil added to water of 1,333 ppm.

CHRONIC EXPOSURE TO OIL SPILLS

Fifteen lisa *(Mugil curema)* were selected at random, weighed and placed in each of six 48-liter aquaria with constantly flowing lake water. For five of the tanks, amounts of South Tia Juana Medium Crude Oil was poured into each of the vacuum funnels where the water entered the tank, mixing with the water and dispersing into small droplets beneath the surface of the water. A sixth tank contained controls. Some of the fish were observed to eat some of the oil droplets during this time. After the oil had disappeared from the water, the fish were fed commercially prepared food at a rate of 3% per total weight of fish. The fish were weighed and counted weekly.

After one week three fish in the control tank had died. One mortality occurred in the tank exposed to 30 milliliter crude oil per day. In the fourth week of the experiment there were three fatalities, one in each of the 15 milliliter and 20 milliliter of petroleum per day tanks and one more in the 30 milliliter per day tank. One mortality occurred in the 20 milliliter per day tank in the seventh week and another in the eighth.

The fish were compared on an average weight basis because of the mortalities in some tanks. Figure 101 shows the growth rate over the 11-week period. There is no significant difference in the rates.

Selected samples of water from the exposure were analyzed for light hydrocarbon contents (Table 24). These samples were taken during the first two days of the experiment. The results showed that very little aromatic hydrocarbon was present in the water column, even immediately after the spill had taken place. At the termination of the experiment, the fish were removed from the tank and submitted for analysis of hydrocarbon contents. The analytical results are given in Table 25. These data indicate no significant difference between the hydrocarbon contents of the fish exposed to the spill and the control fish.

Table 24
Monocyclic aromatics in water column after oil spills

Tank Number	Ml Oil Spilled	Min After Spill	Monocyclic Aromatics thru C_8 (mg/ℓ)
6	control	5	Trace
1	15	5	0.02
2	20	5	Trace
3	30	5	0.01
4	40	5	0.03
5	50	5	0.05
5	50	480	—
2	20	5	0.03
6	control	5	—
5	50	5	0.03

Table 25
Hydrocarbon analysis of lisa exposed to daily spills of oil

Milliliters of Oil Spilled per Day	Saturate Hydrocarbons Found (µg/g)	Aromatic Hydrocarbons Found (µg/g)	Total (µg/g)
50	69.1	31.3	100.4
20	75.4	33.1	108.5
control	94.6	37.0	131.6

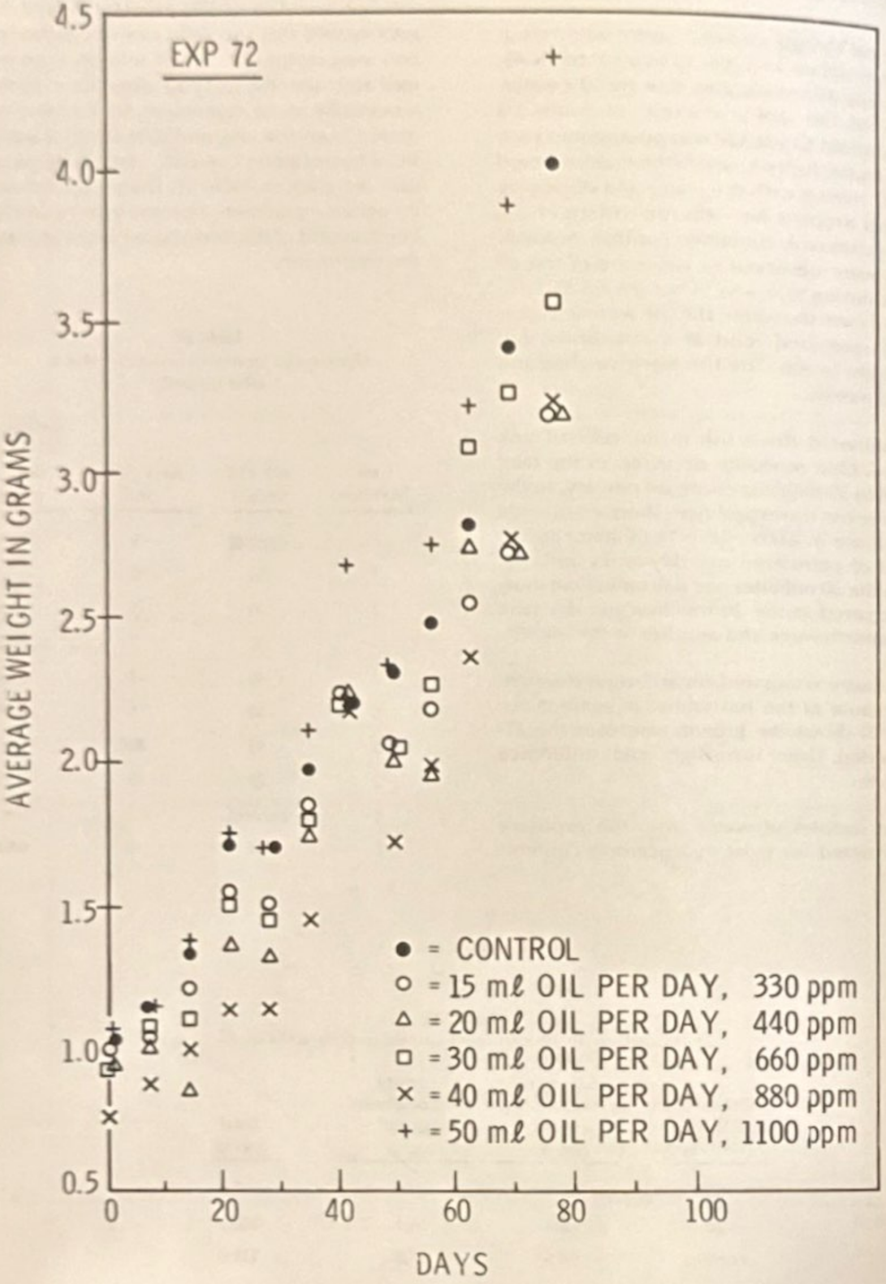

Figure 101
Lisa, *Mugil curema*, exposed to daily oil spills of varying concentrations over an 11 week period

SUMMARY

- A single dose of oil floating on the surface of a volume of water which is being continuously exchanged at 3000 milliliters per minute does not appear to be toxic to lisa and white shrimp at initial volume ratios of up to 18.1 liters of oil to 340 liters of water.

- Agitation of an oil-water mixture extracts toxic components from the oil rendering the water phase lethal to white shrimp, robalo, and lisa.

- The type of mixing influences the toxicity of the water extract. Agitation by inverting an oil-water mixture (140 milliliters oil/15ℓ water) 20 times resulted in no toxicity when the water extract was bioassayed. Identical levels of oil in water resulted in highly toxic water extract when the mixture was agitated for 10 minutes with a magnetic mixer.

- Using as values for oil toxicity the amount of oil added to the water, it was found that the 24-hour median tolerance limit (TLm$_{24}$) for white shrimp (*Penaeus schmitti*) for South Tia Juana Crude Oil was 1507 ppm with 95% fiducial limits of 1133 ppm and 1667. For lisa (*Mugil curema*), the value obtained was 2400 (2325-2465) ppm. Robalo was found to be much more resistant with a calculated TLm$_{24}$ of 10,667 ppm.

- A dehydration chemical commonly used in the production of crude oil did not increase the toxicity of oil but apparently decreased the induction time of the toxic response.

- Under the experimental conditions used, was an important variable; Tia Juana Light Crude Oil mixed poorly with lake water giving erratic response, and resulting in no evident correlation of amount of oil contacted with the amount added. On the other hand, Tia Juana Heavy Crude Oil mixed well with lake water, but imparted less toxicity than Tia Juana Medium Crude Oil.

- The toxicity of oil spilled on water in a contained system diminishes rapidly with time of exposure to sun and wind. Thick films, approximately 3 millimeters, lost all toxicity after 24 hours. Thinner films lost the toxicity much more rapidly, in less than 90 minutes for a film approaching that which might actually be found in the field situation.

- Total oil in test water as measured by IR does not correlate well with mortality induced in shrimp or robalo. The monocyclic hydrocarbon content of aqueous extracts correlates well with mortality, however. Weathering of crude oils substantially reduces the amounts of aromatic compounds extracted into lake water and these results also correspond very well with reductions in mortality.

- The extractable organic material in lake water samples was less than 0.5 ppm in 90% of the water samples. This is many orders of magnitude smaller than the levels of concentration which cause mortality to fish and shrimp.

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APPENDICES

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APPENDIX A

PROCEDURES FOR ANALYSIS OF HYDROCARBONS IN TISSUE, SEDIMENT, AND WATER

APPENDIX A

PROCEDURES FOR ANALYSIS OF HYDROCARBONS IN TISSUE, SEDIMENT, AND WATER

ANALYSIS OF TISSUE SAMPLES

Samples of fish arrived in the laboratory whole, and in an eviscerated, frozen condition. When selected for analysis, the sample was thawed using moderate heat from a lamp, and then skinned using a knife and forceps previously washed in carbon tetrachloride. Care was taken to prevent contact of the sample with fingers, or with any other possible source of hydrocarbon. Once the skin was removed, the flesh was dissected from the skeleton, cut into pieces (1-3 cm^3), and the pieces randomized. The flesh was then divided into 50-gram lots, placed in jars and refrozen prior to analysis.

Shown in Figure A.1 is a summary schematic of the procedure for isolation of hydrocarbons from tissue samples. Fish tissue samples (50 g ± 0.5 g) were placed in a round bottom flask (500 ml), with standard taper ground glass neck and mixed with 95 percent ethanol (150 ml), several boiling chips, and KOH (10 g). The mixture was refluxed at approximately 80°C on a heating mantle and under a Friedrich reflux condenser for one hour. After cooling to ambient temperature, the digested material was transferred to a Teflon® stoppered separatory funnel (1 liter) using distilled H_2O (80 ml) and two portions (50 ml each) of CCl_4 or hexane. Use of more than 80 ml distilled H_2O tended to reduce the extraction efficiency by forming a stable solvent emulsion. The mixture was shaken by hand for approximately one minute and the solvent and aqueous phases were allowed to separate. The two phases were drained into separate flasks and the aqueous phase was extracted with two additional aliquots (50 ml each) of CCl_4 or hexane.

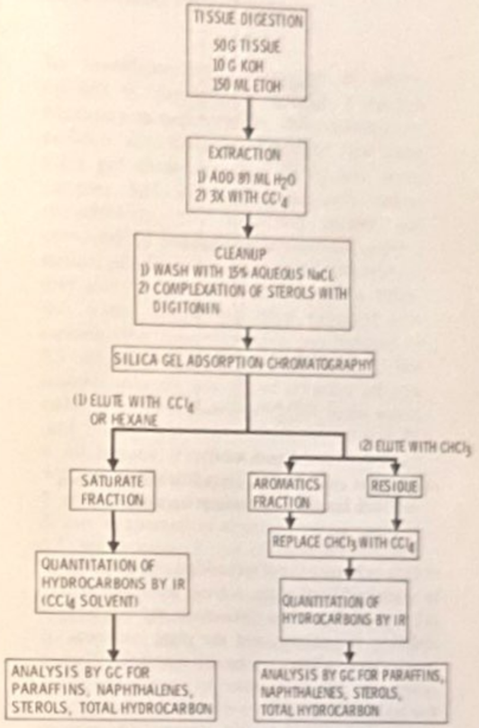

Figure A.1

Scheme for analysis of tissue samples for hydrocarbons

The combined solvent extracts were washed three times with distilled H_2O (500 ml each) containing 15 percent NaCl. The washed extracts were transferred to an Erlenmeyer flask (300 ml). To remove water, anhydrous Na_2SO_4 (10-20 g) was added and the extracts let stand overnight. The extracts were then filtered through filter paper previously extracted overnight with solvent. Then they were concentrated to approximately 50 ml under a stream of N_2 in a water bath (50-60°C), transferred to a conical tube (50 ml) and concentrated to a final volume of approximately 5 ml. The solution was quantitatively transferred to an Erlenmeyer flask (50 ml) containing distilled water (1.5 ml), Hyflo Super-Cel (1.5 g), and digitonin (1.0 g). This mixture was allowed to stand approximately 12 hours to complex sterols.* The sample "slurry" was transferred to a 1 cm glass column with fritted glass disc and eluted with the appropriate solvent (CCl_4 or hexane 20 ml). The sample was concentrated to 2.0 ml and transferred to a modified FIA Chromatographic column, Figure A.2, containing silica gel activated at 250°C. After the sample (2 ml) reached bed level in the column, an additional aliquot

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*After the procedure of Katz, I. and M. Keeney. 1967. **J. Dairy Science 50,** 1764-68.

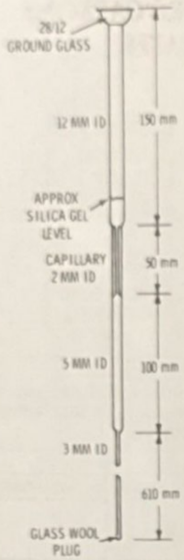


Figure A.2

Modified FIA column for separation of hydrocarbons into saturate and aromatic fractions

of CCl_4 or hexane (2 ml) was added and allowed to reach bed level again. A fresh layer of silica gel (1 cm) followed by chloroform (10 ml) was added to the column and the glass joint was attached to a nitrogen pressure manifold. The system was then pressurized to approximately five p.s.i. with N_2 to force the solvent through the small bore glass tubing. When CCl_4 was used, the first 2.5 ml to elute from the column contained the saturate fraction and the next 2.5 ml contained the aromatic fraction. Where hexane was used as the extraction solvent, the total elution after the 2.5 ml saturate fraction was collected to assure complete removal of the aromatics from the column.

When carbon tetrachloride was used as the extracting solvent it was possible to analyze for total hydrocarbon concentration using infrared spectrophotometry. Chloroform was removed from the aromatic and saturate fractions by evaporating in a water bath to ca. 1 ml, diluting to 10 ml with CCl_4 and repeating the dilution-evaporation procedure twice more. Final volume of CCl_4 was adjusted to 5 ml, and IR scans between 3200 cm^{-1} and 2750 cm^{-1} were run, using 1 cm quartz cells. The absorbance at 2930 cm^{-1} was measured, subtracting baseline absorbance when necessary, and the concentration of hydrocarbon determined using coefficients of 1.76 ℓ/g-cm for aromatic extracts and 3.33 ℓ/g-cm for saturate extracts. These coefficients were determined from Beers-law calibration curves using the saturate and aromatic fractions of Tia Juana Medium Crude Light Gas Oil.

The saturate and aromatic fractions were concentrated to 0.5 ml and analyzed by gas chromatography. The instruments used were a Packard Model 1704 and a Varian Aerograph Model 2860 equipped with flame ionization detectors and columns (183 cm length x 2 mm I.D.) packed with 3 percent SE 30 on 100-200 mesh gas chrom Q. Operating conditions were as follows: N_2, 30 ml/min; H_2, 30 ml/min; air, 300 ml/min; injector temperature, 185°C; detector temperature, 265°C, column temperature, 50°C programmed at a rate of 5°C per minute to 250°C. Maximum instrument sensitivity utilized was 1×10^{-11} amps which provided a minimum detectable level of approximately 0.007 µg/g of fish tissue for individual compounds in the saturated fraction.

Qualitative identification of the hydrocarbons was achieved by comparison of their retention times to retention times of known standards and spiking of tissue extracts with pure hydrocarbons to evaluate peak heights and peak conformation.

A quantitative determination of total hydrocarbons in the separated fractions was obtained by measuring total area of resolved peaks and the unresolved envelope occurring under the peaks. A procedural blank was used to determine background contamination, and was subtracted from the appropriate fractions. The total hydrocarbon concentration in the fractions was calculated with instrument response factors for n-alkane hydrocarbons because the complexity of the samples precluded examination of individual compounds.

The gas chromatograms of the extract fractions were examined for the presence of the following hydrocarbon compounds by comparison to the retention time of pure compounds: C_{12} through C_{27} n-alkanes, pristane, phytane, cholesterol, and the following methyl substituted naphthalenes; 1-methyl and 2-methylnaphthalene, 1,2-dimethylnaphthalene, 1,3-dimethylnaphthalene, and 2,6-dimethylnaphthalene.

ANALYSIS OF LAKE WATER SAMPLES USING INFRARED SPECTROPHOTOMETRY

Samples of lake water were collected in 1 liter jars in triplicate and frozen at the Las Morochas Laboratory. Although some breakage occurred during the freezing and subsequent thawing of water samples, this procedure was generally successful and has eliminated the previous problem of sample contamination. Samples received at Richland were kept frozen until 24 hours prior to analysis, when they were thawed overnight. The samples were then placed into 1 liter Teflon® stopcocked separatory funnels, 1 ml 50% HCl added, and extracted with two successive 10 ml portions of Burdick and Jackson carbon tetrachloride. The volume of water extracted was measured and recorded.

After standing one hour or more to allow small droplets of water to coalesce, the carbon tetrachloride extracts were placed in a 1 cm quartz cell and scanned from 3200 cm^{-1} to 2750 cm^{-1} versus pure CCl_4 in the reference cell. The absorbance at 2930 cm^{-1} (methylene stretching frequency) was recorded, and when necessary background absorbance subtracted using baseline correction. The concentration of extractable organics/oil was determined by reference to a Beers-law calibration chart constructed by plotting the absorbance of Tia Juana Medium Crude Oil versus concentration in mg/ℓ in carbon tetrachloride. If sample absorbance was greater than 1.0, an aliquot of the sample was diluted to a known volume with solvent and rescanned. The concentration of oil in water was calculated as follows:

$$C = \frac{P \times E \times D}{V}$$

where C = concentration of oil in water expressed in mg/ℓ

P = concentration of oil in CCl_4 as determined from the Beers-law calibration curve

E = volume of CCl_4 used for extraction (20 ml)

V = volume of water extracted (ml)

D = dilution factor.

EXAMINATION OF THE HYDROCARBON COMPOSITION OF LAKE WATER EXTRACTS BY GAS CHROMATOGRAPHY

To investigate the composition of solvent extracts of Maracaibo lake water, it was first necessary to segregate the hydrocarbon components according to compound type using silica gel chromatography. If the lake water samples had been extracted with carbon tetrachloride, the extracting solvent was removed by evaporating to dryness under a stream of nitrogen below room temperature, and was replaced with 0.5 ml hexane. When the water sample had been extracted with hexane, the solvent volume was reduced to 0.5 ml by evaporation under nitrogen. The hexane solution was placed on a 3 x 240 mm column of deactivated silica gel (6.9% water) and eluted with 1.5 ml hexane (Fraction 1), 6 ml hexane (Fraction 2), and then 4 ml of 1:1 benzene-methanol (Fraction 3). Fractions 1 and 2 were evaporated to 0.5 ml. Fraction 3 was evaported to dryness and taken up in 0.5 ml hexane. 5 µℓ of each fraction was injected onto a 6' x 1/8" column of 3% SE30 on Chromosorb W. A single-column gas chromatogram of each fraction was obtained from 50 to 280°C at a programming rate of 6°C/min. Initial instrument sensitivity was 4×10^{-11} amps.

The results of an investigation of the resolving power of the liquid chromatographic system used is given in Table A.1, which gives recovery data from the silica gel chromatography of 6.1 mg n-octane, 2.4 mg 1-eicosene, 9.3 mg n-octylbenzene, and 7.5 mg 1-methylnaphthalene. The recovery results, obtained by gas chromatography, show that paraffins and long chain monoolefins can be expected to be found in Fraction 1. Methylnaphthalene was found exclusively in Fraction 2, and the octylbenzene split more or less evenly between Fractions 1 and 2. Thus, Fraction 1 has been labeled the "saturate/olefin" fraction, Fraction 2, the "aromatic" fraction, and Fraction 3 the "polar" fraction.

Table A.1

Elution of n-octadecane, 1-eicosene, n-octylbenzene, and 1-methylnaphthalene on deactivated silica gel[1] with n-hexane

	Separation A		Separation B	
Compound	Fraction 1 (1.5 ml)	Fraction 2 (6.0 ml)	Fraction 1 (1.5 ml)	Fraction 2 (6.0 ml)
n-octadecane, %	100	—	100	—
1-eicosene, %	100	—	100	—
n-octylbenzene, %	44	56	70	30
1-methylnaphthalene, %	—	100	—	100[2]

[1] Gel deactivated with 6.9% water.

[2] All of the 1-methylnaphthalene was found in the first 3 milliliters of this sample.

ANALYSIS OF LAKE MARACAIBO SEDIMENT SAMPLES

The procedure illustrated in Figure A.3 has been adopted for routine extraction and analysis of petroleum hydrocarbons in marine sediments. The method, originally developed by Exxon Research and Engineering Company, Houston, Texas, to evaluate sedimentary rock as a potential source for hydrocarbons, uses a solvent mixture of benzene and methanol to extract organic matter from sediment by ultrasonic dispersion. The extracted organic matter is recovered by evaporation and solubilization in pentane. Asphaltene is removed by column filtration. The solubilized fraction is separated into (1) saturates, (2) aromatics, and (3) nitrogen, sulfur, and oxygen containing compounds (NSO) by elution from a silica gel-alumina column with pentane, benzene, and benzene-methanol, respectively. The recovered fractions are gravimetrically quantitated.

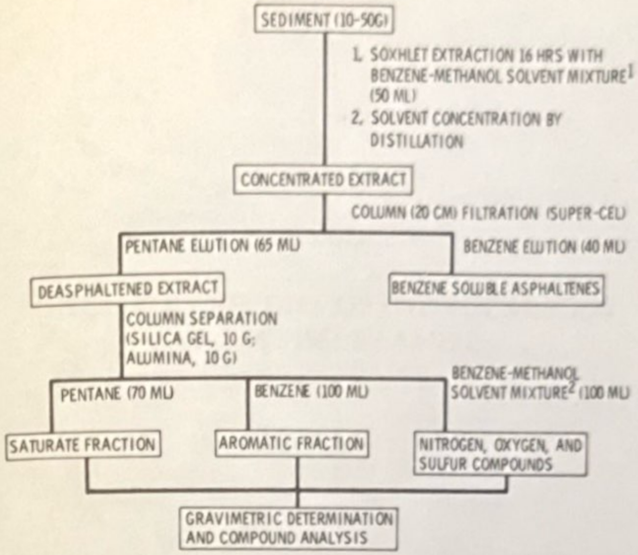
Sediment Preparation

Water content must be less than about 30 percent to obtain complete extraction of organic matter from sediment. The sediments were freeze-dried for 24 hours in a 10 micron vacuum to reduce potential for biodegradation of oil during drying.

Sediment Extraction

The sediment sample (10-50 g) was placed in a 40 x 130 mm pyrex Soxhlet thimble with a coarse fritted glass bottom. The thimble was placed in a 200 ml Soxhlet extractor equipped with an Allihn condenser and round bottom 1000 ml flask. The flask contained 60.4 percent benzene and 39.6 percent methanol for a total volume of 500 ml. Assembled apparatus was placed in a heating mantle and allowed to reflux for 16 hours (about 10 min/reflux cycle). A heating mantle and water cooled vacuum condenser were used to concentrate the extracts to approximately 125 ml by distillation. Distillation was enhanced by a weak vacuum from a water aspirator. After distillation the extracts were cooled to near ambient temperature and transferred to a clean, tared, beaker. They were evaporated to dryness at room temperature. The dry weight was recorded as total organic extract.

Separation of Asphaltenes. The extract was dissolved in 25 ml pentane and covered with a watch glass. One hour was allowed for precipitation of asphaltenes. The pentane solution was transferred to a Chromaflex column which had been previously washed with 50 ml pentane and was packed with 15 to 20 cm of Fisher Hyflo Super-Cel. The 30 x 1 cm colum was equipped with a 100 ml reservoir, a Teflon® stopcock and a 50 ml sidearm Erlenmeyer flask. The residue was washed with three 10 ml portions of pentane which were added individually to the column when the solvent reached filter bed level. The column was eluted with an additional 10 ml pentane and the total eluate, including a 5 ml washing of the collector's flask, was transferred to a 50 ml beaker. The pentane was evaporated at room temperature to approximately 5 ml, transferred to a tared aluminum foil weighing boat, and evaporated to dryness. The weight was recorded as deasphaltened extract.



[1] BENXENE (60.4%), METHANOL (39.6%)

[2] BENZENE (50%), METHANOL (50%)

Figure A.3
Scheme for analysis of sediments

The residue remaining in the beaker after removal of pentane solubles was dissolved with three 10 ml portions of benzene and transferred to the column. An additional 10 ml benzene solution was carried out. The eluate was transferred, with washings (5 ml), to a 150 ml beaker and evaporated at room temperature to approximately 5 ml. It was then transferred to a tared aluminum foil weighing boat, evaporated to dryness and the weight recorded as benzene soluble asphaltenes.

Chromatographic Separation of Asphaltene-Free Extract. The asphaltene-free extract, dissolved in 10 ml pentane, was transferred to a Chromaflex column. The column was packed with 10 g Davison Grade 923 silica gel which had been activated at 150°C for 16 hours under 10 g Alcoa F-20 alumina activated at 400°C for 16 hours. The column was washed with 50 ml pentane. The beaker was rinsed with two, 2 ml portions of pentane. These washings were transferred to the column when the first charge reached bed level. The column was eluted within an additional 56 ml of pentane and the total eluate was collected in a 150 ml beaker. The eluate was evaporated to approximately 5 ml at room temperature. It was then transferred to a tared aluminum foil weighing boat and evaporated to dryness. The weight was recorded as the saturate fraction.

The column was eluted with benzene (two 3 ml and one 94 ml portions). Collection was as described above. The eluate was evaporated at room temperature and the weight recorded as the aromatic fraction.

Final elution was with a 1:1 solution of methanol and benzene. Two 3 ml and one 94 ml portions were used. Collection and evaporation were as for the aromatics. The weight was recorded as nitrogen, sulfur, and oxygen containing compounds (NSO fractions).

APPENDIX B

RECOVERY STUDIES OF HYDROCARBONS FROM TISSUE SAMPLES

APPENDIX B

RECOVERY STUDIES OF HYDROCARBONS FROM TISSUE SAMPLES

Investigations were conducted to evaluate the recovery of standard hydrocarbons added to tissue samples using the procedure described in Appendix A. Shown in Tables B.1 to B.3 are recovery data of n-alkanes C_{12} to C_{28} and the following methylnaphthalenes; 2-methylnaphthalene, 1-methylnaphthalene, 2,6-dimethylnaphthalene, 1,3-dimethylnaphthalene, and 1,2-dimethylnaphthalene from curvina and robalo tissue samples when added at 0.004 to 0.02 µg/g of tissue. At the high instrument sensitivity necessary for these low concentrations, C_{12} n-alkane was eluted high on the solvent tail and could not be quantitated. The n-alkanes C_{17} and C_{28} were masked by large concentrations of pristane and cholesterol, respectively, in the samples. Four 50-gram tissue samples were amended with hydrocarbon for each recovery experiment. For comparison, a tissue-free sample (containing hydrocarbons at the same level as the tissue samples) was also carried through the procedure.

The recovery data from robablo tissue, Table B.1 was conducted at a concentration approaching the minimum detectable level, with a tissue containing the largest amount of interfering background material of any of those previously investigated and thus represents more adverse conditions in the 4 to 10 parts per billion range. Recoveries of higher than 100% reflect the influence of background material. Most components were recoverable within a range of 1/2 to 2 times their amended concentration, although 2,6-dimethylnaphthalene is an exception; none were recovered from the tissue-free samples, and a fourfold excess was reported in the tissue samples.

A twofold higher concentration of paraffin and naphthalene standards were used in a curvina recovery experiment using CCl_4. Also added was pyrene at 3 ppm. The results are in Table B.2. The lower background material encountered with curvina tissue and the higher concentrations (8 to 20 parts per billion) resulted in more consistent data. The recovery without tissue ranged from 0 to 120% and with curvina tissue, 20 to 136%.

To provide a basis of comparison of the chromatographic column efficiency between CCl_4 and hexane solvents, four samples of curvina tissue with standard hydrocarbons added were extracted with hexane and analyzed. Trace contamination of paraffin hydrocarbons were found in the hexane concentrate at concentration levels approaching the standard hydrocarbons. These interfered with the recovery experiment; therefore, the paraffin recovery data is not reported. The recovery data consisting of methyl-substituted naphthalenes and pyrene from the hexane extracted tissue is shown in Table B.3. Recoveries of monoaromatics were consistent with the previously described CCl_4 experiment. However, the recovery of pyrene was improved 52% with hexane as opposed to 20% when extracted from CCl_4.

For the detailed recovery studies using CCl_4 extraction of curvina and robalo tissues, parallel extractions were performed on tissue samples which had no standard hydrocarbons added. In each case, no reportable paraffin or methylnaphthalene hydrocarbons were found in the control samples.

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Table B.1

Recovery of standard hydrocarbons from robalo tissue samples[1]
(concentrations reported as nanograms, 10^{-9} g, per 50 g of tissue)

	Wt. HC's Added, ng	Recovery, ng (No Tissue)	Recovery, % (No Tissue)	Recovery from Tissue Samples, ng				Avg. Recovery from Tissue, ng	Avg. Recovery from Tissue, (%)	Rela. Std. Devia. (%)
C12	369	—	—	—	—	—	—	—	—	—
C13	381	268	70	435	194	413	629	415	109	± 42.64
C14	375	229	61	556	288	539	572	487	130	± 27.56
C15	461	249	54	352	222	273	488	334	72	± 34.78
C16	464	256	55	256	192	297	431	294	63	± 34.35
C17	413	—	—	—	—	—	—	—	—	—
C18	421	247	59	334	387	328	523	393	93	± 22.97
C19	493	273	55	569	602	454	602	557	113	± 12.57
C20	300	215	72	764	794	700	751	752	251	± 5.33
C22	300	413	138	284	484	403	548	429	143	± 26.56
C24	330	427	129	0	266	358	252	219	66	± 69.94
C28	300	—	—	—	—	—	—	—	—	—
2, MN	193	147	76	544	221	447	488	425	221	± 33.31
1, MN	249	166	67	346	158	338	427	317	127	± 35.67
2,6-DMN	188	0	0	1413	725	1313	1500	1237	658	± 28.27
1,3-DMN	225	311	138	534	259	405	563	440	196	± 31.55
1,2-DMN	223	143	64	281	138	239	287	236	106	± 29.14

[1] Samples extracted with CCl_4 after digestion.

Table B.2

Recovery of standard hydrocarbons from curvina tissue samples[1]
(concentrations reported as nanograms, 10^{-9} g, per 50 g of tissue)

	Wt. HC's Added, ng	Recovery, ng (No Tissue)	Recovery, % (No Tissue)	Recovery from Tissue Samples, ng				Avg. Recovery from Tissue, ng	Avg. Recovery from Tissue (%)	Rela. Std. Devia. (%)
C12	738	677	92	369	492	225	369	364	49	±30.06
C13	762	652	86	435	466	273	444	405	53	±21.87
C14	750	583	78	438	634	281	402	439	59	±33.45
C15	922	666	72	580	710	341	505	534	58	±28.83
C16	928	659	70	618	956	405	523	626	67	±37.84
C17	826	633	77	897	1164	562	839	866	105	±28.52
C18	842	664	79	804	741	461	—[2]	669	79	±27.23
C19	986	754	76	890	854	518	835	774	79	±22.26
C20	600	513	85	753	490	354	776	593	99	±34.64
C22	600	836	139	885	826	561	984	814	136	±22.25
C24	660	720	120	818	—[2]	556	971	781	130	±26.82
C28	600	—	—	—	—	—	—	—	—	
2,MN	386	179	46	165	225	121	130	160	42	±29.46
1,MN	498	287	58	244	317	148	230	235	47	±29.27
2,6-DMN	375	100	27	192	225	125	0	181	48	±73.42
1,3-DMN	450	83	19	464	433	333	167	349	78	±38.36
1,2-DMN	446	134	30	216	259	163	139	194	44	±26.67
Pyrene[3]	3,000	—	—	600	600	490	750	610	20	±17.57

[1] Samples extracted with CCl_4 after digestion.

[2] Background too large for quantitation of this hydrocarbon in this sample.

[3] Concentrations determined by UV.

Table B.3

Recovery of standard hydrocarbons from curvina tissue samples[1]
(concentrations reported as nanograms, 10^{-9} g, per 50 g of tissue)

	Wt. HC's Added, ng	Recovery from Tissue Samples, ng				Avg. Recovery from Tissue, ng	Avg. Recovery from Tissue, (%)	Rela. Std. Devia. (%)
2,MN	386	146	73	108	73	100	26	2.2
1,MN	498	269	164	254	169	214	43	3.5
2,6-DMN	375	251	229	311	124	229	61	5.2
1,3-DMN	450	194	257	302	234	247	55	2.5
1,2-DMN	446	245	214	335	254	262	59	2.9
Pyrene	3,000	1,590	1,560	1,500	1,560	1,553	52	0.3

[1] Samples extracted with hexane after digestion

APPENDIX C

MASS SPECTROMETRIC ANALYSIS OF HYDROCARBON EXTRACTS

APPENDIX C

MASS SPECTROMETRIC ANALYSIS OF HYDROCARBON EXTRACTS

A preferred method for obtaining compositional information on aromatics fractions of complex hydrocarbon mixtures is to subject the fraction to low voltage (11 eV) mass spectrometry. With this technique, parent ions are generated, permitting analysis of the aromatic ring types by carbon number. However, low voltage MS requires a relatively large sample size because of the small ion currents generated. On the other hand, high voltage (70 eV) methods permit detection of hydrocarbons in the microgram range because of the large number of ions produced. A problem with the high voltage method is that extensive cracking of the compounds being analyzed occurs, and rather than a few easily identified peaks, each compound produces a great number, or envelope, of peaks. Thus, when many different types of molecules are subjected to high voltage MS analysis, there is considerable overlap of mass numbers, and any one mass peak may contain contributions from several different components. Over the past 20 years, methods have evolved for handling this problem of peak overlap, permitting the generation of useful compositional data from high voltage spectra. These methods are based on the fact that certain compound types are major contributors to mass numbers which have relatively little interference from other types. The contributions of other compound types to these principal mass numbers can be estimated from examining other parts of the spectra where these interfering compounds are major contributors. For quantitative work, a series of simultaneous equations is set up, one for each compound type of interest, in which the contribution of each of the types to each mass series is to be solved. These sets of simultaneous equations or matrices are the backbone of the method, and much work has appeared in the literature directed toward assigning coefficients which will give results consistent with the true chemical composition of complex hydrocarbon samples.

Our earliest work with MS analysis of the hydrocarbon contents of environmental samples has shown that the amount of hydrocarbon to be expected from these samples is too small to permit extensive use of low voltage techniques. Therefore, Battelle-Northwest has chosen to develop with Petroleum Analytical Research, a high-voltage analytical method patterned after the techniques reported by Exxon workers.* Samples of sediment extracts separated by chromatography into saturate and aromatic fractions, are shipped to PAR in Teflon® capped vials. The sample is picked up in a 1 mm capillary tube. The capillary is then sealed, introduced into the mass spectrometer glass inlet system, the system pumped down, and the sample tube broken with a striker, admitting the sample into the instrument expansion chamber. Spectra are obtained from masses (m/e) 30 to 400. The normalized intensities of the peaks required for calculation are then recorded and used for the matrix calculations.

Studies relating to the mass spectrometric determination of hydrocarbon types in fish tissue extracts have been carried out with only modest success. Interferences from chlorinated hydrocarbons in carbon tetrachloride solvent, and from cholesterol which could not be completely removed from the tissue extracts, did not permit the application of matrix techniques. Hence, methods of determining the presence of individual heavy aromatic compounds in the aromatic fractions of tissue extracts were investigated. The procedure adopted was to determine the minimum detectable levels of polynuclear aromatic hydrocarbons, and then examine the high voltage spectra of tissue samples for the presence of these hydrocarbon peaks.

Introduction of these samples required a more elaborate procedure than in the case of the sediment samples.

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*Brown, R. A., J. J. Elliott, and T. D. Searl. 1974. Measurement and Characterization of Nonvolatile Hydrocarbons in Ocean Water, **Proceedings, Marine Pollution Monitoring (Petroleum) Symposium and Workshop,** May 13-17, 1974. National Bureau of Standards, Gaithersburg, MD (in press).

The introduction technique was as follows; first, a column of sample in solvent was drawn into a capillary tube (.9-1.0 mm i.d.). One end of the capillary was flame sealed and a slight vacuum applied to the open end of the tube. Care must be taken to avoid losses of liquid sample during this phase. After the solvent was evaporated the other end of the capillary was flame sealed. The capillary was then introduced into the mass spec oven (350°C) and broken which immediately exposed the sample to 350°C heat at 10^{-6} mm Hg. This procedure gives complete vaporization of all compounds boiling up to the 800-1000°F range.

The question of sensitivity for specific compounds or compound types has been approached by comparing limited empirical data to published data. Standards were prepared in CCl_4 and spectra obtained using the method described above. Ten compounds were studied with three separate determinations on each compound. The results are in Table C.1.

Although there is a large relative deviation in these values, the general level appears reasonable. This data also indicates the repeatability and reproducibility of the results. It would appear, in general, that condensed ring polynuclear aromatics with no side chain substitutions can be detected at the 1 µg level. The only substituted standard had about twice the detectability level of the others. The substituted species have higher limits of detectability, perhaps at about the 2-3 µg level. This statement can be partially substantiated by looking at the sensitivity data in Table C.2. Included in this table is a listing of available published relative sensitivities. All data is taken at 70 V, with n-Butane m/e 43 taken as having a sensitivity of 87.

From the sensitivities given in Table C.2, it can be seen that generally as substitution increases, sensitivity decreases. This is because the substituted side chain cracks off the molecule leaving the aromatic rings minus one hydrogen atom. For example, phenanthrene has a molecular weight of 178. Its mass spectrum has the most abundant peak at mass 178. Methyl phenanthrene with molecular weight 192 has its most abundant peak at 191. For polysubstituted compounds the most abundant peak varies. For this reason only the parent peak can be used for sensitivity and calculation purposes. The lack of published sensitivities available makes the task of quantitating these compounds more difficult.

Given in Table C.2 are the results of examination of seven aromatics fractions of tissue extracts. Each extract represents an initial wet sample weight of about 50 grams. The data are reported in terms of number of micrograms detectable under the operating conditions of the instrument during the run. Detectability levels for benzpyrenes were on the order of 0.05 µg per grain of tissue, a fairly high value when compared to other methodologies. No aromatic compounds were detected in these samples using the mass spectrometric method.

Table C.1

Limits of detectability of polynuclear aromatic hydrocarbons by MS

Compound	Run 1 Sensitivity (µg)	Run 2 Sensitivity (µg)	Run 3 Sensitivity (µg)
Chrysene	.5	.08	.2
Methylanthracene	none detected	2.0	.9
Pyrene	.09	.25	.18
Benzpyrene	.26	.24	.31
Phenanthrene	.25	.27	.25
Benzanthracene	.5	.5	.2
Perylene	.24	.41	.34
Triphenylene	.2	.2	.2
Anthracene	.02	.20	.31
Dibenzanthracene	.2	.5	.6

Table C.2

Mass spectra analysis of tissue aromatic extracts

Z*	Compound	m/e	Sensitivity	Manamana (Station 32)	Robalo (Station 16)	Lisa (Station 2)	Grays Harbor Perch	Meridan Trout	Bocachico	Shrimp
							µg			
6	Benzene	78	107	<1.2	<2.4	<1.2	<2.0	<2.4	<2.4	<2.2
6	Toluene	92	94	<1.4	<2.7	<1.4	<2.3	<2.7	<2.7	<2.5
8	Styrene	104	94	<1.4	<2.7	<1.4	<2.3	<2.7	<2.7	<2.5
6	C_8 Alkyl Benzenes	106	82	<1.6	<3.1	<1.6	<2.6	<3.1	<3.1	<2.9
10	Indene	116	63	<2.0	<4.1	<2.0	<3.4	<4.1	<4.1	<3.8
8	C_9 Indanes	118	96	<1.3	<2.7	<1.3	<2.2	<2.7	<2.7	<2.5
6	C_9 Alkyl Benzenes	120	84	<1.5	<3.1	<1.5	<2.6	<3.1	<3.1	<2.8
12	Naphthalene	128	177	<0.7	<1.4	<0.7	<1.2	<1.4	<1.4	<1.4
10	C_{10} Indenes	130	68	<1.9	<3.8	<1.9	<3.1	<3.8	<3.8	<3.5
8	C_{10} Indanes	132	32	<4.0	<8.1	<4.0	<6.7	<8.1	<8.1	<7.4
6	C_{10} Alkyl Benzenes	134	42	<3.0	<6.1	<3.1	<5.1	<6.1	<6.1	<5.6
12	C_{11} Naphthalenes	142	131	<1.0	<2.0	<1.0	<1.6	<2.0	<2.0	<1.8
8	C_{11} Indanes	146	24	<5.4	<10.7	<5.4	<9.0	<10.7	<10.7	<9.9
6	C_{11} Alkyl Benzenes	148	99	<1.3	<2.6	<1.3	<2.1	<2.6	<2.6	<2.4
16	Acenaphthalene	152	175	<0.7	<1.5	<0.7	<1.2	<1.5	<1.5	<1.4
14	Acenaphthene	154	185	<0.7	<1.4	<0.7	<1.1	<1.4	<1.4	<1.3
12	C_{12} Naphthalenes	156	114	<1.1	<2.2	<1.1	<1.9	<2.2	<2.2	<2.1
8	C_{12} Indenes	160	21	<6.1	<12.3	<6.1	<10.2	<12.3	<12.3	<11.3
6	C_{12} Alkyl Benzenes	162	36	<3.6	<7.1	<3.5	<5.9	<7.1	<7.1	<6.6
14	C_{13} Acenaphthenes	168	108	<1.2	<2.4	<1.2	<2.0	<2.4	<2.4	<2.2
6	C_{13} Alkyl Benzenes	176	10	<12.9	<25.8	<13.0	<21.5	<25.8	<25.8	<23.5
18	Phenanthrene	178	215	<0.6	<1.2	<0.6	<1.0	<1.2	<1.2	<1.1
16	C_{14} Acenaphthalenes	180	122	<1.0	<2.1	<1.1	<1.8	<2.1	<2.1	<1.9
14	C_{14} Acenaphthenes	182	64	<2.0	<4.0	<2.0	<3.36	<4.0	<4.0	<3.7
12	C_{14} Naphthalenes	184	46	<2.8	<5.6	<2.8	<4.7	<5.6	<5.6	<5.1
8	C_{14} Indanes	188	18	<7.2	<14.3	<7.2	<11.9	<14.3	<14.3	<13.1
18	C_{15} Phenanthenes	192	130	<1.0	<1.9	<1.0	<1.7	<1.9	<1.9	<1.8
22	Pyrene	202	108	<1.2	<2.4	<1.2	<1.9	<2.4	<2.4	<2.2
18	C_{16} Phenanthrenes	206	33	<3.9	<7.8	<3.9	<6.5	<7.8	<7.8	<7.2
24	Chrysene	228	77	<1.7	<3.4	<1.7	<2.8	<3.4	<3.4	<3.1
18	C_{18} Phenanthrenes	234	26	<5.0	<9.9	<5.0	<8.3	<9.9	<9.9	<9.1
24	C_{19} Chrysenes	242	87	<1.5	<3.0	<1.5	<2.5	<3.0	<3.0	<2.7
28	Benzpyrene	252	104	<1.2	<2.5	<0.2	<2.1	<2.5	<2.5	<2.3
24	C_{20} Chrysenes	256	53	<2.4	<4.9	<2.4	<4.1	<4.9	<4.9	<4.5
30	C_{22} Picenes	278	75	<1.7	<3.4	<1.7	<2.9	<3.4	<3.4	<3.2
40	Trinaphthalenes	352	6	<21.0	<43.0	<21.0	<36.0	<43.0	<43.0	<39.4

APPENDIX D

STUDIES OF BITUMEN RECOVERY FROM SEDIMENTS AND ANALYSIS OF ANALYTICAL PRECISION

APPENDIX D

STUDIES OF BITUMEN RECOVERY FROM SEDIMENTS AND ANALYSIS OF ANALYTICAL PRECISION

To determine the accuracy and sensitivity of the sediment analytical procedure described in Appendix A, the following recovery experiment was performed. A composite sample consisting of the residual sediment from analysis of Stations 12, 13, and 14 was exhaustively extracted to remove any residual extractable organic material that would interfere with the recovery study.

South Tia Juana Medium Crude Oil was aged for 56 hours in an open beaker at 80°C. This residual oil is more representative of bituminous material accumulating in Maracaibo sediments than whole crude oil. The bitumen was added to three replicate sediment samples at 2,272 μg/g. This concentration level was selected from knowledge of the distribution of saturate, aromatic, and polar materials in the weathered oil and the amount of hydrocarbons needed to provide valid gravimetric data utilizing this analytical procedure. The recovery of bitumen from the sediment was calculated using the analysis without sediment present. The results of this study are shown in Table D.1. Results indicate at this concentration level that very reproducible data can be obtained. Recoveries of 86 to 104% with a maximum relative standard deviation of 17% were obtained. The high standard deviation observed for the NSO fraction with sediment present would possibly be due to ineffective extraction of polar compounds adsorbed on the sediment.

Shown in Table D.2 are the complete gravimetric results from three replicate analyses of sediment dredge samples from 14 Maracaibo sampling stations. The reproducibility of these data is quite variable, ranging from less than 1 to 73% relative standard deviation. A summary of the standard deviations is provided (Table D.3). The percent relative standard deviation for each sample location was tabulated with the number of samples occurring less than, or greater than, an arbitrarily selected median concentration. The resulting average percent relative standard deviation indicates that, in general, a higher variation is encountered with sediments which contain a large amount of extractable organic material. This could possibly be due to the problem of obtaining a representative subsample of dried sediment for analysis that contains bituminous particles such as the dredge samples obtained from Stations 2 and 13.

Table D.1

Recovery of South Tia Juana bitumen from Maracaibo sediment mineral

	Oil Analysis				% Rel. Std. Dev.	Percent Recovery
Fraction	A	B	C	Average		
		μg/g				
Total Organic Extract	2,282	2,286	2,300	2,289	.4	
Asphaltene-Free Extract	1,677	1,709	1,691	1,692	.8	
Benzene Soluble Asphaltenes	450	418	364	411	10.6	
Saturates	600	682	700	661	8.1	
Aromatics	855	868	873	865	1.1	
NSO Fraction	155	186	182	174	9.7	
	Oil Recovery from Sediment					
	A	B	C	Average		
		μg/g				
Total Organic Extract	2,154	2,332	2,445	2,310	6.3	100.9
Asphaltene-Free Extract	1,659	1,764	1,795	1,739	4.1	102.8
Benzene Soluble Asphaltenes	345	377	341	354	5.6	86.1
Saturates	655	677	736	689	6.1	104.2
Aromatics	736	832	845	804	7.4	92.9
NSO Fraction	136	177	191	168	17.0	96.6

Table D.2

Gravimetric analysis of sediment organic extracts

Sample Designation	Total Organic Extract (%)	Asphaltene Free Extract	Composition of Asphaltene-Free Extract			Benzene Soluble Asphaltenes
			Saturate	Aromatic	NSO	
		μg/g				
Station 6						
A	0.62	3,483	660	1,278	1,034	1,952
B	0.67	3,606	681	1,225	1,080	1,535
C	0.83	3,530	691	1,357	1,101	1,780
Average	0.71	3,540	677	1,287	1,072	1,756
(% rel. std. dev.)	15.4	1.8	2.3	2.3	3.2	11.9
Station 14						
A	0.31	1,679	340	698	488	767
B	0.33	1,785	393	771	491	949
C	0.30	1,413	296	608	371	1,738
Average	0.31	1,626	343	692	450	818
(% rel. std. dev.)	4.9	11.8	14.2	11.8	15.2	14.0
Station 7						
A	0.46	2,705	510	810	943	1,117
B	0.45	2,541	480	779	910	1,164
C	0.47	2,618	496	789	943	1,110
Average	0.46	2,621	495	793	932	1,130
(% rel. std. dev.)	2.2	3.1	3.0	2.0	2.0	2.6
Station 13						
A	2.89	18,969	3,814	7,018	5,162	5,238
B	2.88	18,269	3,797	6,656	5,226	5,695
C	2.58	16,013	3,419	6,165	4,214	5,274
Average	2.78	17,750	3,677	6,613	4,867	5,402
(% rel. std. dev.)	6.3	8.7	6.1	6.5	11.6	4.7
Station 12						
A	2.06	13,310	2,922	5,816	3,896	4,707
B	1.94	12,306	478	884	565	4,054
C	1.99	13,740	2,428	5,837	3,306	3,951
Average	2.00	13,119	1,943	4,179	2,580	4,237
(% rel. std. dev.)	3.0	5.6	47.0	48.3	48.7	6.8
Station 1						
A	0.32	1,832	387	826	529	548
B	0.43	2,789	534	1,162	242	988
C	0.40	2,464	477	1,046	669	914
Average	0.38	2,362	466	1,011	480	817
(% rel. std. dev.)	0.15	20.6	15.9	16.9	45.3	28.8
Station 2						
A	1.51	10,369	1,711	4,143	3,030	3,401
B	1.91	13,417	2,282	5,429	3,699	3,810
C	1.15	6,731	1,278	3,209	1,826	2,844
Average	1.52	10,172	1,757	4,260	2,852	3,352
(% rel. std. dev.)	25.0	32.9	28.7	26.2	33.3	14.5

Table D.2 (Continued)

Sample Designation	Total Organic Extract (%)	Asphaltene Free Extract	Composition of Asphaltene-Free Extract			Benzene Soluble Asphaltenes
		µg/g	Saturate	Aromatic	NSO	
Station 9						
A	0.93	5,350	986	1,801	1,788	2,286
B	1.56	4,985	995	1,718	1,584	2,753
C	1.03	5,050	855	1,697	1,597	2,058
Average	1.17	5,128	945	1,739	1,656	2,366
(% rel. std. dev.)	28.8	3.8	8.3	3.2	6.9	15.0
Station 10						
A	0.63	2,216	392	559	1,050	2,297
B	0.72	2,255	362	526	1,010	2,383
C	0.67	2,198	396	590	991	2,071
Average	0.67	2,223	383	558	1,017	2,250
(% rel. std. dev.)	6.7	1.3	4.8	5.7	3.0	7.2
Station 11						
A	0.75	2,358	303	436	1,200	2,133
B	3.82	2,111	292	427	1,111	2,737
C	1.78	1.925	301	428	913	2,318
Average	2.12	2,131	299	430	1,075	2,396
(% rel. std. dev.)	73.8	10.2	2.0	1.1	13.7	12.9
Station 25						
A	0.85	1,711	358	490	658	4,553
B	0.78	2,398	361	486	1,116	3,565
C	0.48	2,405	360	523	1,153	1,342
Average	0.70	2,171	360	500	976	3,153
(% rel. std. dev.)	27.9	18.4	0.4	4.1	28.3	52.2
Station 26						
A	0.40	1,793	433	854	427	805
B	0.61	1,826	431	760	419	820
C	0.40	2,011	427	919	486	811
Average	0.47	1,877	430	844	444	812
(% rel. std. dev.)	25.8	6.3	0.7	9.5	8.2	0.9
Station 27						
A	0.45	2,841	313	1,327	963	1,168
B	0.45	2,539	276	1,180	982	1,092
C	0.44	2,534	302	1,571	1,349	1,487
Average	0.45	2,638	297	1,359	1,098	1,249
(% rel. std. dev.)	1.3	6.7	6.4	14.5	19.8	16.8
Station 31						
A	0.25	1,310	274	832	442	437
B	0.24	1,342	281	888	378	464
C	0.27	1,469	193	790	444	461
Average	0.25	1,374	249	837	421	454
(% rel. std. dev.)	6.0	6.1	19.6	5.9	8.9	3.2
Station 30						
A	0.38	2,060	327	871	636	876
B	0.37	1,853	298	789	592	876
C	0.44	2,005	304	843	623	1,054
Average	0.40	1,973	310	834	617	935
(% rel. std. dev.)	9.5	5.4	4.9	5.0	3.7	11.0

Table D.3

Summary of relative standard deviations from the gravimetric analysis of sediment organic extracts

Total Organic Extract	0.25-1.0%	1.0-3.0%
Number of samples	10	5
Avg. % rel. std. dev.	9.9	27.4
Asphaltene Free Extract	1300-5000 µg/g	5000-17,750 µg/g
Number of samples	11	4
Avg. % rel. std. dev.	8.3	12.8
Saturate Fraction	250-500 µg/g	500-3700 µg/g
Number of samples	10	5
Avg. % rel. std. dev.	7.2	18.5
Aromatic Fraction	430-1000 µg/g	1000-6600 µg/g
Number of samples	8	7
Avg. % rel. std. dev.	5.6	16.8
NSO Fraction	420-1000 µg/g	1000-4900 µg/g
Number of samples	7	8
Avg. % rel. std. dev.	15.9	17.5
Benzene Soluble Asphaltenes	450-1000 µg/g	1000-5400 µg/g
Number of samples	5	10
Avg. % rel. std. dev.	11.6	14.5

APPENDIX E

ANALYSIS OF TOXICITY TEST WATER FOR LIGHT AROMATIC COMPOUNDS

APPENDIX E

ANALYSIS OF TOXICITY TEST WATER FOR LIGHT AROMATIC COMPOUNDS

An integral part of the experimental design for oil bioassay studies at the Las Morochas Laboratory was the determination of hydrocarbon concentrations in the test media. Previous flow-through bioassay studies by Battelle have shown good correlation between hydrocarbon content of bioassay water, as measured by infrared absorption, and the toxic response elicited. However, application of this analytical technique to the static bioassays conducted as a part of this program has not proven fruitful. The data presented in Table E.1 show poor correspondence between quantity of oil extracted by water and oil concentration in the water as measured by IR. In addition, relationships between IR determined oil concentration and mortality of test biota are not apparent.

Although problems of solvent contamination and high "background" absorbance is recognized as contributing to the data scatter, the change in experimental methodology from flow-through to static regimes had a major influence on analytical requirements. In addition, the use of test oils of widely differing API gravities and the testing of "weathered" oils for toxicity introduced important experimental variables which the simple infrared method is not capable of accounting for.

Battelle-Northwest, in recognition of the physical and chemical complexities of petroleum/water systems, has supported an independent investigation of the bioassay concept as applied to crude oils in seawater systems. The research, principally conducted at Battelle's Marine Research Laboratories at Sequim, Washington, resulted in significant advances in both experimental methodology and analytical technique. One of the important findings of the Battelle study was that a relatively small number of hydrocarbon compounds in petroleum can impart an acutely toxic effect to water which is disproportionately high with respect to their concentrations in the crude oil. These compounds, consisting of the "light ends" of petroleum are readily extracted into water when mixtures of oil and water are agitated.

In view of this result, it was considered appropriate to apply some of the analytical methodology developed for the Battelle studies to the static bioassay studies being conducted at Las Morochas. Duplicate samples of bioassay water were extracted with hexane and with carbon tetrachloride and shipped to the Richland laboratories. The hexane extracts were analyzed for monocyclic aromatic compounds using gas chromatography. A typical gas chromatogram of a hexane extract of bioassay water is shown in Figure E.1. Note that benzene appears as a peak on the trailing edge of the solvent peak. The large peak on the right-hand side of the chromatogram is the internal standard added for purposes of quantitating results. There are a number of small peaks between o-xylene and the internal standard. These peaks have retention times consistent with aromatic compounds in the carbon number range of C_9 to C_{10} and are thus designated "C_9+" aromatics. However, confirmation of these peaks as aromatic compounds has been accomplished to date for only one component. The C_6 to C_8 aromatics in the chromatogram have been confirmed by comparing retention times, sample "spiking", and the use of an alternate separation column. The absence of interfering peaks due to saturated hydrocarbons is explained by the choice of liquid phase in the separation column - carbowax, which does not retain paraffins and cycloparaffins well. Also, higher molecular weight paraffins are poorly soluble in water, and are therefore, not present in the hexane extracts to an appreciable extent.

For two bioassay studies of interest, only carbon tetrachloride extracts were available. These extracts were also analyzed by gas chromatograph. To assure that the results were comparable with those obtained from the hexane samples, both CCl_4 and hexane extracts from two experiments were analyzed. Comparison of results are given in Table E.2. The data show that the values determined for CCl_4 extracts are somewhat lower than those for hexane extracts, but reasonably comparable, with the exception of one test solution in Experiment 50. The lower values for CCl_4 may

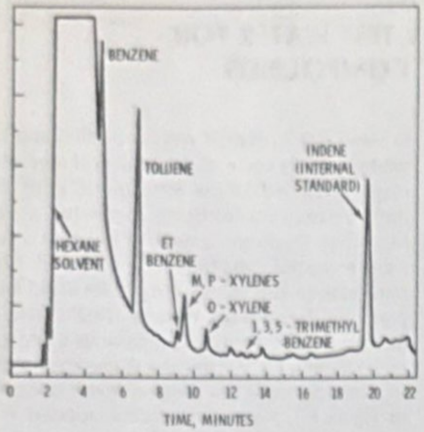

Figure E.1
Gas chromatogram of hexane extracts
of bioassay water samples

be a result of less efficient extraction. In addition, lower values for benzene in CCl_4 extracts can result because separation of benzene from the solvent peak is not particularly good, and thus errors in peak integration occur when benzene concentrations are low.

That the data generated for aromatic content is reasonably consistent with quantity of oil extracted can be seen from an inspection of Table E.3, where the milliliters of oil extracted per liter of water is compared with the total monocyclic aromatics determined by the gas chromatographic technique. A notable exception is Experiment 53, where mortality was also inconsistent with the amount of oil extracted. Table E.4 shows the good correlation between weathering time and quantity of aromatics extracted by water. The complete set of analytical data generated is given in Tables E.5-E.18.

A more comprehensive discussion of the GC analytical method for determination of light ends of petroleum in bioassay water is included in the report on Battelle's bioassay program.*

Table E.1
Comparison of quantity of oil extracted with resulting concentration of oil in water
as determined by infrared spectrometry

		Test #1		Test #2		Test #3		Test #4	
Bioassay No.	Crude Oil	Oil Extracted (ml/ℓ)	Oil in Water (mg/ℓ)	Oil Extracted (ml/ℓ)	Oil in Water (mg/ℓ)	Oil Extracted (ml/ℓ)	Oil in Water (mg/ℓ)	Oil Extracted (ml/ℓ)	Oil in Water (mg/ℓ)
44	STJM	3.00	8.8	2.33	10.4	2.00	8.9	1.67	2.9
49	STJM	2.67	69.3	2.00	12.5	1.33	39.6	0.67	6.7
50	STJM	26.67	17.9	13.33	13.9	6.67	40.4	3.33	14.7
53	TJL	3.33	2.9	2.67	4.0	2.00	16.8	1.33	12.0
55	TJL	3.33	17.9	2.67	7.1	2.00	29.9	1.33	33.1
56	TJH	3.33	24.3	2.67	25.5	2.00	6.5	1.33	13.5
57	TJL	5.56	12.1	4.44	162.5	3.33	58.4	2.22	61.9
62	STJM	26.67	22.4	13.33	18.0	6.67	11.9	3.33	9.2
63	STJM	13.33	14.8	6.67	8.3	3.33	299.1	1.67	5.7

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*Bean, R. M., J. R. Vanderhorst, and P. Wilkinson. 1974. **Interdisciplinary Study of the Toxicity of Petroleum to Marine Organisms,** Battelle, Pacific Northwest Laboratories, Richland, Washington.

Table E.2
Comparison of results obtained from hexane and carbon tetrachloride extracts of bioassay water.
(Component concentrations expressed as mg/ℓ of water.)

Experiment #55

	Mls Oil Extracted per Liter of Water							
	3.33		2.67		2.00		1.33	
	Hexane	CCl_4	Hexane	CCl_4	Hexane	CCl_4	Hexane	CCl_4
Benzene, mg/ℓ	0.57	0.02	1.78	0.60	0.65	—	0.54	—
Toluene, mg/ℓ	1.02	1.17	2.99	2.24	1.43	1.09	1.16	1.05
Et. Benzene, mg/ℓ	0.12	0.11	0.32	0.50	0.17	0.11	0.14	0.10
m, p-Xylenes, mg/ℓ	0.39	0.63	1.24	1.56	0.61	0.64	0.46	0.58
o-Xylene, mg/ℓ	0.13	0.14	0.40	1.15	0.18	0.13	0.14	0.12
"C_9+" mg/ℓ	0.12	0.18	0.52	0.60	0.22	0.13	0.11	0.11
Total, mg/ℓ	2.35	2.25	7.25	6.65	3.26	2.10	2.55	1.96

Experiment #50

	Mls Oil Extracted per Liter of Water							
	26.67		13.33		6.67		3.33	
	Hexane	CCl_4	Hexane	CCl_4	Hexane	CCl_4	Hexane	CCl_4
Benzene, mg/ℓ	3.36	2.70	2.29	1.66	1.61	—	1.08	0.96
Toluene, mg/ℓ	4.63	3.84	3.27	2.73	2.64	0.10	2.32	2.18
Et. Benzene, mg/ℓ	0.43	0.30	0.31	0.23	0.28	0.02	0.28	0.22
m, p-Xylenes, mg/ℓ	1.71	1.28	1.32	1.0	1.15	0.15	1.10	0.96
o-Xylene, mg/ℓ	0.75	0.53	0.50	0.38	0.44	—	0.42	0.35
"C_9+" mg/ℓ	0.97	0.64	0.73	0.60	0.71	—	0.68	0.60
Total, mg/ℓ	11.88	9.29	8.42	6.60	6.83	0.27	5.88	5.27

Table E.3

Comparison of quantity of oil extracted with resulting aromatics concentration in water as determined by gas chromatography

Toxicity Experiment No.	Crude Oil	Test #1 Oil Extracted (ml/ℓ)	Test #1 Aromatics (mg/ℓ)	Test #2 Oil Extracted (ml/ℓ)	Test #2 Aromatics (mg/ℓ)	Test #3 Oil Extracted (ml/ℓ)	Test #3 Aromatics (mg/ℓ)	Test #4 Oil Extracted (ml/ℓ)	Test #4 Aromatics (mg/ℓ)
44	TJM	3.00	1.85	2.33	0.72	2.00	0.95	1.67	0.71
49	TJM	2.67	5.13	2.00	3.45	1.33	1.68	0.67	0.49
50	TJM	26.67	11.88	13.33	8.42	6.67	6.83	3.33	5.88
53	TJL	3.33	1.70	2.67	0.35	2.00	2.51	1.33	2.01
55	TJL	3.33	2.35	2.67	7.25	2.00	3.26	1.33	2.55
56	TJH	3.33	0.26	2.67	Traces	2.00	—	1.33	—
57	TJL	5.56	11.74	4.44	8.19	3.33	9.92	2.22	4.54
62	TJM	26.67	8.59	13.33	7.60	6.67	3.70	3.33	3.60
63	TJM	13.33	7.81	6.67	3.95	3.33	3.24	1.67	1.19

Table E.4

Comparison of weathering time with concentration of aromatics extracted into water*

Toxicity Experiment No.	Test #1 Weathering Time (min.)	Test #1 Aromatics (mg/ℓ)	Test #2 Weathering Time (min.)	Test #2 Aromatics (mg/ℓ)	Test #3 Weathering Time (min.)	Test #3 Aromatics (mg/ℓ)	Test #4 Weathering Time (min.)	Test #4 Aromatics (mg/ℓ)
46	0	1.88	130	2.16	205	1.54	315	0.23
48	0	3.62	120	2.36	240	0.98	480	0.61
54	0	1.97	80	0.48	180	0.05	270	0.04

*50 ml portions of crude oil extracted into 15 liters of lake water after weathering for the indicated times. See bioassay section for methodology.

Table E.5

Experiment #44 - *P. schmitti*

24 Hour Toxicity Test

Tia Juana Medium Crude

Aromatic Analysis: Carbon tetrachloride extracts of bioassay water samples

	Mls oil extracted per liter				
	3.00	2.33	2.00	1.67	0
Benzene*, mg/ℓ	0.05	—	—	—	
Toluene, mg/ℓ	0.95	0.37	0.42	0.36	
Et. Benzene, mg/ℓ	0.10	0.04	0.06	0.05	
m, p-Xylene**, mg/ℓ	0.46	0.23	0.32	0.21	
o-Xylene, mg/ℓ	0.11	0.04	0.06	0.05	
"C_9+", mg/ℓ	0.18	0.04	0.09	0.04	
Total , mg/ℓ	1.85	0.72	0.95	0.71	
Total Oil (IR), mg/ℓ	8.8	10.4	8.9	2.9	
24 Hour Mortality, %	100	94	94	66	0

Table E.6

Experiment #46 - *P. schmitti*

24 Hour Toxicity Tests on Weathered Oil - South Tia Juana Medium

Each test solution prepared by extracting 50 ml weathered oil with 15 liters of lake water.

Calculated oil film thickness over flowing water: 0.6 mm

Aromatic Analysis: Carbon tetrachloride extracts of bioassay water samples

	Weathering Time (minutes)				
	0	130	205	315	415
Benzene*, mg/ℓ	0.07	—	—	—	—
Toluene, mg/ℓ	0.88	0.57	0.58	0.04	0.09
Et. Benzene, mg/ℓ	0.10	0.13	0.09	0.02	0.04
m, p-Xylene**, mg/ℓ	0.46	0.63	0.42	0.16	0.17
o-Xylene, mg/ℓ	0.11	0.16	0.10	0.01	0.05
"C_9+" ,mg/ℓ	0.26	0.67	0.35	—	0.31
Total , mg/ℓ	1.88	2.16	1.54	0.23	0.66
Total Oil (IR), mg/ℓ	7.4	27.8	11.6	11.4	19.3
24 Hour Mortality, %	100	76	28	20	20

*Under the particular analytical conditions, benzene is seriously underestimated using carbon tetrachloride extracts.

**An impurity in the carbon tetrachloride used at the Las Morochas Laboratory interferes with this component - concentrations are thus estimates.

Table E.7

Experiment #48 - *P. schmitti*

24 Hour Toxicity Test on Weathered Oil - South Tia Juana Medium

Eachtest solution prepared by extracting 50 ml weathered oil with 15 liters of lake water.

Calculated oil film thickness over stagnant water: 0.6 mm

Aromatic Analysis: Hexane extracts of bioassay water samples

	Weathering Time (minutes)				
	0	120	240	480	Control
Benzene, mg/ℓ	0.47	0.32	Trace	Trace	
Toluene, mg/ℓ	1.47	0.93	0.52	0.42	
Et. Benzene, mg/ℓ	0.18	0.13	0.07	0.03	
m, p-Xylene, mg/ℓ	0.71	0.47	0.18	0.05	
o-Xylene, mg/ℓ	0.26	0.15	0.07	0.05	
"C_9+", mg/ℓ	0.53	0.36	0.14	0.06	
Total, mg/ℓ	3.62	2.36	0.98	0.61	
Total Oil (IR), mg/ℓ	9.83	26.9	67.1	29.6	
24 Hour Mortality, %	100	68	54	40	4

Table E.8

Experiment #49 - *P. schmitti*

24 Hour Toxicity Test

South Tia Juana Medium Crude

Aromatic Analysis: Hexane extracts of bioassay water samples

	Mls Oil Extracted per Liter of Water				
	2.67	2.00	1.33	0.67	0
Benzene, mg/ℓ	0.74	0.43	0.18	Trace	
Toluene, mg/ℓ	2.24	1.37	0.74	0.36	
Et. Benzene, mg/ℓ	0.29	0.24	0.11	0.02	
m, p-Xylene, mg/ℓ	1.00	0.76	0.41	0.06	
o-Xylene, mg/ℓ	0.29	0.23	0.13	0.05	
"C_9+", mg/ℓ	0.57	0.42	0.11	—	
Total, mg/ℓ	5.13	3.45	1.68	0.49	
Total Oil (IR), mg/ℓ	69.3	12.5	39.6	6.7	
24 Hour Mortality, %	46	68	24	8	10
Mortality After 72 Hr. Depuration, %	52%	68%	82%	12%	12%

Table E.9

Experiment #50 - *Centropomus parallelus*

24 Hour Static Toxicity Test

South Tia Juana Medium Crude

Aromatic Analysis: Hexane extracts of bioassay water samples

	Mls Oil Extracted per Liter of Water				
	26.67	13.33	6.67	3.33	0
Benzene, mg/ℓ	3.36	2.29	1.61	1.08	
Toluene, mg/ℓ	4.63	3.27	2.64	2.32	
Et. Benzene, mg/ℓ	0.43	0.31	0.28	0.28	
m, p-Xylene, mg/ℓ	1.74	1.32	1.15	1.10	
o-Xylene, mg/ℓ	0.75	0.50	0.44	0.42	
"C_9+", mg/ℓ	0.97	0.73	0.71	0.68	
Total, mg/ℓ	11.88	8.42	6.83	5.88	
Total Oil (IR), mg/ℓ	17.9	13.9	40.4	14.7	
24 Hour Mortality, %	100	48	4	22	6

Table E.10

Experiment #53 - *P. schmitti*

24 Hour Static Toxicity Test

Tia Juana Light Crude

Aromatic Analysis: Hexane extracts of bioassay water samples

	Mls Oil Extracted per Liter of Water				
	3.33	2.67	2.00	1.33	0
Benzene, mg/ℓ	0.52	0.09	0.57	0.42	
Toluene, mg/ℓ	0.68	0.21	1.05	0.84	
Et. Benzene, mg/ℓ	0.08	Trace	0.13	0.11	
m, p-Xylene, mg/ℓ	0.27	0.03	0.44	0.37	
o-Xylene, mg/ℓ	0.08	0.02	0.14	0.11	
"C_9+", mg/ℓ	0.07	Trace	0.18	0.16	
Total, mg/ℓ	1.70	0.35	2.51	2.01	
Total Oil (IR), mg/ℓ	2.9	4.0	16.8	12.0	
24 Hour Mortality, %	30	12	80	68	0

Table E.11

Experiment #54 - *P. schmitti*

24 Hour Test on Weathered Oil - South Tia Juana Medium

Each test solution prepared by extracting 50 ml weathered oil with 15 liters of lake water.

Calculated oil film thickness over stagnant water: 0.6 mm

Aromatic Analysis: Hexane extracts of bioassay water samples

	Weathering Time (minutes)				
	0	80	180	270	Control
Benzene, mg/ℓ	0.33	Trace	Trace	—	
Toluene, mg/ℓ	0.81	0.21	0.03	Trace	
Et. Benzene, mg/ℓ	0.11	0.04	Trace	—	
m, p-Xylene, mg/ℓ	0.40	0.13	0.02	Trace	
o-Xylene, mg/ℓ	0.12	0.04	Trace	—	
“C_9+”, mg/ℓ	0.20	0.06	Trace	0.04	
Total, mg/ℓ	1.97	0.48	0.05	0.04	
Total Oil (IR), mg/ℓ	4.5	6.5	13.3	9.6	
24 Hours Mortality, %	76	20	26	36	2

Table E.12

Experiment #55 - *P. schmitti*

24 Hour Static Toxicity Test

Tia Juana Light Crude

Aromatic Analysis: Hexane extracts of bioassay water samples

	Mls Oil Extracted per Liter of Water				
	3.33	2.67	2.00	1.33	0
Benzene, mg/ℓ	0.57	1.78	0.65	0.54	
Toluene, mg/ℓ	1.02	2.99	1.43	1.16	
Et. Benzene, mg/ℓ	0.12	0.32	0.17	0.14	
m, p-Xylene, mg/ℓ	0.39	1.24	0.61	0.46	
o-Xylene, mg/ℓ	0.13	0.40	0.18	0.14	
“C_9+”, mg/ℓ	0.12	0.52	0.22	0.11	
Total, mg/ℓ	2.35	7.25	3.26	2.55	
Total Oil (IR), mg/ℓ	17.9	7.1	29.9	33.1	
24 Hour Mortality, %	4	80	2	2	0

Table E.13

Experiment #56 - *P. schmitti*

24 Hour Toxicity Test

Tia Juana Heavy Crude

Aromatic Analysis: Hexane extracts of bioassay water samples

	Mls Oil Extracted per Liter of Water				
	3.33	2.67	2.00*	1.33*	0
Benzene, mg/ℓ	Trace	—			
Toluene, mg/ℓ	0.19	Trace			
Et. Benzene, mg/ℓ	0.05	—			
m, p-Xylene, mg/ℓ	Trace	Trace			
o-Xylene, mg/ℓ	0.02	Trace			
“C_9+”, mg/ℓ	Trace	—			
Total, mg/ℓ	0.26	—			
Total Oil (IR), mg/ℓ	24.3	25.5	6.5	13.5	
24 Hour Mortality, %	20	18	16	8	4

*Not analyzed.

Table E.14

Experiment #57 - *P. schmitti*

24 Hour Static Toxicity Test

Tia Juana Light Crude

Aromatic Analysis: Hexane extracts of bioassay water samples

	Mls Oil Extracted per Liter of Water				
	5.56	4.44	3.33	2.22	0
Benzene, mg/ℓ	2.10	1.85	1.38	0.84	
Toluene, mg/ℓ	5.08	3.42	4.27	2.04	
Et. Benzene, mg/ℓ	0.50	0.41	0.53	0.24	
m, p-Xylene, mg/ℓ	2.21	1.44	2.13	0.89	
o-Xylene, mg/ℓ	0.79	0.44	0.60	0.28	
“C_9+”, mg/ℓ	1.06	0.63	1.01	0.25	
Total, mg/ℓ	11.74	8.19	9.92	4.54	
Total Oil (IR), mg/ℓ	12.1	162.5	58.4	61.9	
24 Hour Mortality, %	100	82	70	36	6

Table E.15

Experiment #62 - C. *parallelus*

24 Hour Toxicity Test plus 2 week depuration
South Tia Juana Medium Crude

Aromatic Analysis: Hexane extracts of bioassay water samples

	Mls Oil Extracted per Liter of Water				
	26.67	13.33	6.67	3.33	0
Benzene, mg/ℓ	2.44	1.87	0.98	0.48	0
Toluene, mg/ℓ	3.44	3.08	1.42	1.45	
Et. Benzene, mg/ℓ	0.30	0.31	0.18	0.23	
m, p-Xylene, mg/ℓ	1.26	1.23	0.61	0.78	
o-Xylene, mg/ℓ	0.49	0.48	0.19	0.21	
"C_9+", mg/ℓ	0.66	0.63	0.32	0.45	
Total, mg/ℓ	8.59	7.60	3.70	3.60	
Total Oil (IR), mg/ℓ	22.4	18.0	11.9	9.2	
24 Hour Mortality, %	66	34	32	30	30
Mortality After 2 Weeks Depuration, %	84	86	82	82	80

Table E.16

Experiment #63 - C. *Parallelus*

24 Hour Toxicity Test plus 2 week depuration
South Tia Juana Medium Crude

Aromatic Analysis: Hexane extracts of bioassay water samples

	Mls Oil Extracted per Liter of Water				
	13.33	6.67	3.33	1.67	0
Benzene, mg/ℓ	2.11	1.02	0.75	0.25	
Toluene, mg/ℓ	3.16	1.53	1.29	0.46	
Et. Benzene, mg/ℓ	0.29	0.20	0.17	0.07	
m, p-Xylene, mg/ℓ	1.19	0.65	0.58	0.23	
o-Xylene, mg/ℓ	0.48	0.21	0.17	0.07	
"C_9+", mg/ℓ	0.58	0.34	0.28	0.11	
Total, mg/ℓ	7.81	3.95	3.24	1.19	
Total Oil (IR), mg/ℓ	14.8	8.3	299.1	5.7	
24 Hour Mortality, %	23	0	0	0	0
Mortality After 2 Weeks Depuration, %	46	15	20	20	12

Table E.17

Experiment #73 - *P. schmitti*

A. 10 ml of South Tia Juana Medium Crude extracted with 15 liters lake water for toxicity test
Samples of test water removed for analysis at times indicated

(24 Hour Mortality: 8%)

	Time From Initital Exposure, Hr.				
	0	0.5	2.0	6.0	24.0
Benzene, mg/ℓ	0.04	0.03	0.01	—	—
Toluene, mg/ℓ	0.16	0.13	0.08	0.04	—
Et. Benzene, mg/ℓ	0.02	0.02	0.01	0.01	—
m, p-Xylenes, mg/ℓ	0.06	0.05	0.03	0.01	—
o-Xylene, mg/ℓ	0.03	0.02	0.01	0.01	—
"C_9+", mg/ℓ	0.03	0.02	0.01	—	—
Total, mg/ℓ	0.34	0.27	0.15	0.07	none detected

Experiment #73 - *P. schmitti*

B. 20 ml of South Tia Juana Medium Crude extracted with 15 liters lake water for toxicity test
Samples of test water removed for analysis at times indicated

(24 Hour Mortality: 16%)

	Time From Initial Exposure, Hr.				
	0	0.5	2.0	6.0	24.0
Benzene, mg/ℓ	0.19	0.16	0.03	0.02	—
Toluene, mg/ℓ	0.47	0.41	0.14	0.10	—
Et. Benzene, mg/	0.06	0.05	0.02	0.02	—
m, p-Xylenes, mg/ℓ	0.19	0.16	0.05	0.04	—
o-Xylene, mg/ℓ	0.09	0.07	0.02	0.02	—
"C_9+", mg/ℓ	0.18	0.16	0.04	0.04	—
Total, mg/ℓ	1.18	1.01	0.30	0.24	none detected

Table E.17 (Continued)

Experiment #73 - *P. schmitti*

C. 30 ml of South Tia Juana Medium Crude extracted with 15 liters lake water for toxicity test
Samples of test water removed for analysis at times indicated

(24 Hour Mortality: 64%)

	Time From Initial Exposure, Hr.				
	0	0.5	2.0	6.0	24.0
Benzene, mg/ℓ	0.37	0.30	0.18	0.08	—
Toluene, mg/ℓ	0.96	0.67	0.45	0.23	0.01
Et. Benzene, mg/ℓ	0.12	0.08	0.05	0.03	—
m, p-Xylenes, mg/ℓ	0.38	0.24	0.17	0.09	—
o-Xylene, mg/ℓ	0.16	0.11	0.07	0.05	—
"C_9+", mg/ℓ	0.27	0.21	0.13	0.07	—
Total, mg/ℓ	2.26	1.61	1.05	0.55	0.01

Experiment #73 - *P. schmitti*

D. 40 ml of South Tia Juana Medium Crude extracted with 15 liters lake water for toxicity test
Samples of test water removed for analysis at times indicated

(24 Hour Mortality: 68%)

	Time From Initital Exposure, Hr.				
	0	0.5	2.0	6.0	24.0
Benzene, mg/ℓ	0.26	0.19	0.12	0.08	0.02
Toluene, mg/ℓ	0.58	0.46	0.28	0.21	0.04
Et. Benzene, mg/ℓ	0.07	0.05	0.03	0.03	—
m, p-Xylenes, mg/ℓ	0.21	0.18	0.11	0.08	0.01
o-Xylene, mg/ℓ	0.10	0.09	0.05	0.07	0.01
"C_9+", mg/ℓ	0.16	0.15	0.08	0.05	0.01
Total, mg/ℓ	1.38	1.12	0.67	0.52	0.09

Table E.17 (Continued)

Experiment #73 - *P. schmitti*

E. 50 ml of South Tia Juana Medium Crude extracted with 15 liters lake water for toxicity test
Samples of test water removed for analysis at times indicated

(24 Hour Mortality: 76%)

	Time From Initial Exposure, Hr.				
	0	0.5	2.0	6.0	24.0
Benzene, mg/ℓ	0.35	0.32	0.13	0.09	—
Toluene, mg/ℓ	0.72	0.70	0.29	0.22	Trace
Et. Benzene, mg/ℓ	0.09	0.08	0.04	0.03	—
m, p-Xylenes, mg/ℓ	0.26	0.24	0.10	0.08	—
o-Xylene, mg/ℓ	0.11	0.11	0.05	0.04	—
"C_9+", mg/ℓ	0.21	0.21	0.10	0.07	—
Total, mg/ℓ	1.74	1.66	0.71	0.53	Trace

Table E.18

Experiment #74 - *P. schmitti*

A. South Tia Juana Medium Crude weathered for 10 minutes.
50 ml of oil extracted with 15 liters lake water for toxicity test
Samples of test water removed for analysis at times indicated

(24 Hour Mortality: 48%)

	Time from Initial Exposure, Hr				
	0	0.5	2.0	6.0	24.0
Benzene, mg/ℓ	0.10	0.09	0.04	0.04	—
Toluene, mg/ℓ	0.24	0.26	0.15	0.16	—
Et. Benzene, mg/ℓ	0.03	0.03	0.02	0.03	—
m, p-Xylenes, mg/ℓ	0.10	0.10	0.06	0.07	—
o-Xylene, mg/ℓ	0.05	0.05	0.03	0.03	—
"C_9+", mg/ℓ	0.17	0.09	0.06	0.06	—
Total, mg/ℓ	0.69	0.62	0.36	0.39	none detected

Control, Initial Sample: No monoaromatics found.

Table E.18 (Continued)

Experiment #74 - *P. schmitti*

B. South Tia Juana Medium Crude weathered for 20 minutes

50 ml of oil extracted with 15 liters lake water for toxicity test

Samples of test water removed for analysis at times indicated

(24 Hour Mortality: 84%)

	Time from Initial Exposure, Hr				
	0	0.5	2.0	6.0	24.0
Benzene, mg/ℓ	0.16	0.10	0.06	0.04	—
Toluene, mg/ℓ	0.47	0.30	0.18	0.18	0.04
Et. Benzene, mg/ℓ	0.07	0.04	0.03	0.03	—
m, p-Xylenes, mg/ℓ	0.22	0.14	0.08	0.08	0.02
o-Xylene, mg/ℓ	0.10	0.07	0.04	0.04	0.01
"C_9+", mg/ℓ	0.28	0.15	0.07	0.08	0.02
Total, mg/ℓ	1.30	0.80	0.46	0.45	0.09

Experiment #74 - *P. schmitti*

C. South Tia Juana Medium Crude weathered for 40 minutes

50 ml of oil extracted with 15 liters lake water for toxicity test

Samples of test water removed for analysis at times indicated

(24 Hour Mortality: 40%)

	Time from Initial Exposure, Hr				
	0	0.5	2.0	6.0	24.0
Benzene, mg/ℓ	Trace	0.01	Trace	Trace	—
Toluene, mg/ℓ	0.07	0.10	0.09	0.05	—
Et. Benzene, mg/ℓ	0.03	0.02	0.02	0.01	—
m, p-Xylenes, mg/ℓ	0.03	0.06	0.06	0.04	—
o-Xylene, mg/ℓ	0.03	0.03	0.03	0.02	—
"C_9+", mg/ℓ	0.07	0.09	0.08	0.05	—
Total, mg/ℓ	0.23	0.31	0.28	0.17	none detected

Table E.18 (Continued)

Experiment #74 - *P. schmitti*

D. South Tia Juana Medium Crude weathered for 80 minutes

50 ml of oil extracted with 15 liters lake water for toxicity test

Samples of test water removed for analysis at times indicated

(24 Hour Mortality: 36%)

	Time from Initial Exposure, Hr				
	0	0.5	2.0	6.0	24.0
Benzene, mg/ℓ	—	—	—	—	—
Toluene, mg/ℓ	0.03	0.04	0.03	0.01	—
Et. Benzene, mg/ℓ	0.01	0.01	0.01	0.01	—
m, p-Xylenes	0.03	0.04	0.03	0.01	—
o-Xylene, mg/ℓ	0.01	0.02	0.01	0.01	—
"C_9+", mg/ℓ	0.05	0.09	0.07	0.03	—
Total, mg/ℓ	0.13	0.20	0.15	0.07	none detected

APPENDIX F

PLANKTON BIOASSAY STUDIES

APPENDIX F

PLANKTON BIOASSAY STUDIES

ZOOPLANKTON BIOASSAY

One successful bioassay was performed using the zooplankton community. In this experiment, animals were selected at random from lake water and placed in glass petri dishes in 25 ml of filtered lake water. Water phase from 160 ml of oil to 15 liters water was used along with filtered lake water to give desired dilution.

The zooplankton community experienced a sharp decline in mortality below the 1000 ppm concentration (Table F.1).

PHYTOPLANKTON BIOASSAYS

Phytoplankton bioassays were conducted in a static system at ambient temperature 30-33°C out of direct sunlight.

One experiment was conducted using the natural phytoplankton community in an artificial growth medium with various concentrations of crude oil. The phytoplankton community was allowed to develop for five days in 2000 ml of dilute nutrient solution. At this time, crude petroleum was added at a concentration from zero to 16,557 ppm.

This experiment pointed out that only 4281 ppm and above were conclusively inhibitory to photosynthesis (Tables F.2 and F.3).

A third experiment with natural phytoplankton was performed using filtered, sterilized, and nutrient-enriched lake water. The results point out that only concentrations in excess of 10,000 ppm are inhibitory to the natural phytoplankton community as treated in this system (Tables F.4 and F.5).

Crude oil has a stimulatory effect on the phytoplankton of the Ulé laguna (Figure F.1).

There are at least two different algae in the phytoplankton. It is highly probable that the two have different environmental requirements. It has been observed that a fresh sample of the Ulé effluent contains an almost pure *Chlamydomanas spp.* with only a very small percentage of *Stichoccocus bacillaris* but that an old culture (the age of the culture depending primarily on the available nutrients) contains a predominance of *S. bacillaris*. This is an extremely tolerant organism having been found in such diverse habitats as ice and snow, sea water, on flower pots in green houses, and in distilled water marketed in Venezuela. It is not inconceivable that this algae will grow in water overlain by and periodically mixed with crude petroleum (Table F.6).

A final experiment conducted on the plankton community utilized the dissolved oxygen method of determining the productivity (Strickland and Parsons, 1960).[*] In this experiment the lake water was agitated with the desired amount of crude oil for 10 minutes, allowed to separate for 30 minutes, and the water phase dispensed by siphon to five light and two dark bottles. The initial concentration of dissolved oxygen was determined. The bottles were placed in a constantly flowing water bath at ambient lake temperature for 24 hours. From the determination of the 0_2 levels in the water at the end of this period it was possible to determine the gross photosynthesis, net photosynthesis, and respiration. On a short term basis net carbon fixation reaches zero at between 2666 and 5332 ppm of crude oil added. (Table F.7)

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[*] J. D. H. Strickland and T. R. Parsons. 1960. **A Manual of Sea Water Analysis.** Fisheries Research Board of Canada, Bulletin 125.

Table F.1

Twenty-four hour bioassay of South Tia Juana Medium Crude Oil on mixed zooplankton community, the majority of which were cyclopoid copepods

Oil concentration	0	1067 ppm	2130 ppm	3200 ppm	4267 ppm
No. dead	10	31	41	43	40
No. alive	42	25	18	15	15
Total No. animals	52	56	59	58	55
% mortality	19	55	69	74	73

Table F.2

Natural phytoplankton in artificial medium. Percent change of ^{14}C uptake versus time in days and oil concentration. (% of initial ^{14}C uptake)

Oil Conc. ppm	1 day	3 days	5 days	7 days
56	1336	2221	2044	524
193	238	771	53	378
984	80	144	2455	154
1327	41	77	455	385
4281	10	18	23	27
7728	31	21	13	25
0	1183	3162	1447	1476
16,537	172	9	8	8
0	343	2254	4377	7122

Table F.3

Natural phytoplankton in artificial medium. Percent change of chlorophyll-a versus time in days and oil concentration (% initial chlorophyll-a)

Conc. ppm	1 day	3 days	5 days	7 days
56	863	1200	1081	413
193	622	1011	1372	722
984	1200	933	867	867
1327	210	230	260	270
4281	657	671	714	343
7728	1014	571	614	557
0	543	529	743	1625
16,537	83	77	50	67
0	160	640	620	1380

Table F.4

Natural phytoplankton in filtered, sterilized, enriched lake water[1]

Conc. ppm	Initial	1 day	4 days	8 days	9 days	10 days
822	620	160	280	350	980	1150
1225	690	690	1270	580	2880	2740
225	280	120	670	460	1900	2020
10663	690	390	480	[50][2]	320	230
492	600	280	320	1210	810	370
5090	250	90	880	[0][2]	300	120
0	50	220	50	120	120	140
0	320	180	20	320	90	210
0	480	350	20	20	20	50

[1] Values expressed in milligrams chlorophyll-a per liter

[2] Not filterable

Table F.5

^{14}C - uptake data for natural phytoplankton in filtered, sterilized, enriched lake water[1]

Conc.	Initial	1 day	7 days	8 days	9 days
822	100	583	1235	509	3870
1255	100	144	358	2826	906
225	100	242	1204	6466	6877
10665	100	222	731	2	2
492	100	452	908	1629	3037
5090	100	214	1209	3106	5236
0	100	38	978	3065	3188
0	100	55	100	744	1386
0	100	19	360	569	935

[1] Values expressed as percent of value obtained at initiation of experiment

Table F.6

Comparison of chlorophyll-a values. Natural phytoplankton community from Ulé laguna in filtered, sterile, enriched lake water[1]

Conc. ppm	Initial	2 days	4 days	10 days	11 days	12 days	13 days
0	100	158	541	313	167	146	104
124	100	802	2256	2382	2382	0[2]	1504
202	100	201	564	1254	1442	1274	1220
404	100	361	1029	779	624	608	810
800	100	100	937	609	562	167	21
1600	100	401	1317	1833	2241	1693	1003
3350	100	361	1355	703	752	1140	601
6640	100	85	606	534	427	712	783

[1] Values expressed as percent of chlorophyll-a determined at initiation of experiment.

[2] Not filterable

Table F.7
Milligrams of carbon fixed per cubic meter per day

Petroleum Concentration	Gross P.	Net P	Resp.
0	3676	1786	-2260
1000 ppm	4966	2519	-2446
2666 ppm	3913	800	-3113
5332 ppm	3328	-934	-4261
10,666 ppm	1601	-2401	-4002
21,332 ppm	371	-4817	-4817

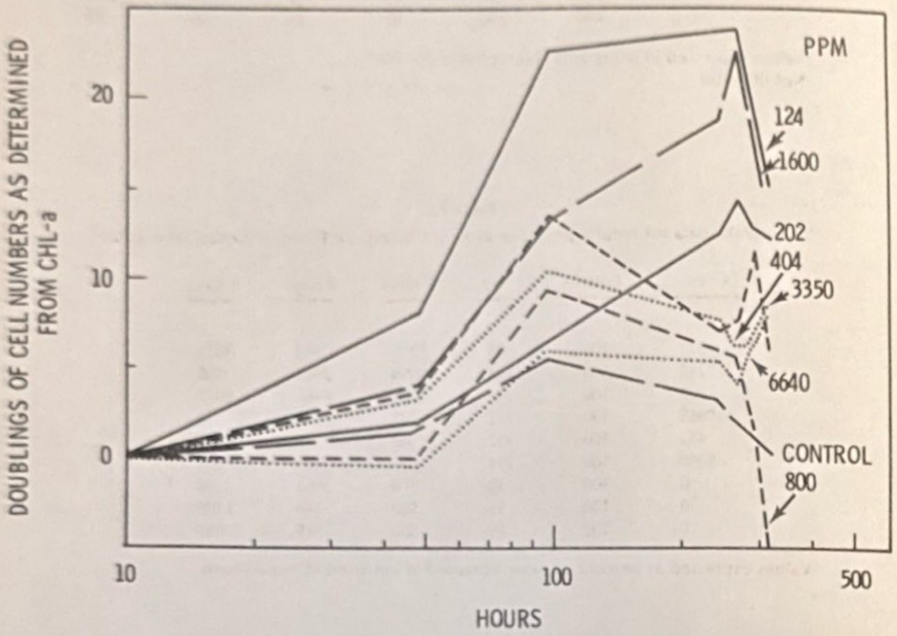

Figure F.1
Growth of Laguna Ulé phytoplankton versus crude petroleum concentration

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APPENDIX G

PRELIMINARY STUDIES OF MICROBIAL DEGRADATION OF OIL

APPENDIX G

PRELIMINARY STUDIES OF MICROBIAL DEGRADATION OF OIL

A number of factors must be considered in a discussion of the microbial degradation of oil occurring in a large relatively well mixed body of water such as Lake Maracaibo. Nutrient concentration no doubt plays a role in the potential of water microbiota to degrade oil. In some bodies of water, phosphate and nitrogen may be limiting; in others, it is conceivable that trace elements may be limiting factors. Complicating evaluation, limiting nutrients may turn over in relatively short periods of time, creating significant changes in the milieu for microbial growth and thus oil degradation. One must also consider the "history" of a given body of water with respect to oil contamination. If a given water source has had frequent spills of oil then it is possible that microbial populations capable of a high degree of oil degradation would be selected for and would eventually reach stable populations at constant concentrations of oil and nutrient; in effect, creating chemostatic conditions. It is unlikely that any selected location in the main body of Lake Maracaibo will have such conditions. Slicks move, sink, and appear to disperse in a noncontinuous fashion. In this same vein, the condition of a given spill may play a role in its microbial degradation. In a still body of water, as the oil weathers and sinks, it will enter into depths of low oxygen concentration where anaerobic and microaerophilic organisms of lesser biological efficiency than surface aerobes will contact the slick. In a large portion of Lake Maracaibo, these conditions are not met, as dissolved oxygen may be quite constant in the water column due to the hydro-dynamic character of the lake, especially in shallow areas subject to wind-induced currents.

One factor with respect to potential microbial degradation of oil that may remain constant as far as Lake Maracaibo is concerned is temperature. The lake's ambient temperature is 30°C, and large water temperature variation is not observed.

All of these considerations limit interpretations of the results of microbiological studies of Lake Maracaibo. Measurements made at a given site provide information regarding the microbial population and the effect of oil on the population at the time of sampling. However, additional measurements would be required if major changes in environmental parameters occurred. With this understood, data will be described which results from onsite studies and a conservative interpretation of the studies will be made.

The following measurements were made:

1. Biochemical oxygen demand in the presence and absence of oil, and microbial population density at six selected sites in Lake Maracaibo.
2. Biochemical oxygen demand in the presence and absence of oil, and microbial population density at different levels of a water column from a site with a history of oil contamination.
3. The effects of the addition of the nutrients phosphate and nitrogen on biochemical oxygen demand in the presence and absence of oil.

In addition to these measurements, two other techniques were developed and utilized at the lake site. These are:

1. A method for the preservation of Lake Maracaibo microbial flora so that a minimal loss of viability would occur during shipment of these samples to Richland, Washington.
2. Isolation of oil oxidizing microorganisms from lake water and oil-bearing waters around the lake.

Methods

Biochemical Oxygen Demand (BOD) Determinations. BOD was measured as a function of incubation time in the presence and absence of oil for sets of water samples from six sites in the lake. The sites were chosen on the following basis: 1) Stations 12 and 13 are in the areas of the lake exposed to oil contamination; 2) Stations 14 and 7 are in areas of the lake exposed both to oil contamination and municipal waste contamination; 3) Stations 8 and 9 are in areas of the lake relatively free from both oil and municipal contamination.

Standard techniques were used to obtain BOD values for both control and oil supplemented lake water samples. BOD in the absence of oil was determined using 300 ml BOD bottles filled to capacity with lake water.

BOD in the presence of oil was determined as follows. Standard BOD bottles were fabricated with a tapered glass tube at the base so that a tygon tubing connection could be made to one liter stoppered jars. One liter of lake water containing 100 ppm crude oil was introduced into the liter jars. The jars were then stoppered and their contents stirred vigorously for 0.5 hour in order to mix and expose the oil to the biomass present in the jar. The water-oil mixture was allowed to separate for 5 minutes and 300 ml of the water was carefully transferred through the tygon tubing to the attached BOD bottle with an oxygen electrode as its stopper. After oxygen concentration was measured, the water was transferred back to the one liter jars until the next reading. For each reading, the rapid stirring, oil-water separation, and transfers were repeated. Readings were taken at daily intervals for 6 to 7 days.

Limitations of Lake Water Nutrients. To determine if Lake Maracaibo water is limiting in either phosphorus and/or nitrogen as nutrients for growth of heterotrophic organisms, similar BOD experiments were conducted using a defined mineral medium (Aaronson, 1970) and lake water supplemented with trace elements (Rodina, 1972) which furnished all the nutrients required for growth of microorganisms except the carbon source. Mineral medium lacking in phosphorus or nitrogen was prepared and different concentrations of the appropriate element added to the medium to determine optimal levels for BOD activity in the presence and absence of 100 ppm crude oil. In these experiments, the mineral medium was prepared at double strength and equal volumes of lake water added to dilute the media to single strength and to introduce the heterotrophic microbial population and nutrients present in the lake water.

Microbial Counts. The total heterotrophic population of aerobic bacteria and fungi at the selected Lake Maracaibo sites was determined by "pour-plating" viable organisms in nutrient medium. Bacto brain heart infusion agar (52g/liter) at pH 7.0 was used for the total aerobic bacterial counts and BBL Littman agar (51g/liter) with 30 µg/ml streptomycin at pH 7.0 was used for the determination of fungi population. The experiments were made with the same lake water samples used for the BOD experiments, overlaid with the appropriate media, incubated for 3 days at 29-30°C and counted. This technique underestimates the total microbial population since it is known that many species of microorganisms will not grow in a media rich with nutrients. However, with the limitation of time, it was considered the most appropriate method to use.

Preservation of Microbiological Samples. The 13th Edition of Standard Methods for the Examination of Water and Wastewater recommends that bacteriological examination of water samples be started promptly after collection to avoid unpredictable changes, with change time limited to 30 hours at temperatures below 10°C. Since these requirements obviously could not be met for water samples shipped from the Lake Maracaibo site to the Richland laboratory, it was considered essential to develop a method that would allow preservation and shipment of the microbiota in the lakewater samples.

A simple method to accomplish this goal was developed. Sterile millipore pre-filters were used to filter 300 ml volumes of lake water and the filtrate was then passed through 0.45 µ millipore filters. On the first day, one set of pre-filters and millipore filters prepared in this manner was reconstituted with 300 ml of millipore sterilized lake water, vigorously mixed with a magnetic stirrer for 0.5 hour to resuspend the microorganisms and the microbial population was determined. Lake water from a given site and set of processed filters from the same site were placed in sterile BOD bottles, sealed with parafilm and refrigerated at 4°C. The processed filters were reconstituted with 300 ml of millipore sterilized lake water after refrigeration for 5 days, pour-plated and counted to determine the percent survival of the microorganisms. The lake water, control refrigerated for 5 days, was also pour-plated and counted at the same time to determine the difference of microbial density in the untreated lake water.

Isolation of Oil Oxidizing Microorganisms. The nitrogen requirement of some microbial species can be met by addition of either ammonium or nitrate to a synthetic medium. Isolation of oil oxidizing organisms was attempted on three different media which varied in nitrogen source and mineral composition. All three were lacking in a source of carbon.

An agar mineral medium containing ammonium supplemented with .05% nitrate, Tauson medium with nitrate and Tauson medium with ammonium were surface streaked with 0.05 ml of lake water or with oily water samples from the Ulé Tank Farm (see Rodina, 1972). The plates were inverted and 0.5 ml crude oil (as carbon source) was added to the cover of the petri dishes. The plates were incubated aerobically and anaerobically at 29-30°C for one month before examination. Since the crude oil is not in direct contact with the inoculated plates, isolates that grow on the plates will be those that can utilize only the volatile fraction of the hydrocarbon as a source of carbon.

Results and Discussion

Assessment of Biochemical Oxygen Demand and Total Microbial Populations at Selected Lake Maracaibo Sites. Biochemical oxygen demand is a relatively indirect measure of two water parameters. These parameters are:

1) Dissolved biodegradable organic matter in the water sample. As oxygen utilization by water microbiota is necessary for metabolism of these compounds, BOD measurements in a comparative sense allow one to compare the degradability of low concentrations of nutrients added to a water sample.

2) Metabolic activity of water microbiota. In a water sample containing no added nutrients, the observed BOD is contingent upon the activity of the microorganisms and the types and amounts of metabolizable materials in the sample.

These criteria are obviously related. If a measure of biodegradable material is desired, water samples can be seeded with known mixed cultures or pure cultures of microbiota. If a measure of microbial activity is necessary, the water sample can be amended with known carbon sources. Addition of either microbiota or nutrients, however, will affect the resident microbial activity and numbers and thus will give little or no information about the constitution of a water sample in terms of nutrient availability and biological activity. In cases where water sampling is performed on a routine basis, and where a base-line of information is available, amendment of the type described is invaluable. In the case of Lake Maracaibo water, however, measurements were made initially on samples that had not been modified in any way. Oil was added as described (see Methods) and BOD determined in its presence. By comparing BOD in the presence of oil versus that in its absence, it was hoped to obtain an index of the effect of oil on the lake's resident microbiota.

Fresh Lake Maracaibo water collected at one meter depths at the six sampling stations was used to determine the kinetics of BOD and to measure the effect of added oil on BOD (Table G.1). Four out of the six test stations showed little or no difference in BOD in the presence or absence of oil (ratio BOD + oil/ BOD - oil = 1), implying that the oil had little or no effect on the numbers of microbiota or on their biological activity and was not utilized to an appreciable extent in our test system. Station 14 had low microbiological activity, with both control and oil sample having a value of less than one ppm. Station 13, however, had a high level of dissolved oxygen and a relatively high microbial population density and thus was selected as the most promising area for additional BOD studies and for the possible isolation of oil-oxidizing microorganisms. The dissolved oxygen content at Station 14 was only 0.8 ppm, sufficient for growth of micoorganisms, but insufficient to support fish life. Station 7 (located approximately 0.5 kilometers offshore from Station 14), Station 12 (oil contaminated area) and Stations 8 and 9 (areas relatively free from contamination) showed 6 day BOD activity similar to readings recorded at free-flowing sites on the Columbia River, a relatively uncontaminated river, and have dissolved oxygen concentrations more than sufficient to support both microbial and fish life in the lake.

Data on BOD and microbial populations demonstrate no consistent pattern as a function of change in site (Table G.1). Stations 12, 7, 8 and 9

Table G.1

Effects of 100 ppm Venezuelan crude oil on the biochemical oxygen demand of Lake Maracaibo water sampled from 1 meter depths at 6 stations

	Station	Zero Day ppm O_2	6 day BOD, mg/ℓ		$\left(\frac{\text{BOD + oil}}{\text{BOD - oil}}\right)$	Microbial Counts/ml	
			Control	100 ppm Oil		Bacteria	Fungi
Stations closest to oily water effluent	12	8.5	3.5	3.0	0.85	4.8 x 10^3	600
	13	9.8	9.0	3.6	0.40	2 x 10^4	2.45 x 10^3
Stations close to oily water and sewage effluents	14	0.8	0.8	0.4	0.50	5 x 10^5	3.3 x 10^4
	7	8.2	2.4	2.4	1.0	4 x 10^3	<1
Control area of lake	8	8.0	2.0	1.7	.85	1.4 x 10^3	<1
	9	8.0	2.8	2.8	1.0	2.8 x 10^3	2.8 x 10^3

are similar in BOD and total number of aerobes, differing only in fungal population; which appears to vary with times of sampling (see Table G.2). Although these sites differ greatly in geographical location and history of contamination, they cannot be distinguished based upon the measured microbial activity. Station 14, located in an inlet and continuously exposed to oil and municipal sewage is distinct from the other test sites.

The variability that is observed within stations may be more a function of time of sampling than any property of the site itself. For example, counts of total aerobic bacteria and BOD measurements taken at weekly intervals over approximately three weeks at Station 13 vary considerably (Table G.2).

BOD + oil shows an approximate twofold change in the first interval; examined in this interval, number of total aerobes has dropped by 1/2 and fungi by an approximate factor of four. (Note, however, that the sample with lower microbial counts has a higher BOD + oil and a lower BOD - oil). This variability of similar degree at different sites, suggests that analyses at single time intervals for a number of lake sites will be of limited use in describing the lake's ability to remove oil by microbial metabolism over extended time periods.

Biochemical oxygen demand tests without crude oil were run with water samples obtained from varying lake depths at Station 13. The test was designed to determine the depth in the water column with the highest BOD activity and microbial population density. Water samples from the surface, 1, 2, 3, and 4 meter depths showed BOD activity and the concentration of the heterotrophic population to be relatively similar. Peak BOD activity at all depths occurred at 3 days and decreased suggesting a depletion of nutrients in the water samples and gradual cessation of growth. The slightly lower BOD activity at the 4 meter depth, near the floor of the lake, is coupled with lower microbial counts, and the higher surface microbial counts may be associated with better aerobic conditions from wind and wave action in the surface water.

Table G.2

BOD with and without oil and microbial counts at Station 13 at intervals of time (BOD concentrations expressed in ppm)

Sampling Date	6 Day BOD, mg/ℓ		Microbial counts/ml	
	Control	100 ppm oil	Bacteria	Fungi
1/29/73	9.0	3.6	2 x 10^4	2.45 x 10^3
2/06/73	6.3	6.1	1.4 x 10^4	600
2/14/73	6.1	—	2.6 x 10^4	670

The water samples obtained at varying depths from Station 13 indicated that zero day dissolved oxygen content of the water, 6 day BOD's with and without oil, and microbial counts were generally reduced with increasing depth (Table G.3). The highest activity, in all cases, occurred at the 1 meter level. The BOD activity at the 1 meter depth was slightly lower than that found in the previous experiment, and considerably lower at all other depths. These data suggest: 1) there is a considerable exchange of water and heterotrophic microorganisms at the lower depths; 2) BOD activity at Station 13 may vary markedly from day to day; 3) routine BOD determinations and microbial population density measurements must be made at frequent intervals to determine if any definite pattern exists and; 4) the inoculation of 100 ppm oil supplement into the static BOD culture did not enhance microbial activity during the limited test period. It should be noted that the introduction of a crude oil supplement to lake water under the static conditions of a BOD test may be toxic to many of the non oil-oxidizing microbial species present in the test bottles. Furthermore, since the complete or near complete degradation of crude oil by oil oxidizing microorganisms is at best a slow process, time course BOD tests over a 1 week period may give only a suggestion of the natural degradation capabilities in any given area or depth in the lake. Low molecular weight water soluble components may be expected to be degraded most rapidly. These may have considerable ecological significance. The results suggest that future microbial studies in Lake Maracaibo should be correlated with chemical measurements of the degradation of individual crude oil components.

Effects of Inorganic Phosphorus and Nitrogen as Possible Limiting Nutrients in Lake Maracaibo Water. It is known that most microbial populations can attain maximal growth rates at less than 1 ppm dissolved oxygen provided all other required nutrients are present. It therefore appears highly unlikely that oxygen can be a limiting factor in obtaining good microbial growth in the Lake Maracaibo water samples. Past experience indicates, however, that nitrogen and possibly phosphorus have the potential for limiting microbial growth in natural waters. To test this hypothesis, BOD tests were run with water from Station 13 diluted 1:1 with a defined mineral media containing no carbon source and supplemented independently with inorganic phosphorus and inorganic nitrogen (Table G.4). Reducing the resident heterotrophic population by 50% by dilution with mineral media may have initially limited BOD activity in both phosphorus and nitrogen tests. The addition of larger concentrations of phosphorus enhanced BOD activity in the presence and absence of oil, suggesting that phosphorus was a limiting nutrient in lake-water media.

The addition of larger concentrations of nitrogen enhanced BOD activity in the presence and absence of oil, indicating that nitrogen was also a limiting nutrient in the lakewater/mineral medium mixture.

Supplementation of Station 13 water/mineral medium mix with oil and PO_4-P or NH_4-N results in a BOD substantially increased relative to control minus oils (Table G.4). As in these (and in all samples from other stations) the ratio BOD + oil/BOD - oil was one or less in the

Table G.3

Effects of depth and 100 ppm crude oil on the biochemical oxygen demand of Lake Maracaibo water. Station 13 (BOD concentrations expressed as ppm)

Depth	Zero Day ppm O_2	6 Day BOD, mg/ℓ		$\left(\frac{\text{BOD + oil}}{\text{BOD - oil}}\right)$	Microbial Counts/ml	
		Control	100 ppm Oil		Bacteria	Fungi
1 M	9.4	6.3	6.1	.96	1.4 x 10^4	600
2 M	9.3	5.3	3.1	.58	1.3 x 10^4	400
3 M	8.5	2.7	1.1	.40	5.7 x 10^3	300
4 M	8.4	2.4	1.9	.79	5.4 x 10^3	200

Table G.4
Effects of Station 13 water diluted 1:1 with mineral media containing no carbon source and supplemented with PO_4 and NH_4 independently.

Effects of PO_4-P			6 Day BOD, mg/ℓ		
% KH_2PO_4	Molar P	Zero Day ppm O_2	Control	100 ppm Oil	$\left(\frac{\text{BOD + oil}}{\text{BOD - oil}}\right)$
0.05	.003	8.2	1.2	3.3	2.75
0.1	.006	8.2	1.0	1.0	3.6
0.2	.0128	8.2	2.7	2.7	1.0
0.5	.032	8.2	4.2	6.0	1.42
Effects of NH_4-N					
% NH_4CL	Molar N				
.025	.005	8.2	.5	3.0	6.00
.05	.009	8.2	.7	3.6	5.14
.1	.019	8.2	1.0	3.4	3.4
.5	.094	8.2	2.5	7.8	3.12

absence of PO_4-P or NH_4-N, nitrogen or phosphate or both may be needed to promote utilization of crude oil as a carbon and energy source in this test system. In addition, these data suggest that addition of oil plus the two added nutrients to the water samples results in a massive depletion of available oxygen to levels similar to those found in Station 14. This was the only site sampled for microbial studies which exhibited dissolved oxygen levels below 1 ppm.

Preservation and Shipment of Lake Maracaibo Microbiological Samples. One of the problems encountered when water samples are held for more than a day is an alteration in viability of the microbial population, depending upon the nutrient content of the water. A simple filtration technique was found to be effective in preserving microbial populations for at least 5 days. The results (Table G.5) suggest that refrigeration of the processed filters for 5 days at 4°C does not appreciably alter the measured microbial population. Lake water stored at 4°C for 5 days, however, showed a reduction in the total aerobic bacteria and fungi population. If the technique can be demonstrated to preserve microbial populations for up to 10 days, it can be used to process Lake Maracaibo water on site for future study at other locations.

Isolation of Oil-Oxidizing Microorganisms. Possible oil-oxidizing microorganisms were isolated from the Ulé tank farm and from Station 13 in the lake. The inoculum from the tank farm was obtained from three locations: the bottom of the water layer in the oil separation pond; the oil plus sediment mixture from the skimming tank and; the pond discharge water. Station 13 inoculum was obtained from the surface, and from 1, 2, 3, and 4 meter depths. The growth of microorganisms on a defined mineral media with crude oil as the only source of carbon and energy is a slow process. After 30 days incubation, six aerobic oil oxidizers and at least two anaerobic oil oxidizers have been isolated from lake water and the Creole oil-water separation pond. Tentatively, we have identified four of the aerobic microorganisms as *Pseudomonas, Aeromonas, Bacillis* and fungi. The two other aerobic species were not identified. *Pseudomonas* and fungi were isolated from all four depths at Station 13, suggesting that these microbial species and possibly other oil oxidizing microorganisms may be relatively common in this area of Lake Maracaibo.

Table G.5
Effects of filtration and refrigeration on preservation of microbial populations in Lake Maracaibo water samples.

	Total Aerobic Counts/ml After 3 Day Incubation at 29-30°C		
	Zero Day Filters	5 Day Filters (4C)	5 Day Lake Water (4C)
Bacteria	1.45×10^4	1.56×10^4	2.2×10^3
Fungi	2.6×10^3	4.1×10^3	300

Summary

While local areas vary widely on a day to day basis in terms of measured parameters, the lake is remarkably homogeneous at locations differing in history and geology. The recent history of a site with respect to contamination by oil or other contaminants is probably of much greater importance than whether or not a site is located in an area which has a history of more frequent spillage. Thus, no evidence suggestive of geographic site-specific oil decomposing activity has been found. There was no evidence of large changes in microbial population as a function of location.

The studies suggest that oil degradation by lake water can be accelerated by the addition of phosphate or nitrogen. However, this is a result from one station at one time interval and verification is required.