UNITED STATES DEPARTMENT OF THE INTERIOR

GEOLOGICAL SURVEY

A Technique for the Analysis of Xylem Tracheid Cells in Sediments from the 106-Mile Dump Site off New York: An Indicator of Sewage Sludge Contamination

by

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This report is preliminary and has not been reviewed for conformity with U. S. Geological Survey editorial standards and stratigraphic nomenclature.

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ABSTRACT

This report presents details of a modified method for determining the concentration of xylem tracheid cells in marine sediment samples. These cells have been used as an indicator of sewage sludge and the modified technique has been applied to sediments and suspended matter beneath the 106-mile dump site southeast of New York City. Although the technique seems to be less sensitive than that of silver or *Clostridium perfringens*, also indicators of sewage sludge, we find high concentrations of xylem tracheids in samples from sediment traps and measurable concentrations in bottom sediments at two locations near the dump site. Low densities and settling velocity may partially explain the absence or low concentrations of this sludge tracer in surficial sediments affected by sludge accumulation.

I INTRODUCTION

Several indicator techniques have been used to identify sewage-sludge contamination in bottom and suspended sediments from the New York Bight. The indicators include spores of the bacterium *Clostridium perfrigens* (Cabelli and Pederson, 1982; Cabelli and others, 1984), the steroid coprostanol (Hatcher and McGillivary, 1979), polychlorinated biphenyls (West and Hatcher, 1980), heavy metals such as silver (Gross, 1971), and xylem tracheid cells which originate in the vascular tissue of terrestrial plants (Freudenthal and others, 1984). Of these sludge indicators, analyses of xylem tracheid cells is probably the fastest and least expensive with respect to equipment required.

The rapid sewage indicator technique (RSIT) outlined by Freudenthal and others (1984) describes a method of preparing samples to identify xylem tracheid cells using a polarizing microscope. We have included the RSIT report as an appendix to this paper because of its limited availability elsewhere and because of its importance in providing information on the basic procedure. In this report we describe procedures modified to accommodate suspended- and bottom- samples collected in and near the 106-mile dump site on the continental rise in water depths of 2500 - 2700 m (see Figures 1 and 2 for station locations). An objective of this study is to improve the existing methodology for the analysis of xylem tracheid cells and assess the sensitivity of the method in comparison with other indicators at an open-ocean dump site. Samples of bottom sediments were collected by means of box coring in August of 1990 during RV Oceanus Cruise 222. Suspended sediment was collected by means of sediment traps moored within 105 m of the bottom at stations 3 and 4 (moorings 337 and 336, respectively) from September, 1989 until August, 1990. We report the results of the cell counts as numbers of xylem tracheid cells per gram of sample.

II METHOD OF SAMPLE PREPARATION FOR XYLEM TRACHEID ANALYSIS

"Fluff" Samples From Box Cores

These surficial-sediment samples consist of loosely consolidated material re-suspended from the upper 1 to 3 mm of the box core. The water overlying the sediment in one of the compartments or sub-cores of the box core was agitated using a hand-held paddle to suspend the upper sediment layer. This water and sediment mixture was collected into liter-sized bottles, preserved with sodium azide, and refrigerated until analysis.

The procedures for determining xylem tracheid cells included the following steps:

1. The bottles were removed from the refrigerator while being careful not to disturb the sediment which had settled on the bottom.
2. The clear overlying water was syphoned off and saved for future analysis. The volume of the sediment plus water in the bottle was reduced to less than 40 ml.
3. While maintaining a well-mixed suspension in the bottle, 15 - 25 ml of the suspension (depending on the concentration of solids) was poured into a graduated 50 ml beaker and brought up to the 40 ml mark with distilled water.
4. The salt-free dry weight of a 10 ml aliquot of the 40 ml suspension was determined in the following manner:
   a. While mixing the sediment in the beaker into suspension, two 10 ml aliquots were pipetted into two pre-weighed 50 ml centrifuge tubes.
   b. The tubes were filled with distilled water mixing the sediment and water together; The tubes were centrifuged at 2300 G's (relative centrifugal force) for 1 minute.
   c. Most of the overlying water was pipetted out of the tubes, being careful not to disturb the sediment at the bottom.
   d. Steps b. and c. were repeated two more times to wash away the salt and sodium azide.
   e. The sediment in the tubes was dried overnight in a 60°C oven.
   f. The weight of the dry sediment in each tube was determined by weighing the sediment in the tubes and then subtracting the weight of the tubes.
   g. The salt-free dry weight of a 10 ml aliquot of the water-sediment mixture was calculated by averaging the weights of sediment determined in Step 4f.

An alternative to the centrifuging technique above is to wash the 10 ml aliquots onto pre-weighed filters. The sediment can be flushed with enough distilled water to wash away the salt. The weight of the dry sediment is determined by weighing the dry sediment on the filter and subtracting the weight of the filter.

5. An aliquot for xylem tracheid analysis was prepared in the following manner:

   a. While mixing the sediment in the beaker into suspension (during Step 4a. above), a 10 ml aliquot was pipetted into a 10 ml graduated cylinder.
   b. The RSIT sample preparation procedure (Freudenthal and others, 1984) was followed beginning at Step 4.

   The RSIT preparation procedure was followed through to the end. However, the number of xylem tracheid cells found on three microscope slides were totaled together, not averaged. The calculations were modified to accommodate this change so that the number of xylem tracheid cells per aliquot could be calculated. The RSIT procedure outlined by Fruedenthal and others (1984) reports xylem tracheid data per volume (ml) of wet sediment. We have modified the procedure to determine the weight of separate aliquots (known volumes) of sample mixture to calculate and report our data as xylem tracheid cells per gram of salt-free dry sediment.

Sediment Samples From Box Cores

Sub-cores from each of the box cores were returned to our laboratory facility and stored in the freezer. Sub-cores from Box Cores 3, 5, 10, and 14 were removed from the freezer and partially thawed. The top 1 cm of sediment from each of the sub-cores was removed, placed into 125 ml, screw-capped, plastic containers and reserved for xylem tracheid analysis. The top 1 cm of sediment included the 1 to 3 mm surficial ("fluff") layer mentioned in the analytical procedure previously described.

1. The sediment samples were analyzed for xylem tracheid cell concentrations as follows:

   a. The wet sediment was thoroughly mixed in the container, and a 2 ml split of the sample was placed into a pre-weighed 10 ml graduated cylinder using a 3 ml plastic syringe. (The end of the syringe was cut off so that it could be used as a sediment sampler.)
   b. The cylinder containing the wet sediment was weighed on a top-loading balance, and the weight of the wet sediment was determined by subtracting the weight of the cylinder.
   c. The RSIT sample preparation procedure (Freudenthal and others, 1984) was followed beginning in step 4.

   As mentioned in the preparation of the re-suspended sediment samples above, the number of xylem tracheid cells found on three slides were totaled together, and the data was reported as xylem tracheid cells per gram of salt-free dry sediment. The salt-free dry weight of the sample used for xylem tracheid

counting was calculated by multiplying the wet (salt-in) sediment weight by a measured dry-weight (salt-free) to wet-weight (salt-in) ratio. This ratio was determined from separate splits of the same sample and is detailed in Step 2 below.

2. The dry-weight (salt-free) to wet-weight (with salt) ratio of a known weight of wet sediment was determined in the following manner:

   a. The sediment and water was thoroughly mixed in the container, and two 2 ml splits of the same sediment sample were placed into two pre-weighed 50 ml centrifuge tubes using a 3 ml plastic syringe.
   b. The tubes containing the wet sediment were weighed on a top-loading balance, and the weight of the wet sediment was determined by subtracting the weight of the tubes.
   c. The tubes were filled with distilled water, mixing the sediment and water together, and then the tubes were centrifuged at 2300 G's (relative centrifugal force) for 1 minute.
   d. Most of the overlying water was pipetted out of the tubes, being careful not to disturb the sediment at the bottom.
   e. Steps c. and d. were repeated two more times to remove the salt.
   f. The sediment in the tubes was dried overnight in a 60°C oven.
   g. The weight of the dry sediment in each tube was determined by weighing the sediment in the tubes and then subtracting the weight of the tubes.
   h. The salt-free dry weight (salt-free) to wet-weight ratio for each of the sediment splits was calculated and then averaged together.

Suspended Sediment Collected in "Tube" Traps

Some of the sediment traps used to collect suspended materials consisted of plastic cylinders 6.6 cm I.D. x 50 cm long and are known as "tube" traps. On recovery, most of the overlying water was removed from the trap, and the sediment was re-suspended in the remaining water and poured into a liter-sized bottle. A preservative consisting of sodium azide and sodium chloride was added to the sediment trap during deployment using a solid dispenser (Bothner and others, 1988). The bottles were returned to our laboratory facility and stored in a refrigerator to allow the sediment to settle. After settling, additional overlying water was removed and the sediment was re-suspended in the remaining water. The suspension was then divided into four equal-sized splits using a rotary splitter and placed into 125 ml, screw-capped, plastic containers. A split from one of the containers was washed free of salt, dried and weighed. Another split was set aside for xylem tracheid analysis.

The samples reserved for xylem tracheid analysis were processed in the following manner:

1. The containers were removed from the refrigerator while being careful not to disturb the sediment settled on the bottom.
2. The clear overlying water was syphoned off and saved for future analysis.
3. The sediment was thoroughly mixed with the remaining water and the mixture was poured into a 50 ml centrifuge tube.
4. The tubes were filled with distilled water mixing the sediment and water together and the tubes were centrifuged at 2300 G's (relative centrifugal force) for 1 minute.
5. Most of the overlying water was pipetted out of the tubes, being careful not to disturb the sediment at the bottom.
6. The sediment was washed out of the centrifuge tube into a 10 ml graduated cylinder and the volume was brought up to 10 ml with distilled water.
7. The RSIT sample preparation procedure (Freudenthal and others, 1984) was followed beginning at Step 4.

As mentioned in the preparation of the "fluff" samples, the number of xylem tracheid cells found on three slides were totaled together, and the data was reported as xylem tracheid cells per gram of salt-free dry sediment. This was determined from the xylem tracheid counts measured in one split and the salt-free dry weight measured on another equal-sized split.

Anderson Trap Samples

Anderson traps consist of funnels .5 m wide at the mouth tapering to a 3 cm I.D. plastic tube that is 75 cm in length (Bothner and others, 1988). During the 9 month deployment, a column of sediment up to 32 cm long was collected. Sodium azide and sodium chloride was added to the sediment trap by means of a solid dispenser (Bothner and others, 1988). In the laboratory, the refrigerated sediment was carefully extruded from the tube and sub-sampled at selected intervals along the tube. Each sub-sample was homogenized and divided into a number of splits which were placed into 125 ml screw-capped, plastic containers, and refrigerated. A split of each sub-sample was reserved for xylem tracheid analysis.

The sub-samples were processed for xylem tracheid analysis in the following manner:

1. The sediment was washed off the walls of the container with distilled water to bring the volume of the sediment-water mixture up to 40 ml.
2. The method used to determine the number of xylem tracheid cells per gram of salt-free dry sediment is the same as that described in steps 4 and 5 of the procedure for the "fluff" samples.

III RESULTS AND DISCUSSION

The results of the xylem tracheid analysis for samples collected on RV Oceanus cruise 222 from 11 stations in and near the site 106-mile site are listed in Table 1. Two control samples (table 1) were collected from locations on the Continental Slope up-current from the dump site and contained no xylem tracheid cells. The highest concentration of xylem tracheid cells were found in sediment traps at Station 4 and Station 3 on the western edge of the dump site. Among the re-suspended or "fluff" samples from box cores, xylem tracheid cells were found only at Stations 3 and 11 (13 and 27 xylem tracheid cells per gram, respectfully). As a check on the reliability of our method, additional preparations of fluff samples from Stations 3, 5, 7, 10, and 11 were analyzed, because elevated silver had been measured at all of these stations (Bothner, unpublished data). The re-analyses confirmed that xylem tracheid cells were found only at Stations 3 and 11. No xylem tracheid cells were found in samples representing the 0 - 1 cm depth interval in any of the box cores analyzed.

Because these stations had elevated concentrations *Clostridium perfringens* spores (Hill and others, 1993) and silver (Bothner and others, in press; Bothner, unpublished data), we are convinced that sewage sludge is present and would expect all stations to have measurable numbers of xylem tracheid cells. We were only able to detect significant numbers of xylem tracheid cells in samples that were collected from the water column by tube or Anderson traps that were attached to moorings. One explanation may be that the density of a xylem tracheid cell is very similar to that of water and it may not readily settle out of the water column or remain on the water-sediment interface when even weak bottom currents are present. However, the sediment traps in the water column provide a protected environment in which the xylem tracheid cells can accumulate. Cabelli and others (1984) have suggested that the reason *Clostridium perfringens* spores settle close to the area of sewage dumping or discharge may be because they are fecal in origin and are found in the larger sewage sludge particles. Most of the xylem tracheid cells found in sewage sludge dumped at site 106 may come from paper products such as toilet tissue, facial tissue, and hand towels and not from digested vegetable residue in fecal matter (Hunt and others, 1992). This would explain why *Clostridium perfringens* spores may be present in the bottom sediments, but not xylem tracheid cells. Silver, like *Clostridium perfringens,* was also measurable over a wide area to the southwest of the dump site. Silver may be associated with larger and heavier sludge particles than the xylem tracheid cells.

IV CONCLUSIONS

The method used to identify xylem tracheid cells, as described in Freudenthal and others (1984) and in this report, is very effective in identifying cells present in suspended sediment collected by means of sediment traps at the 106-mile site. We were not able

to identify xylem tracheid cells in the bottom sediments, except for the minor concentrations found in samples from Stations 3 and 11. Repeat analyses performed on the same bottom samples were consistent with the original data. However, the presence of *Clostridium perfringens* spores and elevated silver concentrations in some of the same samples indicate that the bottom sediments are contaminated with sewage sludge. Therefore, we conclude that the method for xylem tracheid analysis is not as sensitive as other more expensive and time consuming methods commonly used. If xylem tracheid cells are present in the sediments at the dump site, they are present at levels that are below the limit of detection of the method. These low levels may be because the density of the xylem tracheid cell is close to that of water, and the cells do not settle to the bottom very rapidly or, if currents are present, may not settle at all. One way to increase the sensitivity of the method is to process a larger amount of sample at the expense of increasing the processing time.

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[1]Because of the limited availability of this paper, it has been included as an appendix to this report..

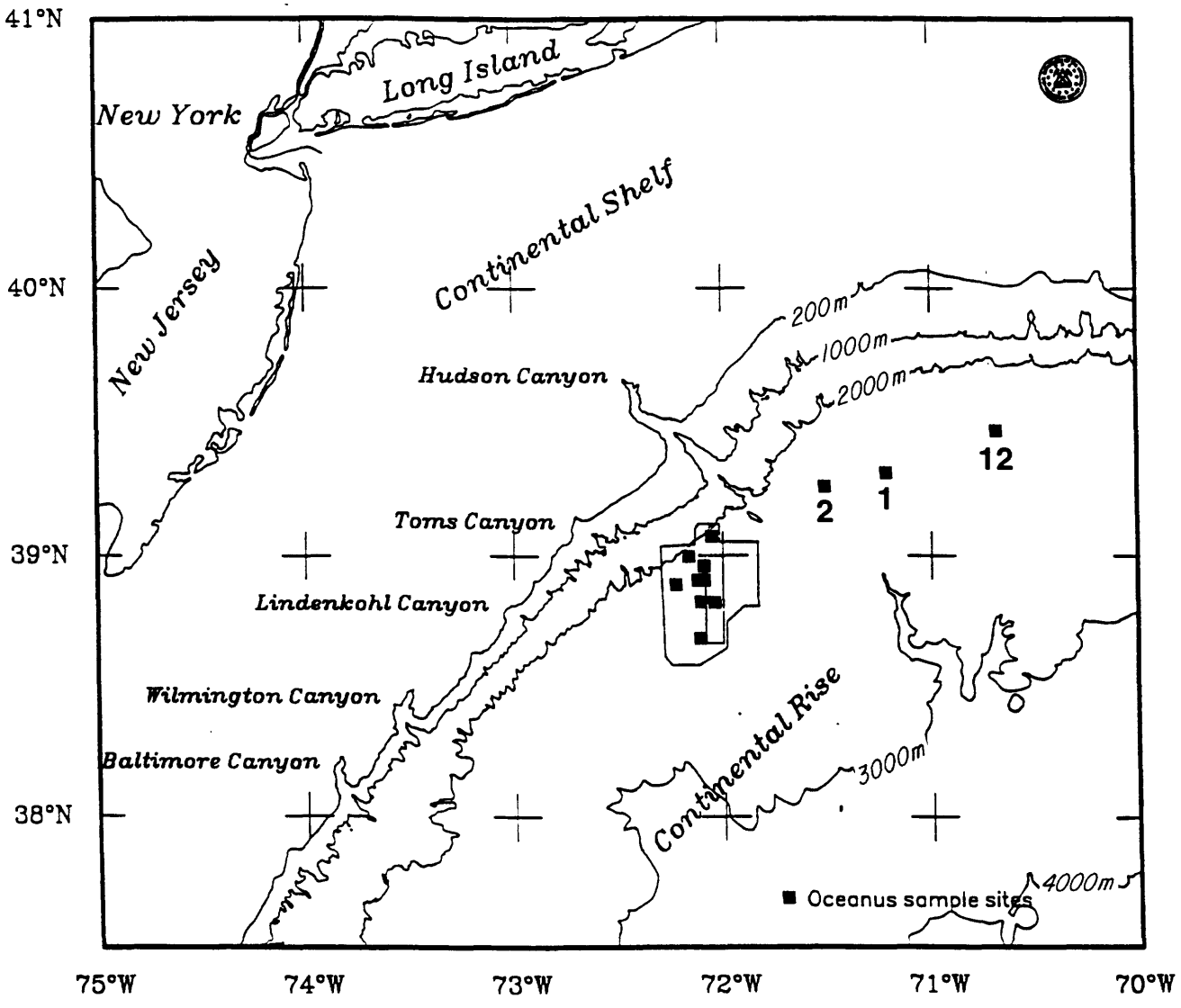

Figure 1. Index map of continental rise area showing the location of 106-mile dump site off New York (irregularly shaped box). Squares denote station locations where samples were collected during RV Oceanus cruise 222. See Figure 2 for detail on sample locations in the dump site.

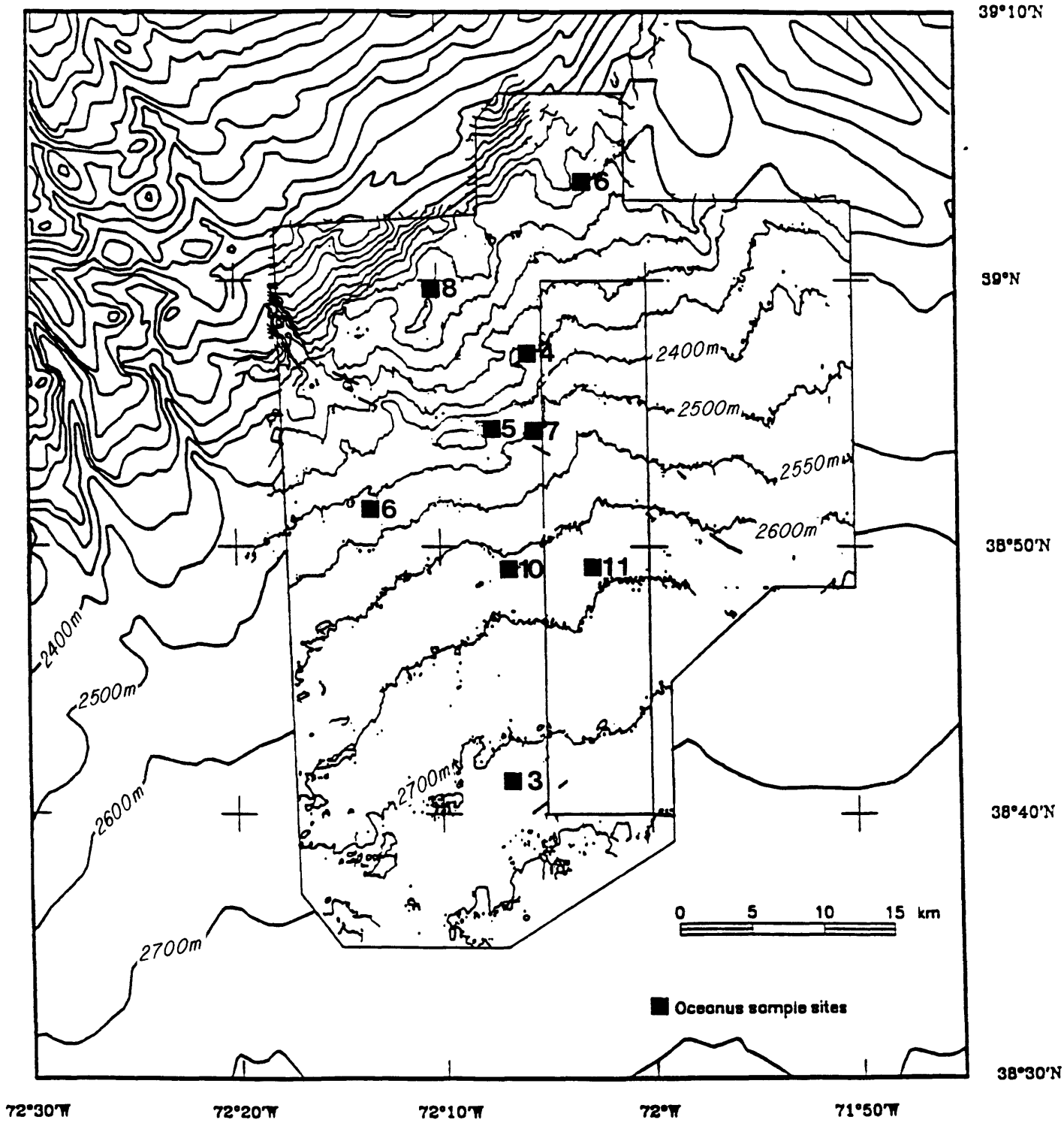

Figure 2. Station locations of samples collected in 106-mile dump site during RV Oceanus cruise 222. Seabeam contours (50-m interval) are shown within the study area; older NOAA contours (100-m interval) are shown around the study area. Sediment traps were deployed at Stations 3 and 4.

Table 1: Xylem Tracheid Data, Oceanus Cruise 222 - Modified RSIT Method

SAMPLE NO.	STATION NO.	LOCATION LAT	LONG	WATER DEPTH (METERS)	XYLEM TRACHEIDS PER GM DRY SED. (SALT-FREE)	COMMENTS
1105	4	38°57.30'N	72°05.62'W	2409	283	Sample from tube trap, Mooring 336. 5 meters above bottom.
1106	4	38°57.30'N	72°05.62'W	2409	138	Sample from tube trap, Mooring 336. 12 meters above bottom.
1107	4	38°57.30'N	72°05.62'W	2409	231	Sample from tube trap, Mooring 336. 75 meters above bottom.
1108	4	38°57.30'N	72°05.62'W	2409	0	Sample from tube trap, Mooring 336. 105 meters above bottom.
1109	3	38°41.50'N	72°06.19'W	2719	339	Sample from tube trap, Mooring 337. 5 meters above bottom.
1110	3	38°41.50'N	72°06.19'W	2719	197	Sample from tube trap, Mooring 337. 12 meters above bottom.
1111	3	38°41.50'N	72°06.19'W	2719	1111	Sample from tube trap, Mooring 337. 75 meters above bottom.
T1103 0-3.5	4	38°57.30'N	72°05.62'W	2409	561	Sample from Anderson trap, 0 - 3.5 cm, Mooring 336. 104 meters above bottom.
T1103 3.5-7.0	4	38°57.30'N	72°05.62'W	2409	354	Sample from Anderson trap, 3.5 - 7.0 cm, Mooring 336. 104 meters above bottom.
T1103 7.0-10.0	4	38°57.30'N	72°05.62'W	2409	0	Sample from Anderson trap, 7.0 - 10.0 cm, Mooring 336. 104 meters above bottom.
T1103 10.5-14.0	4	38°57.30'N	72°05.62'W	2409	111	Sample from Anderson trap, 10.5 - 14.0 cm, Mooring 336. 104 meters above bottom.
T1104 0.0-2.0	3	38°41.50'N	72°06.19'W	2719	820	Sample from Anderson trap, 0.0 - 2.0 cm, Mooring 337. 103 meters above bottom.
T1104 2.0-4.0	3	38°41.50'N	72°06.19'W	2719	933	Sample from Anderson trap, 2.0 - 4.0 cm, Mooring 337. 103 meters above bottom.
T1104 4.0-7.0	3	38°41.50'N	72°06.19'W	2719	944	Sample from Anderson trap, 4.0 - 7.0 cm, Mooring 337. 103 meters above bottom.
BC1-SC8E	1	39°18.83'N	71°12.38'W	2560	0	**CONTROL SAMPLE** Fluff sample from Boxcore 1
BC2-SC14	2	39°15.16'N	71°30.07'W	2548	0	**CONTROL SAMPLE** Fluff sample from Boxcore 2
BC3-SC7E	3	38°41.17'N	72°06.78'W	2712	13	Fluff sample from Boxcore 3
BC3	3	38°41.17'N	72°06.78'W	2712	0	Top layer of sediment (0 - 1cm interval) from Boxcore 3
BC4-SC8	4	38°57.30'N	72°05.62'W	2410	0	Fluff sample from Boxcore 4
BC5-SC4	5	38°54.56'N	72°07.39'W	2495	0	Note on Bottle: Part of sample lost during spindown prep. Therefore not processed. Fluff sample from Boxcore 5
BC5	5	38°54.56'N	72°07.39'W	2495	0	Top layer of sediment (0 - 1cm interval) from Boxcore 5
BC6-SC15	5	38°54.58'N	72°07.39'W	2495	0	Fluff sample from Boxcore 6
BC7-SC20	5	38°54.52'N	72°07.38'W	2495	0	Fluff sample from Boxcore 7
BC8-SC2	7	38°54.43'N	72°05.40'W	2492	0	Fluff sample from Boxcore 8
BC9-SC3	7	38°54.44'N	72°05.38'W	2492	0	Fluff sample from Boxcore 9
BC10-SC15	7	38°54.45'N	72°05.43'W	2492	0	Fluff sample from Boxcore 10
BC10	7	38°54.45'N	72°05.43'W	2492	0	Top layer of sediment (0 - 1cm interval) from Boxcore 10
BC11-SC9	8	38°59.71'N	72°10.20'W	2207	0	Fluff sample from Boxcore 11

Table 1(Continued): Xylem Tracheid Data, Oceanus Cruise 222 - Modified RSIT Method

SAMPLE NO.	STATION NO.	LOCATION LAT	LONG	WATER DEPTH (METERS)	XYLEM TRACHEIDS PER GM DRY SED. (SALT-FREE)	COMMENTS
BC12-SC9	9	39°03.94'N	72°02.95'W	2178	0	Fluff sample from Boxcore 12
BC13-SC4	10	38°49.16'N	72°06.70'W	2642	0	Fluff sample from Boxcore 13
BC14-SC7	11	38°49.29'N	72°02.77'W	2625	27	Fluff sample from Boxcore 14
BC14	11	38°49.29'N	72°02.77'W	2625	0	Top layer of sediment (0 - 1cm interval) from Boxcore 14
BC15-SC8	12	39°27.52'N	70°40.12'W	2498	0	**CONTROL SAMPLE** Fluff sample from Boxcore 15
848		39°50.55'N	70°01.38'W	1220	0	**CONTROL SAMPLE** Sample from sediment trap. 25 meters above bottom.
853		39°50.55'N	70°01.38'W	1220	0	**CONTROL SAMPLE** Sample from sediment trap. 10 meters above bottom.

Appendix

NASSAU COUNTY
NEW YORK

PILOT PROJECT

RAPID SEWAGE

INDICATOR TECHNIQUE

NASSAU COUNTY DEPARTMENT OF HEALTH
240 Old Country Road
Mineola, New York 11501

Francis T. Purcell
County Executive

John J. Dowling, M.D., M.P.H.
Commissioner

FRANCIS T. PURCELL
COUNTY EXECUTIVE

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COMMISSIONER

NASSAU COUNTY
DEPARTMENT OF HEALTH
240 OLD COUNTRY ROAD, MINEOLA, N.Y. 11501

January 30, 1985

Mr. Frank Steimle
Supervisory Fishery Biologist
United States Department of Commerce
NOAA - National Fisheries Service
Northeast Fisheries Center
Sandy Hook Laboratory
Highlands, New Jersey 07732

Dear Mr. Steimle:

We are herewith transmitting ten copies of the Rapid Sewage Indicator Technique Pilot Project Report, in fulfillment of the requirements of NOAA Contract No. NA-83-FA-C-00038.

This report details the validation of the Rapid Sewage Indicator Technique (RSIT) as a reliable indicator when used as a gross screening technique. While the full potential of the RSIT has not yet been determined, due to the limited scope of this pilot project, the study results indicate that the RSIT is useful qualitatively, and should be further tested to determine its potential for quantitative use.

This grant has enabled us to refine a simple, easily taught, microscopic technique for both on-site monitoring of emergency situations, and to direct routine monitoring efforts by defining the leading edge and distribution of sewage sludge. The development of a rapid and reliable identification procedure is an important development in the study of offshore disposal of sewage sludge.

We greatly appreciate your guidance in this effort and the support of the National Marine Fisheries Service of NOAA, United States Department of Commerce.

Sincerely,

John J. Dowling, MD

John J. Dowling, M.D., M.P.H.
Commissioner

JJD:ARF:ms

NASSAU COUNTY
NEW YORK

PILOT PROJECT

RAPID SEWAGE

INDICATOR TECHNIQUE

NASSAU COUNTY DEPARTMENT OF HEALTH
240 Old Country Road
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RAPID SEWAGE INDICATOR TECHNIQUE
PILOT PROJECT
NASSAU COUNTY, NEW YORK

Prepared by
NASSAU COUNTY DEPARTMENT OF HEALTH
July 1984

Anita R. Freudenthal, Ph.D.
Project Director

Scientific Staff
John Jacobs, M.S.
Alma Hyman, M.S.

NOAA Contract No. NA-83-FA-C-00038

FOREWORD

This study was conducted by Anita R. Freudenthal, Ph.D., Chief, Marine Ecology Section, with funds provided by NOAA Contract No. NA-83-FA-C-00038. The Marine Ecology Section is under the supervision of Theodore B. Burger, Ph.D., P.E., Director, Bureau of Water Pollution Control.

Fifty-five samples were provided by Frank Steimle of the National Oceanic and Atmospheric Administration-Northeast Fisheries Center, Sandy Hook Laboratory. The remaining samples were collected by Nassau County Department of Health. Chemical and bacteriological analyses of these samples were performed by the Division of Laboratories and Research, Nassau County Department of Health.

This report is part of the Department's continuing programs in environmental protection and control which are administered within the Division of Environmental Health under the direction of Francis V. Padar, P.E., M.C.E., Deputy Commissioner.

This report should be cited as follows:

> Freudenthal, A. R., Jacobs, J., Hyman, A., 1984. A Rapid Sewage Indicator Technique: Use of xylem tracheids as an indicator of sewage contamination in the marine environment. Pilot project prepared by Nassau County Department of Health for the National Oceanic and Atmospheric Administration.

Special acknowledgement is due to Frank Steimle for his guidance and assistance during this study.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

EXECUTIVE SUMMARY OF THE RAPID SEWAGE INDICATOR TECHNIQUE (RSIT) PILOT PROJECT

1. The purpose of this study is to validate the use of the microscopic presence of xylem tracheids as an indicator of sewage/sludge contamination in the marine environment.

2. The use of this indicator is based on the premise that the fiber content of terrestrial vascular plants is not digested by the human body. The microscopically identifiable components of this undigested roughage are not normally found in the marine environment and are, therefore, an indicator of sewage contamination.

3. In order to validate the technique, 96 water column and sediment samples from the following study sites were collected by the Marine Ecology staff or provided by the National Marine Fisheries Service:

   - Uncontaminated and contaminated estuarine and nearshore ocean
   - Uncontaminated and contaminated ocean
   - Sewage sludge dump site - New York Bight Apex
   - Raw and processed sewage from sewage treatment plants

4. These samples were analyzed using a rapid sample-to-slide technique developed for the RSIT. The entire quantitative procedure from sample to slide, including analysis, takes 45 minutes to one hour and can be easily taught to non-technical staff. For rapid, gross screening purposes for sewage contamination, a determination can be made within five minutes.

5. Preliminary results demonstrate that the RSIT is a valid indicator for determining, qualitatively, categories of sewage/sludge contamination from highly impacted to slightly contaminated. Quantitative examination of the 96 samples reveals a non-random pattern which correlates descriptively with existing dumpsite data.

6. The study demonstrated that the technique is effective for fresh, frozen and preserved samples. The length of preservation (with formaldehyde) was tested for both short-term (months) and long-term (up to six years).

7. The full potential of the RSIT has not yet been determined because of the limited scope of this pilot project. The results of the study indicate, however, that the Rapid Sewage Indicator Technique should be further tested to determine its potential for quantitative application.

I. INTRODUCTION

Nassau County is the western territory of Long Island, just to the east of New York City. The southern beach barrier ocean shoreline forms one boundary of the New York Bight where disposal of sewage sludge has occurred 12 miles offshore for 60 years since 1924. This is the nations' largest ocean dumping operation, receiving 7.2 million tons of sewage sludge in 1980 alone from 18 million people. During the last decade there has been heightened interest and awareness in the fate, movement and environmental impact of this material.

The effects of contaminant inputs into marine environments have been clearly documented and include: "buildup of organic carbon and metals in sediments; altered benthic (bottom) communities consisting of a few pollution-tolerant species; increased incidence of finrot compared to less impacted areas; closure of shellfishing grounds due to high bacterial counts; and development of tolerance to high concentrations of trace metals and antibiotics in some bacteria" (Reid, O'Reilly, Zdanowicz, Eds., 1982). However, the complexity of contaminant sources, including sewage sludge, dredge spoils, estuarine runoff, vessel discharges, local outfalls, and atmospheric fallout, preclude effective management of marine resources. Not enough

is known about the fates and effects of various contaminants to enable a clear definition of cause and effect for each contaminant source.

Sophisticated monitoring techniques have been developed in an attempt to determine the effects of using the marine environment as a respository of sewage related material. Many of these techniques, while accurately assessing the effects and paths of the raw sewage or sewage sludge, are often time consuming and demand expensive equipment operated by highly trained technicians. A need exists, therefore, during monitoring, and especially in times of sewage related emergency, for an indicator that can be used rapidly and on-site to determine if, and to what degree, areas of the marine environment are contaminated.

A microscopic technique has been developed using as an indicator the appearance in samples of easily identifiable traces of the vascular tissues of terrestrial plants, specifically the secondary walls of xylem tracheids. The indicator does not naturally exist in the marine environment; its primary source is sewage related material.

This technique has been used by the Nassau County Marine Biologist during sewage-related crises situations: to direct clean-up efforts after sludge pipe breaks and a sludge storage tank explosion; to determine which beaches should be closed after two New York City black-outs sent

untreated raw sewage into Nassau County waters; and for the past ten years to determine if numerous complaints of sewage/sludge on beaches or in the marine environment were valid.

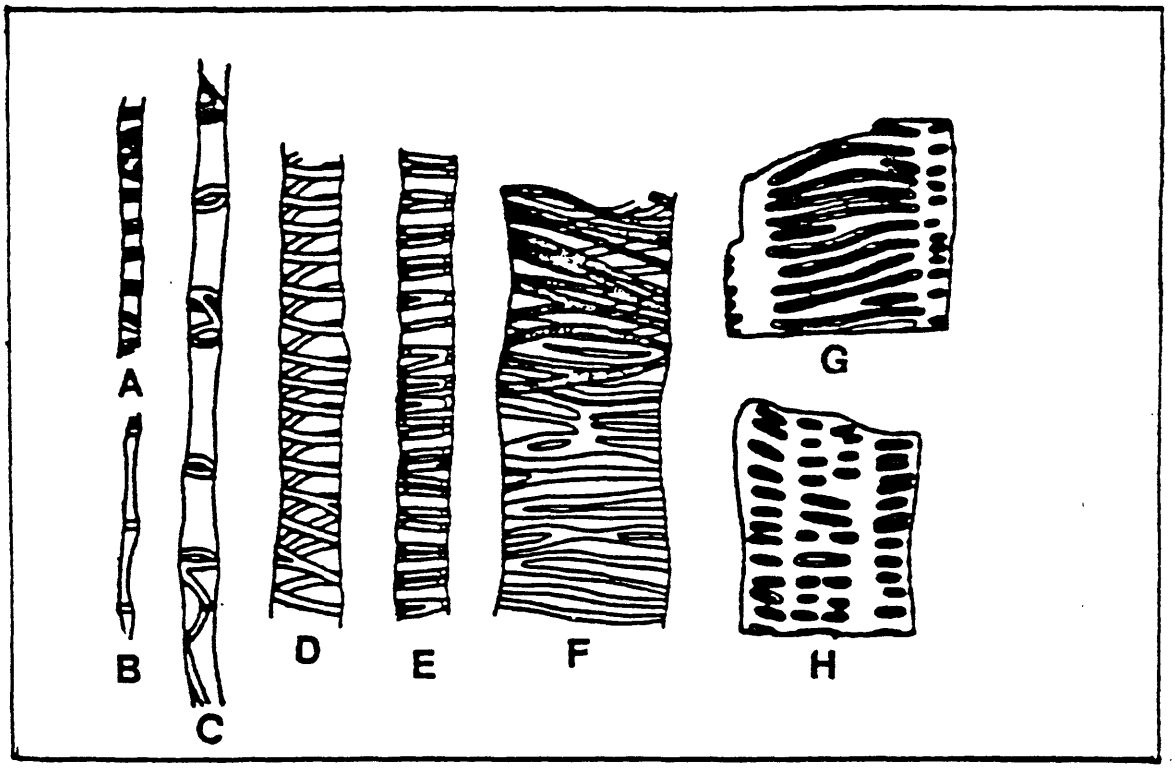
In cases, such as those cited above, the inherent delay in traditional testing was unacceptable. Decisions had to be reached immediately in order to protect the health of the public and to avoid economic losses which would be caused by undirected clean-up efforts or unnecessary beach closures. In each case, the accuracy of rapidly reached decisions based on the microscopic indicator was later confirmed by correlation with traditional tests.

The same Rapid Sewage Indicator Technique (RSIT) is useful not only in emergency situations, but also as a means to direct routine monitoring efforts by defining the leading edge of the sludge. Contamination could be detected within minutes of sample collection and sampling grids could be adjusted on-site and stations added or deleted, depending on the actual, known, path of the sewage related material. In addition, immediate knowledge of the gross degree of contamination of stations might alter sampling strategy, especially in the vicinity of ocean dumpsites.

II. STUDY CONCEPTS

The purpose of this study is to validate and describe the use of xylem tracheids as indicators of sewage/sludge presence in the marine environment. This technique is based on the following premises:

1. The vascular system of food plants is composed of xylem, the principal water conducting tissue, and phloem, the food-conducting tissue. The xylem also functions as the mechanical support mechanism of the plant. The cells of the xylem have enduring rigid walls composed primarily of cellulose which is further strengthened by a secondary lignified wall. The lignin is deposited on the inner surface of the cell wall, replacing most of the cellulose. On the basis of the manner in which lignin becomes deposited, the secondary walls may appear as spirals, rings, parallel bars, irregular networks or pits, (Figure 1), (Bold 1977, Esau 1965).
2. The xylem tissue, composed of cellulose, hemicellulose, and lignin is the most important component in dietary fiber, that fraction of food resistant to attack by the mammalian digestive enzymes. Most fruits and vegetables are included in fiber-contributing food groups. Examples of food that have high fiber content are carrots, cabbage, lettuce, celery and fennel. Fiber is minimally degraded in the digestive process. Cellulose may be partly degraded by the



A. Annular
B. Annular extended
C. Annular, transitional to helical
D. Helical
E. Helical
F. Reticulate
G. Scalariformly pitted
H. Oppositely pitted

From: K. Easu, Plant Anatomy (2nd edition), John Wiley and Sons, Inc. New York, P. 233

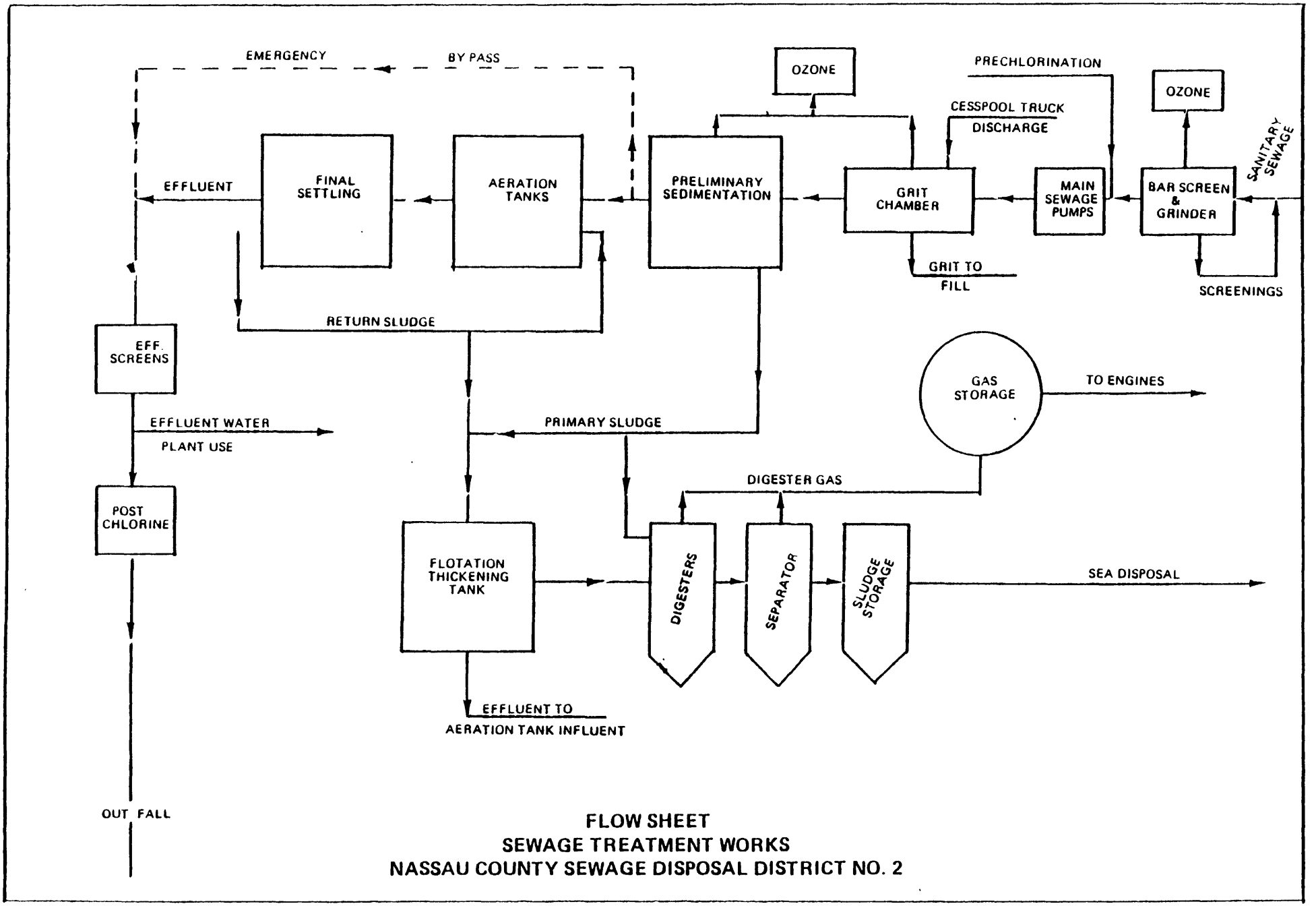
Figure 1: XYLEM SECONDARY WALL STRUCTURE

colon microflora of man, but lignins are very resistant to colonic degradation and chemical attack. The human body passes the undigested remains of the food fiber out of the body as the roughage component of fecal material (Davenport, 1977, Inglett, 1979).

3. Sewage treatment may alter, but does not destroy, the identifiable fiber remains present in raw sewage. Primary sewage treatment consists of mechanical separation and settling of solids from wastewater, while the secondary stage consists of biological treatment of the separated components of the raw sewage (Figure 2). The solids removed from wastewater in both primary and secondary treatment units, together with the water removed with them, constitute wastewater sludge. The fiber settles during primary treatment and remains as part of the sludge, through secondary treatment, until disposal (Freudenthal, 1976).

4. Sewage material enters the marine environment as discharged raw sewage, as a result of sewage-related accidents, from illegal dumping, or by the purposeful marine disposal of sewage sludge. The remains of the secondary plant cell walls of terrestrial vascular plants thus enter the marine environment (O'Connor, Stanford, Eds., 1979).

5. The helical and spiral patterns of the lignified secondary cell walls of xylem tissues do not naturally exist in the marine environment. Although a few vascular plants exist in the marine environment, they do not exhibit the same



FLOW SHEET
SEWAGE TREATMENT WORKS
NASSAU COUNTY SEWAGE DISPOSAL DISTRICT NO. 2

Figure 2: SEWAGE TREATMENT PROCESS

structural features as terrestrial plants. "The marine angiosperms all resemble Elodea in lacking true vessels and in being almost totally devoid of any vascular elements with even annular thickenings" (Sculthorpe 1967, p. 144).

The foregoing premises are the basis for the following conclusion: That microscopically identified helical or spiral remains of the secondary cell walls of the xylem tissues in the marine samples indicate the presence of untreated sewage or sewage sludge in the marine environment.

The RSIT pilot project was designed to test this conclusion. Therefore, the objectives of the project were to demonstrate that:

- The indicator is not present in uncontaminated ocean areas.
- Samples from contaminated areas contain the indicator.
- The quantity of indicator present reflects the level of contamination.
- The analysis technique is rapid, cost-effective, and uncomplicated.
- The procedure is effective for fresh, frozen, and formaldehyde preserved (at least through six years) samples.
- A potential exists for further refinements to the technique.
- A potential exists for further applications of the technique.

III. MATERIALS AND METHODS

III.1 Study Areas

Sampling study areas were selected to reflect specific objectives of the RSIT project. A total of 96 samples were analyzed from the following categories:

1. Contaminated and uncontaminated estuarine and nearshore ocean: 8 sediment and 8 water column samples were taken from 8 uncontaminated areas in order to determine whether the indicator is naturally present. For comparison purposes, 15 water column samples were collected from estuarine areas believed to be contaminated by a sewage pipe break.
2. Ocean samples: 38 sediment samples were taken from the New York Bight area in order to document the presence and quantity of xylem tracheids. Seventeen water column samples, collected one-meter above the bottom from the New York Bight in the spring and fall of 1978, were analyzed to document the presence and quantity of xylem tracheids in contaminated and uncontaminated open ocean water samples. These also validate the use of the technique with preserved samples.
3. Sludge dumpsite: 6 sediment samples were taken from the New York Bight sludge dumpsite in order to document the presence and quantity of xylem.

4. Raw unprocessed sewage: 1 sample was taken from the Cedar Creek Water Pollution Control Plant influent in order to document the presence, quantity and appearance of the xylem tracheids before sewage treatment.
5. Processed sewage/sludge: 3 samples of processed sewage/sludge from 3 sewage treatment plants were analyzed to document the presence, quantity and appearance of the xylem tracheids before ocean dumping.

Fifty five (55) samples were provided by NOAA for the purposes of this study. The remaining 41 samples were collected by the Nassau County Department of Health. Table 1 describes the study area, the agency source, and processing of the samples.

III.2 Sample Collection Methods

The 7 sediment samples collected by the Marine Ecology Section were taken using a Smith McIntyre grab. A $0.1M^2$ surface sediment sample was taken from the surface of grab samples for the following analysis:

200 grams for microscopic analysis. Sample refrigerated in whirlpack

500 grams for heavy metal analysis. Placed in heavy metals analysis bottles

250 grams for bacteriological analysis. Sample refrigerated in whirlpacks.

The method used by the Nassau County Department of Health for the 15 estuarine water column samples was to dip a 100-ml bottle

TABLE 1

STUDY AREAS

Study Area	No. of Samples	Samples Provided by Nassau County Department of Health	Samples Provided by National Oceanic and Atmospheric Administration	Condition of Sample Fresh	Condition of Sample Frozen	Condition of Sample Preserved
1. Estuarine and nearshore	31	15 (water)	16 (8 water, 8 sediment)	15		16 (formaldehyde)
2. Ocean	55	22 (17 water, 5 sediment)	33 (sediment)	5*	33	17 (formaldehyde)
3. Sludge dump site	6	2 (sediment)	4 (sediment)	2*	4	
4. Unprocessed sewage	1	1		1*		
5. Processed sewage/ sludge	3	1	2	1*	2	
TOTALS	96	41	55	24	39	33

* Refrigerated

below the surface of the water, fill, cap, and refrigerate until analysis.

The method used for the collection of the 17 water column samples from 1978 was to lower a Van Dorn sampler to one-meter above the bottom and fill. The water sample was then transferred to a one-liter bottle, stained with rose bengal, and then preserved with 10 percent formalin.

The sample collection method used by NOAA for the 37 sediment samples collected from the Ocean Pulse Monitoring Cruise, No. DL 82-06 was to use a NOAA standard stainless steel Smith McIntyre grab with special flaps to prevent excessive wash off. The top surface centimeter of the grab sample was skimmed with a stainless steel spoon and put into a 3-ounce jar until it was half filled. The samples were than frozen until analysis.

III.3 Sample Sites

Figure 3 (a & b) illustrates the locations of the NOAA-provided samples. Figure 4(a) shows the seven New York Bight locations sampled by Nassau County Department of Health in 1983; Figure 4(b) shows the 17 New York Bight locations sampled by the Nassau County Department of Health in spring and fall of 1978.

Remaining samples were from:

1. Water Pollution Control Plants

   a. Cedar Creek, Wantagh, New York
   b. Sayreville, New Jersey
   c. Ridgewood, New Jersey

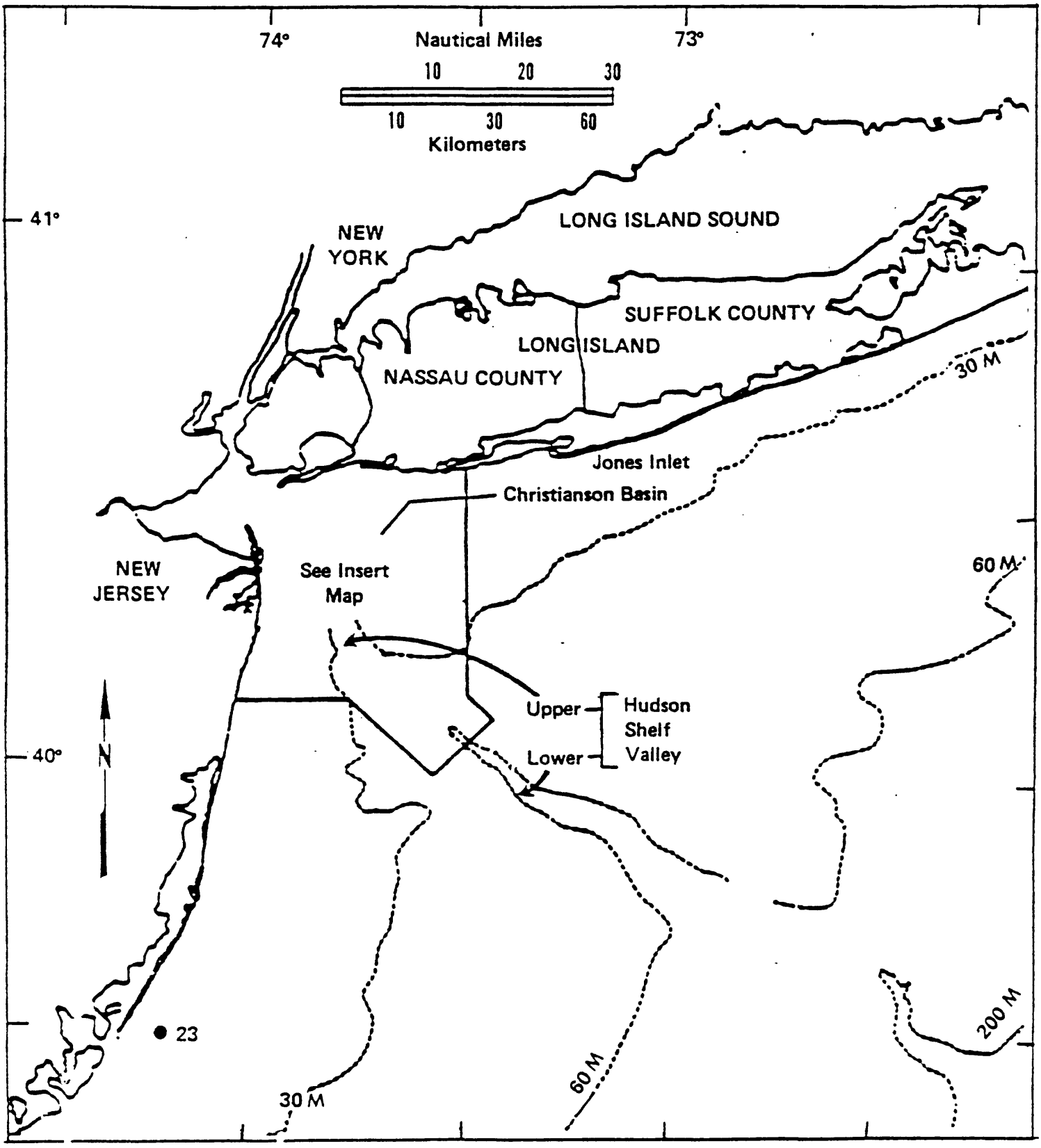


Figure 3a: MAP SHOWING NOAA SAMPLE LOCATION "23". OTHER SAMPLE LOCATIONS ARE ON INSERT MAP. (see figure 3b)

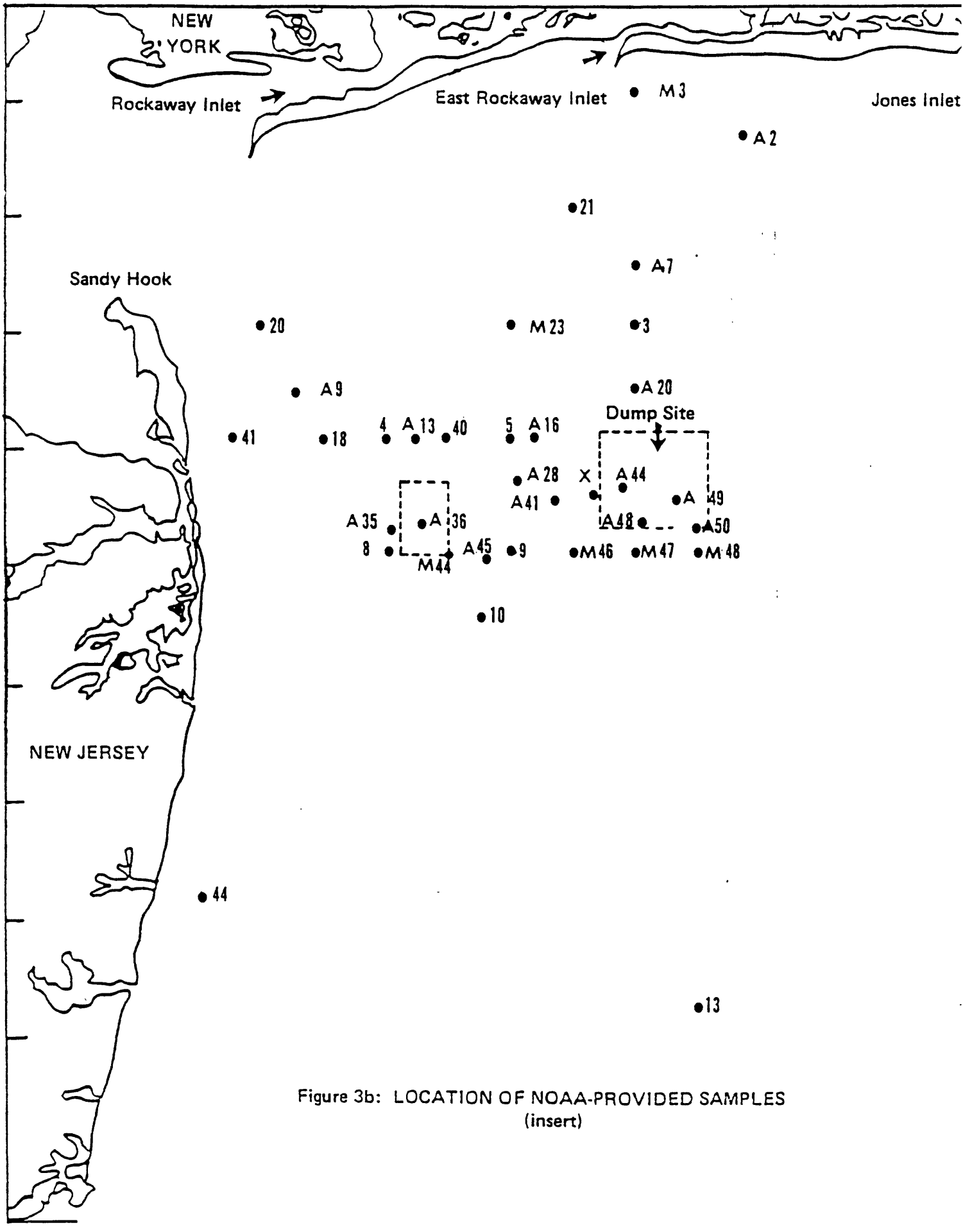


Figure 3b: LOCATION OF NOAA-PROVIDED SAMPLES
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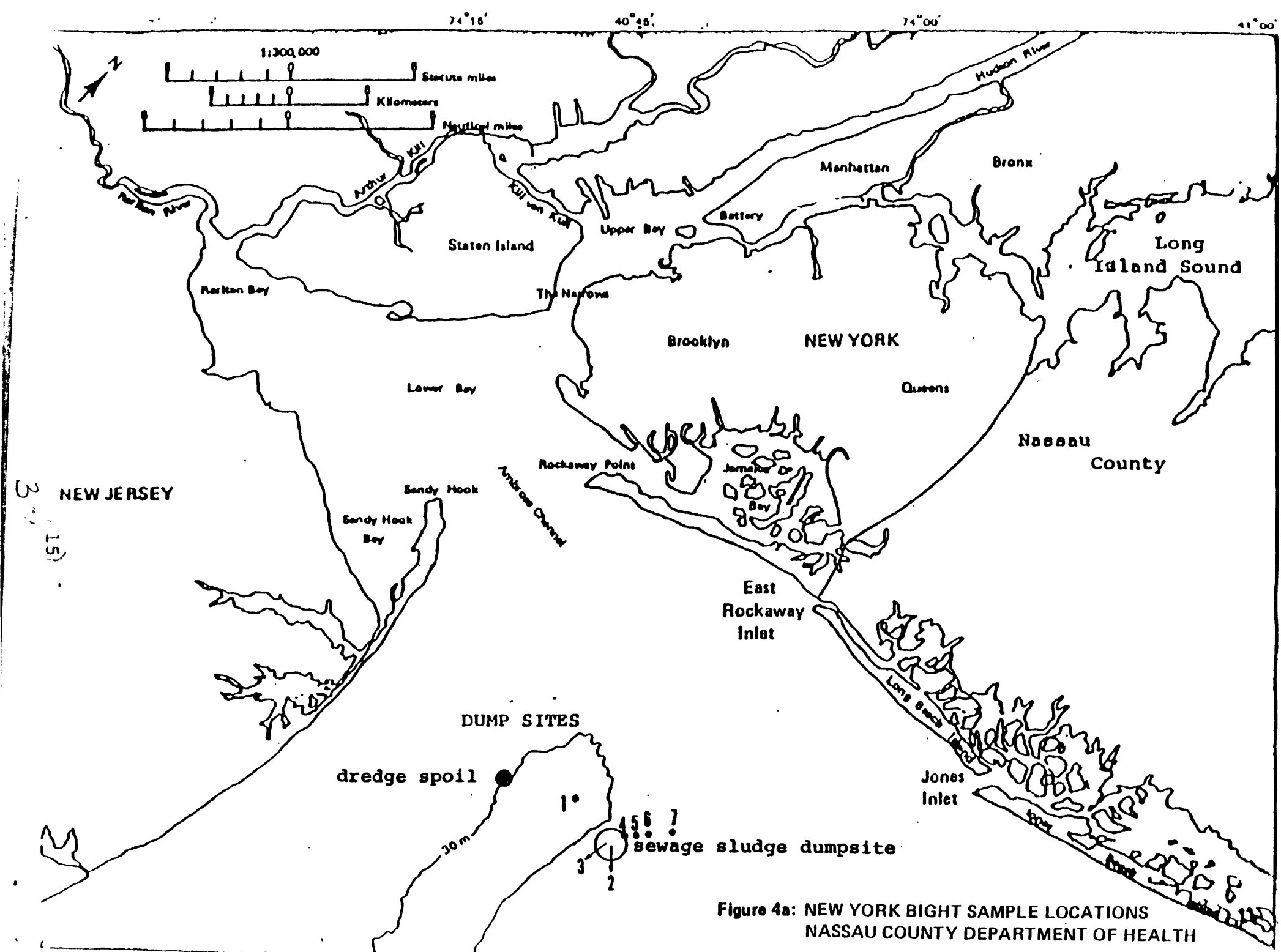


Figure 4a: NEW YORK BIGHT SAMPLE LOCATIONS
NASSAU COUNTY DEPARTMENT OF HEALTH

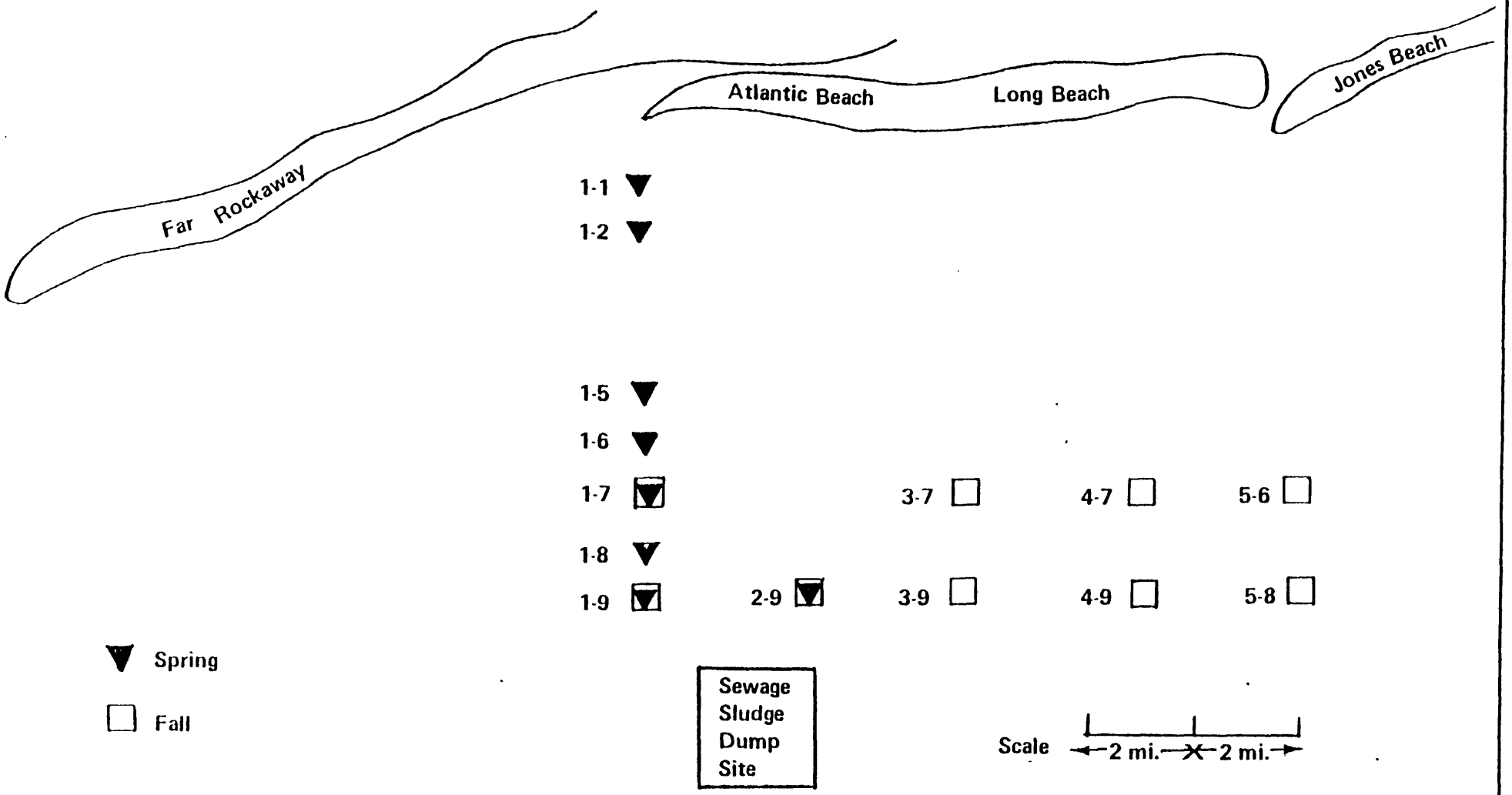


Figure 4b: 1978 NEW YORK BIGHT SAMPLING LOCATIONS
NASSAU COUNTY DEPARTMENT OF HEALTH

2. Estuarine Samples

a. Bass River: Under Parkway Bridge (Delaware)
b. Cape May: Delaware Bay
c. Ocean View: A branch of Indian River Bay (Delaware)
d. Assateague Island: Tom's Cove
e. Albemarle: Currituck Sound
f. Oregon Inlet: Pamlico Sound
g. Oyster Creek: Davis, North Carolina
h. Radio Island: Beaufort, North Carolina

3. Water Column

Hempstead Harbor, Long Island, New York

III.4 Analysis of Samples

III.4a Companion Tests

For comparison purposes various chemical and bacteriological analyses were to be performed on a majority of the sediment samples. Specific test results for the NOAA-provided samples have not been completed and are not available for this report.

Companion analysis conducted on the seven New York Bight sediment samples, collected by the Nassau County Department of Health, were: arsenic, cadmium, chromium total, copper, lead, nickel, selenium, zinc, total coliform, and fecal coliform (Table 2(a)). These tests were conducted using the 15th edition, Standard Methods for Examination of Water and Wastewater, 1980. The coliform analyses were performed using the Multiple Tube Method, pp. 786-805. The metal tests were conducted using a Perkin-Elmer Model 5,000 Atomic Absorption Spectrophotometer with Graphite furnace modification

TABLE 2 (a)

COMPARISON OF XYLEM COUNTS AND COMPANION TESTS RESULTS

Station No.	Xylems/ 10 ml	Total Coliform	Fecal Coliform	Vibrio (Bottom Water)	Chemical Analyses (mg/kg dry)						
					Arsenic	Cadmium	Chromium	Copper	Lead	Nickel	Zinc
3	4875	240,000.0	24,000.0	427	12.8	8.5	208.0	338.0	396.0	20.6	515.0
5	2940	240,000.0	110,000.0	144	5.7	3.0	52.3	80.5	92.5	8.2	151.0
4	1620	4,300.0	980.0	113	5.2	2.0	40.6	71.2	89.0	7.9	129.0
1	795	46,000.0	3.0	40	10.9	2.0	29.7	32.3	51.5	49.5	99.0
7	585	46,000.0	280.0	76	17.7	2.0	21.4	12.6	32.0	5.8	102.0
6	540	46,000.0	240.0	124	3.0	2.0	11.6	11.0	27.5	3.5	30.5
2	170	9,300.0	9.1	-	8.8	2.0	12.4	10.0	22.6	6.0	35.8

TABLE 2(b)

COMPARISON OF XYLEM COUNT AND COMPANION TEST RESULTS

1978 WATER COLUMN SAMPLES

Station Number	Xylems	Salinity	Water Temperature	Total Coliform	Fecal Coliform	Dissolved Oxygen
Spring:						
1-1B 1	0	31.00	10.2	3	3	9.4
1-2B	0	31.05	10.1	3	3	9.0
1-5B	0	31.27	9.5	3	3	9.2
1-6B	0	31.29	9.1	3	3	9.4
1-7B	0	31.28	8.9	3	3	9.2
1-8B	20	31.30	8.7	9.1	3	9.2
1-9B	12	31.36	8.5	9.1	3	9.0
2-9B	6	31.38	8.6	3	3	9.2
Fall:						
1-7B	14	32.4	15.0	15	3	6.2
1-9B	2	32.4	15.1	9.1	3	5.8
2-9B	2	32.4	15.2	23	3	5.8
3-7B	0	32.4	16.5	3	3	7.2
3-9B	8	32.3	16.5	43	9.1	6.6
4-7B	0	31.9	16.2	9.1	3	7.8
4-9B	3	31.9	16.1	3	3	8.4
5-6B	0	31.8	16.2	3	3	7.8
5-8B	4	31.8	16.2	3	3	8.0

TABLE 2(c)

COMPANION TEST RESULTS - 1978 SEDIMENT SAMPLES

Station Number	eH	TC	FC	% Vol. Solids	% COD	%KJL_N	Cadmium	Chromium	Copper	Lead	Nickel	Zinc
Spring:												
1-1B	+75	3.6	3	.5	.49	110	.03	10.0	1.3	2.7	4.7	23.0
1-2B	+100	43	3	1.0	.69	190	.05	4.7	4.7	4.7	3.0	23.0
1-5B	+175	3	3	2.1	.43	130	.02	16.0	4.0	40.0	12.0	67.0
1-6B	0	93	21	1.2	.60	430	.04	14.0	4.0	4.2	8.0	45.0
1-7B	-325	930	3	2.5	3.16	690	.97	45.0	40.0	60.0	13.0	87.0
1-8B	-300	43	3	3.7	5.17	1300	1.3	68.0	68.0	90.0	17.0	140.0
1-9B	-325	4300	3	2.1	2.69	610	1.1	40.0	40.0	53.0	9.7	80.0
2-9B	-225	23	3	1.1	.85	410	.20	19.0	9.7	3.2	4.7	40.0
Fall:												
1-7B	-450	23	3	2.5	3.35	1000	1.2	50.0	40	61	13	90
1-9B	-380	3.6	3	1.8	2.37	1400	.92	27.0	23	43	8	72
2-9B	-445	93.0	9.1	1.5	1.67	8.0	.27	25.0	20	32	8	55
3-7B	-170	240	3.6	.4	.15	1.3	.05	3.8	1.8	1.8	1.3	12.0
3-9B	-	240	3.0	.4	.17	1.3	.02	4.8	1.3	2.8	1.3	14.0
4-7B	+ 30	3	3	1.0	.13	100	.02	15	1.3	2.7	8	40
4-9B	+ 90	23	3	.4	.12	100	.02	4.3	1.3	1.0	3	10
5-6B	-210	3	3	.6	.31	110	.05	9.2	1.7	3.5	3.0	23
5-8B	-280	3	3	.8	.67	250	.05	11.0	2.5	3.0	1.2	18

(part 300, beginning p. 141).

Companion analyses conducted on the 17 New York Bight water column samples collected in 1978 by the Nassau County Department of Health were: salinity, water temperature, total coliform, fecal coliform, and dissolved oxygen (Table 2(b)). In addition, sediment samples collected simultaneously from these same sites were analyzed for: eH, total coliform, fecal coliform, percent volatile solids, percent C.O.D., percent Kjeldahl nitrogen, cadmium, chromium, copper, lead, nickel, and zinc (Table 2(c)).

III.4b RSIT Sample Analysis Procedure

The samples were prepared using the Sample to Slide procedure described in Table 3. The procedure for the analysis of the slides is as follows:

1. Scan each prepared slide - Scan each prepared slide, under 200x magnification, for presence of xylem spirals (Figure 5). Phase contrast illumination is preferable. A skilled viewer can benefit from the judicious use of polarized light.
2. Count and record the number of xylem spirals on each slide - This will result in the total number of xylem spirals in each drop of sample. To keep the counts consistent, the following rules apply:

   a. Do not count if identification is uncertain
   b. A cluster of xylem spirals is counted as one spiral
   c. Each xylem spiral in each field is counted as one

TABLE 3

SAMPLE PREPARATION PROCEDURE FOR QUANTITATIVE RESULTS

Equipment	Sediment Procedure	Water Column Procedure
10 ml graduated cylinder	1. Fill 10 ml graduated cylinder with 8 ml of room temperature water (tap)	None
Plastic syringe with tip removed Sediment	2. Draw 2 ml of sediment into plastic syringe	None
	3. Add sediment to cylinder bringing total volume in cylinder to 10 ml	
Stopper for cylinder	4. Stopper cylinder and shake by inverting from top to bottom until well mixed (approximately one minute). Remove stopper	
	5. Let stand exactly 10 seconds	
Pipette fitted with rubber bulb	6. Immediately draw off supernatant with pipette and reserve	
	7. Add water to residue to bring total to 10 ml and repeat steps 4 to 6. Do this twice reserving supernatant	
20 micron nytex filter	8. Discard residue and filter reserved supernatant through 20 micron nytex filter, discarding filtrate	Filter 500 ml of water through 20 micron nytex filter, discarding filtrate
Squirt bottle with tap water	9. Thoroughly flush residue remaining on filter with squirt bottle containing tap water	Same
Beaker	10. Wash residue remaining on filter into a beaker	Same

TABLE 3--continued

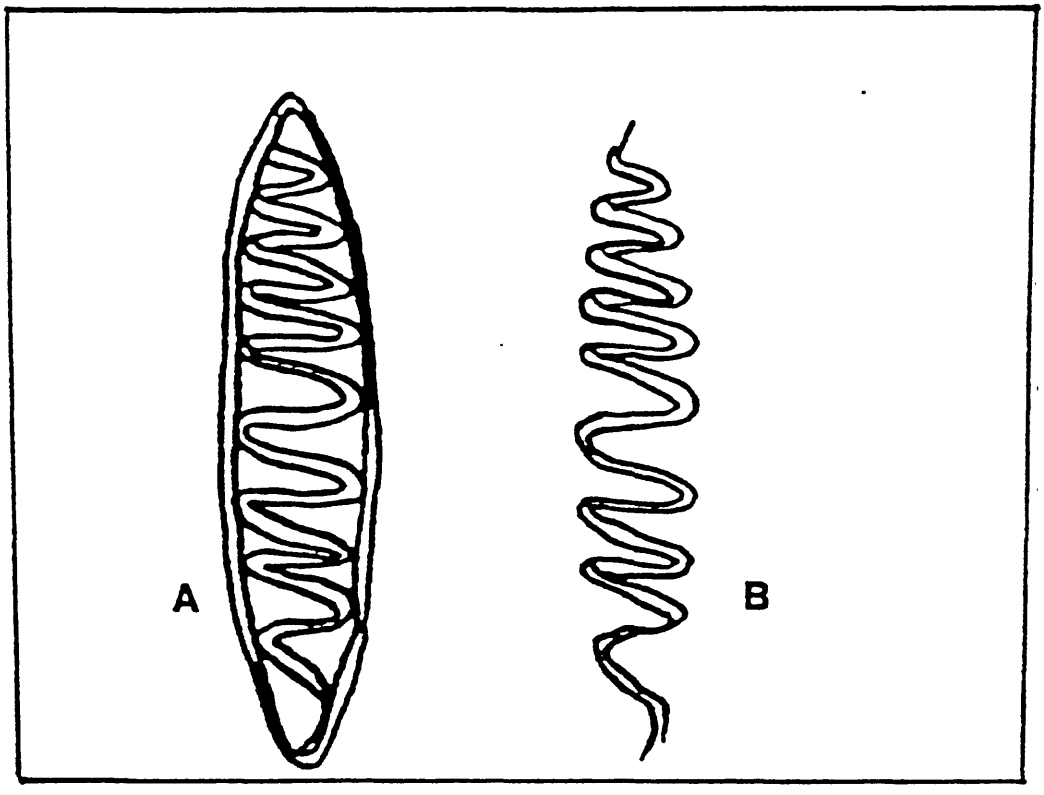
Sample Preparation Procedure for Quantitative Results

Equipment	Sediment Procedure	Water Column Procedure
Pipette with bulb 15 ml centrifuge tube with graduated markings to 0.1 ml Centrifuge	11. Draw off slurry in beaker with pipette and place in centrifuge tube	Same
	12. Centrifuge for one minute forming pellet	Same
	13. Measure and record volume of pellet	Same
Pipette (1 ml)	14. Draw off supernatant until double the volume of the pellet is left. For example, if the pellet measures .4 ml, draw off all but .4 ml of supernatant leaving a total volume of .8, pellet and supernatant	Same
	15. Stir residue and remaining supernatant, with pipette	Same
Tap water, slide	16. Using the pipette place one drop of solution on microscope slide. Add two drops of water	Same
Microtip probe	17. Using probe, mix drops of solution and water to evenly space out residue	Same
Coverslip	18. Cover with coverslip	Same

3. Follow 1 and 2 above for three slides from each sample - Average the number of xylems recorded for the three slides. To determine the number of xylem tracheids per 10 ml use the following calculations:

   a. Divide the volume of the pellet obtained in step 13 by the volume of one drop of solution in step 16 (Table 3)

   b. Take the number obtained above, then multiply by the average count of the three slides
   c. Double this number to account for the dilution in step 14 (Table 3)
   d. The result is the number of xylems per 2 ml. To extrapolate to 10 ml simply multiply by 5



A. Xylem - helical secondary wall thickening

B. As the food substance passes through the digestive and sewage systems to the marine environment, the outer primary wall disintegrates leaving the helical (or spiral) thickenings. These spirals are the identifiers in the RSIT method.

Figure 5: XYLEM IDENTIFICATION

IV. RESULTS

Using the sample-to-slide technique and the analysis procedures discussed in the previous section, samples were examined quantitatively for the presence of xylem. All of the samples were examined, and findings recorded, without reference to the source of the sample or its contamination potential. When the examination of the 96 samples was completed, findings were then organized as to site locations and study areas. Table 4 lists the sediment samples by study areas in quantitatively descending order of xylem observations per 10 ml. The samples range from a high of 4,875 xylem observations in a dumpsite sample to none in all the estuarine and nearshore samples.

Figure 6 illustrates the 20 percent of the sediment sample locations with the highest xylem observations. The greatest concentrations of xylem observations are to the north and northwest of the dumpsite (no samples were collected east of the dumpsite). The southern boundary of the dumpsite, Stations No. 2, A50, M46, M47, and M48 have xylem concentrations in the lower 50 percent of all samples and lowest quarter of all ocean samples. The xylem concentrations define the pattern of the sludge path to the north and west of the dumpsite in a crescent shape (Figure 7). A second smaller area of elevated counts coincides with the

TABLE 4

SEDIMENT SAMPLES RANKED IN QUANTITATIVE ORDER

Sample No.	Study Area	Total Number/10 ml	Rank
3	Dump Site	4,875	1
A-44	Dump Site	3,400	2
5	Ocean (N.Y. Bight)	2,940	3
A-20	Ocean (N.Y. Bight)	2,935	4
A-35	Ocean (N.Y. Bight)	2,745	5
A-49	Dump Site	2,585	6
003	Ocean (N.Y. Bight)	2,475	7
A-41	Ocean	2,445	8
X	Ocean	2,315	9
A-28	Ocean	2,075	10
4	Ocean	1,620	11
A-16	Ocean	1,575	12
010	Ocean	1,510	13
M-23	Ocean	1,355	14
009	Ocean	1,250	15
A-36	Ocean	1,225	16
005	Ocean	1,000	17
A-09	Ocean	865	18
M-44	Ocean	840	19
1	Ocean	795	20
A-48	Dump Site	725	21
7	Ocean	585	22
0-40	Ocean	555	23
6	Ocean	540	24
A-7	Ocean	450	25
013	Ocean	450	26
018	Ocean	430	27
A-45	Ocean	385	28
A-38	Ocean	340	29

TABLE 4--continued

SEDIMENT SAMPLES RANKED IN QUANTITATIVE ORDER

Sample No.	Study Area	Total Number/10 ml	Rank
A-13	Ocean	330	30
M-48	Ocean	325	31
A-50	Dump Site	290	32
021	Ocean	180	33
M-46	Ocean	175	34
2	Dump Site	170	35
008	Ocean	125	36
A-02	Ocean	115	37
M-47	Ocean	85	38
004	Ocean	60	39
020	Ocean	15	40
041	Ocean	5	41
M-03	Ocean	5	42
044	Ocean	5	43
23	Ocean	0	44
Pamlico Sound	Estuarine and Nearshore	0	45
Bass River	Estuarine and Nearshore	0	46
Radio Island	Estuarine and Nearshore	0	47
Cape May	Estuarine and Nearshore	0	48
Assateague Island	Estuarine and Nearshore	0	49
Indian River Bay	Estuarine and Nearshore	0	50
Oyster Creek	Estuarine and Nearshore	0	51
Currituck Sound	Estuarine and Nearshore	0	52

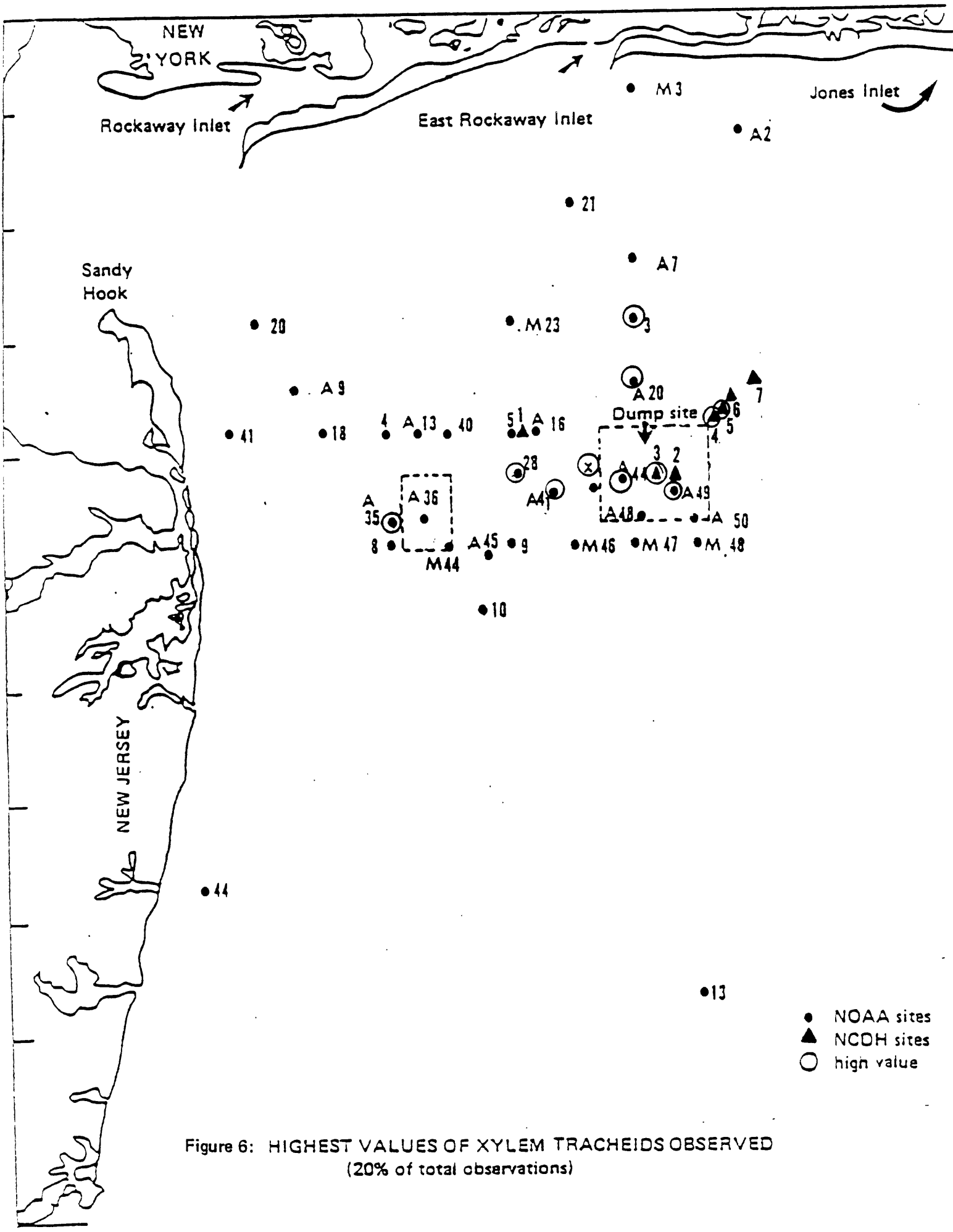


Figure 6: HIGHEST VALUES OF XYLEM TRACHEIDS OBSERVED
(20% of total observations)

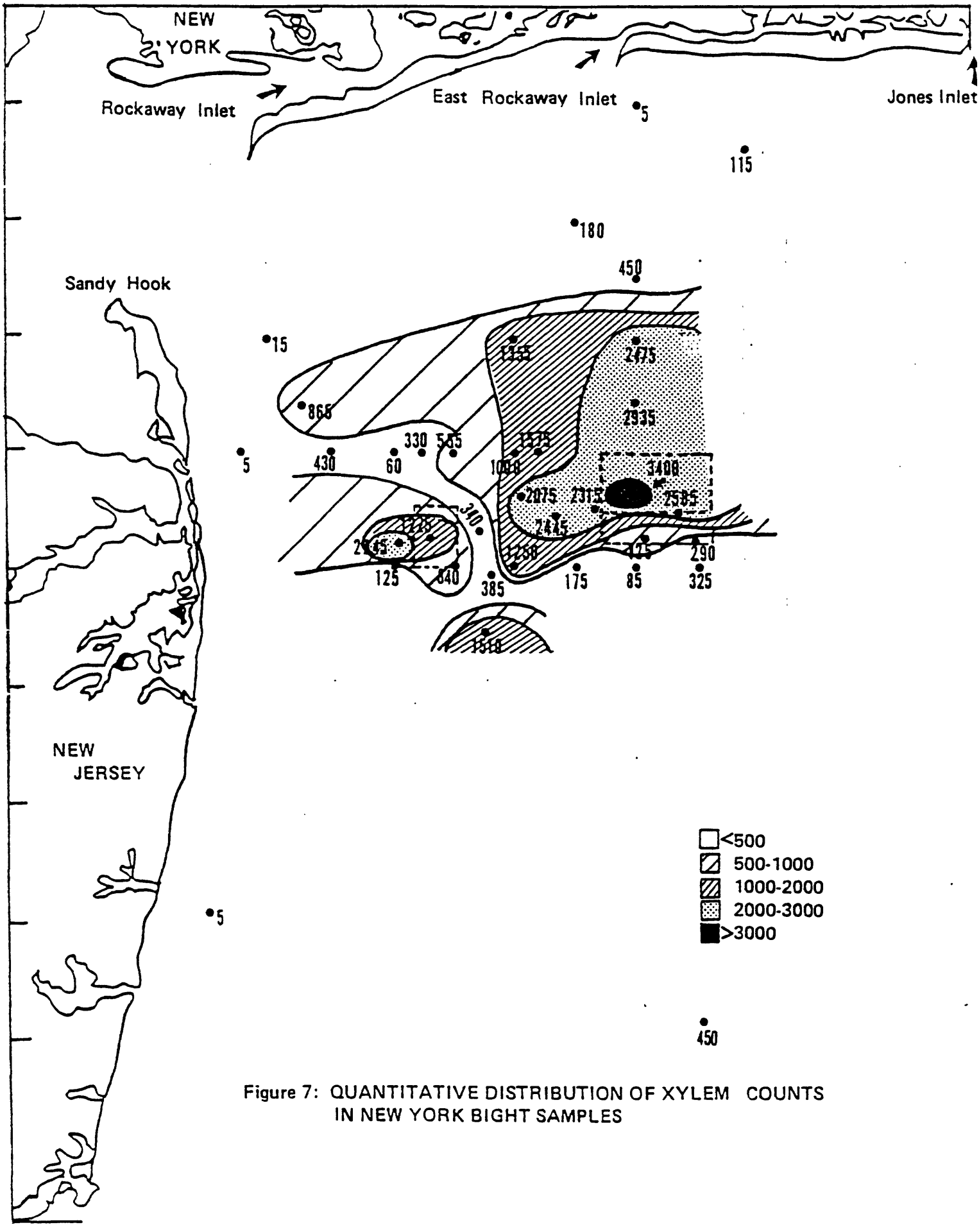


Figure 7: QUANTITATIVE DISTRIBUTION OF XYLEM COUNTS IN NEW YORK BIGHT SAMPLES

"cellar dirt" dumpsite southeast of the sludge dumpsite. Again, contamination lessens radially.

It is possible, as shown on Table 4, that by xylem counts alone, an area can be broadly identified as to its sludge contamination level, from very high to uncontaminated. These broad categories conform to what is known of the study areas. A 1980 study, Contaminants in the New York Bight and Long Island Sound Sediments and Demersal Species, and Contaminant Effects on Benthos, Summer 1980, concludes that "Concentrations of organic carbon, metals, PCBs, PAHs, and coprostanol in sediments were all at, or near, maximum values in the area of sewage sludge accumulation just west of the sludge dumpsite. Fecal coliform bacteria and Clostridium perfringens were also elevated here, and all macrofauna indices pointed to a highly impacted condition in eastern Christiaensen Basin." Samples near the dumpsite (except directly south) during this study showed the highest presence of xylem, while those from the uncontaminated estuaries and nearshore showed no xylem presence.

Estuaries are an interesting phenomenon. There was some doubt at the beginning of the study if the marsh plants or plant detritis from terrestrial runoff would effect the integrity of the RSIT as an indicator of sewage/sludge contamination in nearshore areas. The marsh plants were tested using a phloroglucin and hydrochloric acid staining

technique for lignin content. They were found to be high in lignin, but without observable xylem tracheids, thus eliminating them as a factor in RSIT analysis. The existence of terrestrial plant detritus poses possible misleading results and needs to be investigated further before the RSIT can be used as a positive indicator of sewage contamination in nearshore areas known to have leaf detritus. However, preliminary results are encouraging, for in every estuarine and nearshore sediment sample examined for this study, there was no indication of xylem presence, although there was observable plant material.

Forty water column samples (See Tables 2b, 5) were examined to verify that the RSIT procedure was a valid indicator for water column contamination. Of the eight estuary water column samples provided by NOAA, two showed minimal water column xylem presence (Indian River - 2 xylems/500 ml and Currituck Sound - 15 xylems/500 ml). Companion bacteriological data were not available from NOAA for these samples. The fifteen Hempstead Harbor samples, collected from waters possibly contaminated by a sewer pipe break showed three positive xylem results. Total coliform levels were from 240 to 930 for these samples. Three other samples also registered total coliform counts of 240 with no xylems present. The remaining

TABLE 5

ESTUARINE WATER COLUMN QUANTATIVE XYLEM COUNTS
AND COMPANION BACTERIOLOGICAL RESULTS

Study Area and Location	Total Xylems/500 ml	Total Coliform	Fecal Coliform
Pamlico Sound	0		
Bass River	0		
Radio Island	0		
Cape May	0		
Assateaque Island	0		
Indian River Bay	2		
Oyster Creek	0		
Currituck Sound	15		
Hempstead Harbor 4*	0	23.0	23.0
Hempstead Harbor 5	0	43.0	23.0
Hempstead Harbor 6	0	240.0	240.0
Hempstead Harbor 7	5	240.0	240.0
Hempstead Harbor 8	5	240.0	23.0
Hempstead Harbor 9	5	930.0	240.0
Hempstead Harbor 10	0	240.0	43.0
Hempstead Harbor 11	0	240.0	43.0
Hempstead Harbor 12	0	93.0	3.0
Hempstead Harbor 13	0	43.0	3.6
Hempstead Harbor 14	0	43.0	3.0
Hempstead Harbor 15	0	43.0	7.3
Hempstead Harbor 16	0	23.0	3.0
Hempstead Harbor 17	0	23.0	3.6
Hempstead Harbor 18	0	43.0	3.0

* All Hempstead Harbor samples were 100 ml in volume.

samples had very low coliforms and no xylems were observed. While these results are encouraging, they are based on a very small sample size. The xylem samples were only 100 mls in volume; better results are obtained with one liter samples.

A third set of water column samples were analyzed. Seventeen bottom water samples were collected in the spring and fall of 1978 as part of a NCDH monitoring program of the waters from the 12-mile dump site to the Nassau County coastline. The companion bacteriological and chemical results (from both bottom water column and simultaneous sediment samples) and the xylem results show the water to be minimally contaminated in a few places. However, because of these low levels, it is not possible to fully judge the validity of the RSIT tech- for water column samples.

Past experience within the NCDH has shown the RSIT to be of value for water column samples in times of extensive and moderate contamination. Therefore, further study with samples known to be contaminated at various levels will further justify this use. A valuable observation is that the xylems were unchanged after six years of preservation in formalin.

Comparison of raw sewage to processed, but undumped, sewage sludge, showed no obvious structural change in xylem tracheids. However, there is reason to suspect that there are chemical changes in the cellular content of the xylem tracheids between raw sewage and processed sludge. The experiments necessary to determine the chemical changes in the xylem tracheids were beyond the scope of this study but should be encouraged because these chemical changes may provide a means of distinguishing among origins of sewage contamination.

The final step in the analysis of the xylem tracheid data would have been a statistical comparison of the xylem observations to microbiological and chemical analysis of the samples. In this way a correlation between the RSIT and traditional tests could have been accomplished. However, the companion tests were not available for a majority of the NOAA-provided samples and the samples from the Nassau County Department of Health did not cover a large enough range of contamination to yield useful, conclusive results.

V. CONCLUSIONS

The objective of this pilot project was to validate that the RSIT, as developed by the author, and in use for ten years by the Nassau County Department of Health, is a reliable screening method for identifying sewage impact on the marine environment. The study results indicate that the RSIT is a valid technique for both identifying areas of the marine environment which are impacted by sewage/sludge contamination and for determining broad categories of sewage contamination. These are vital inputs to the effective management of marine resources.

Because the technique is rapid and cost-effective, without the delays inherent in traditional testing methods, the RSIT is an important addition to the procedures available for determination of sewage/sludge presence in the marine environment. The limits of the RSIT procedures, however, have not been determined. Its use in estuaries and with the water column samples needs further study as do the chemical changes in the vascular tissue from wastewater treatment and/or length of time in the marine environment.

At this point in the refinement of the technique, the greatest benefit of the RSIT is its ease and accuracy in defining the pattern and path of sewage/sludge sediment contamination. This is useful both in emergency public

health situations where the delays in receiving the results of traditional tests are unacceptable, and as a guide to designating sampling stations during routine sampling. In order to allow a more cost effective use of boat time and microbiological and chemical test procedures, quick on-board screening of samples performed between designated stations could determine if one, two or a series of sub-stations should be sampled.

A significant factor in the use of the RSIT is the ease with which it can be taught. Section III-4(b) of this report illustrates the simplicity of sample preparation and analysis. With minimal training and access to common laboratory materials, pollution control personnel can conduct their own preliminary analyses and thereby effect management decisions by adding to available information. In times of sewage related emergency or in vital decision-making processes such as sludge dump-site location, effective, valid and timely information is vital and can be augmented by the use of the RSIT.

This RSIT Pilot Project effectively proved the validity of the technique, but did not explore the full extent of its usefulness in defining marine sewage/sludge contamination.

VI. FUTURE APPLICATIONS

The advantages of a rapid, low-cost technique for determining sewage/sludge contamination should not be limited to only indicating broad, qualitative levels of contamination. Its potential to quantitatively distinguish between levels of contamination and to distinguish between sources of contamination should be investigated. By analyzing additional samples from specifically chosen sites with known levels of contamination as shown by traditional tests, a quantitative scale of contamination could be developed with product approximating Figure 7 (based on results from this study), which could illustrate the path of the sludge contamination.

The technique should prove useful in:

a. delineating the fresh impact zone at newly established virgin dump sites such as the 106-mile site south of the New York Bight;

b. tracking the movement of existing sludge deposits;

c. tracking the sludge plume released during disposal operations; and

d. monitoring the possible recovery of sites to be abandoned such as the 12-mile dump site in the New York Bight.

The RSIT can be utilized as a cost-effective means to establish the distance from the dump site that sludge can be identified in any of the layers. Costlier and more time-consuming sophisticated tests could then be saved for the positive sites.

It has been shown that the sludge "accumulation" area is often offset from the sludge "dumpsite" area, thus the RSIT can also be used to find the distribution and abundance of sludge reaching the seafloor. A series of small core samples could be used to map affected areas and levels of contamination. The RSIT appears to be effective even after the sludge is mixed with ambient bottom material. It is also a good tracer because ambient concentrations of xylem tracheids would be zero, and any xylem presence would indicate contamination.

The results of the Pilot Project suggest that by developing innovative laboratory analysis procedures it may be possible to distinguish indicator patterns as a first step in determining differing sources of sewage/sludge contamination.
In addition, training materials should be developed to fully disseminate the RSIT procedures, and by producing a training package, the RSIT would be ready for nationwide usage and would be a significant addition to techniques available for determining the extent, patterns, and path of sewage/sludge impact on ocean habitats.

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