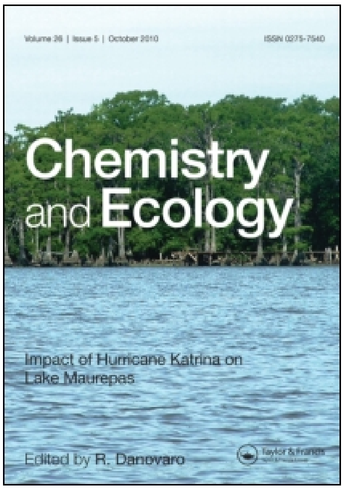

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Anita R. Freudenthal[ab]
[a] Bureau of Water Pollution Control, Nassau County Department of Health, Chief (ret.), Office of Marine Ecology, Mineola, NY [b] Departments of Biology and Enviromental Sciences, C.W. Post College of Long Island University, Brookville, NY

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SUMMARY OF 25 YEARS OF XYLEM TRACHEIDS AND SEWAGE/SLUDGE IN THE SEA

ANITA R. FREUDENTHAL

Chief (ret.), Office of Marine Ecology, Bureau of Water Pollution Control, Nassau County Department of Health, Mineola, NY 11501, Departments of Biology and Enviromental Sciences, C.W. Post College of Long Island University, Brookville, NY 11548

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The Rapid Sewage/Sludge Indicator Technique (RSIT), developed during the early 1970's, responded to critical needs within the Bureau of Water Pollution Control. It enabled quick detection of contamination in ocean water and bottom sediments during sewage-related emergencies and routine monitoring. RSIT became an invaluable tool for resource management, in determining where and to what extent marine environments are impacted, and when immediate information is necessary for public health decisions.

The RSIT utilizes microscopic examination for terrestrial plant vascular tissue, especially the xylem tracheid elements, as identifiers of sewage/sludge. Not normally found in ocean waters, this material, in toilet paper and the roughage of the human diet which passes through the digestive system intact, is an indicator of human faecal contamination. Minimal training and common laboratory materials allow gross screening in five minutes. New studies by the United States Environmental Protection Agency (USEPA), United States Geologic Survey (USGS) and National Oceanic and Atmospheric Administration (NOAA), most recently at the 106-mile dump site off the New York Bight, revalidated the technique as a rapid, inexpensive, easy to use, semi-quantitative indicator both for water column and sediment samples.

Keywords: Sewage sludge; indicator method; xylem tracheids; terrestrial plant vascular tissue

INTRODUCTION

The purposeful use of the marine environment as a repository for sewage-related material, plus the accidental introduction and illegal dumping of sewage and sewage sludge, necessitated the development of monitoring and detection techniques in an attempt to determine location, quantity, and effects. Many of these techniques, while accurately assessing sites and paths of contaminants, are highly sophisticated, time-consuming, require expensive equipment operated by highly trained technicians and results are not immediately available. A need existed, therefore, during routine monitoring, and especially in times of sewage-related emergency, for an indicator that could be used cost-effectively, rapidly and on-site to determine if, and to what extent, areas of the marine environment are impacted.

The Rapid Sewage/Sludge Indicator Technique (RSIT) was developed in the early 1970's during activity for me as the marine biologist for the County Department of Health. Nassau County, with a population of one and one-half million, is the western portion of Long Island, New York, just to the east of New York City. The southern barrier beach Atlantic Ocean shoreline forms the northern boundary of the New York Bight, where disposal of sewage sludge at the 12-mile dumpsite started in 1924. This was the largest ocean dumping operation in the nation, serving almost 20 million people until 1988. The new Deep-water Municipal Sewage Sludge Dump-site (106-Mile Site) was adopted in 1988 and closed in 1992.

The RSIT uses as an indicator the appearance in sediment and water column samples, of easily identifiable traces of the vascular materials of higher terrestrial plants, especially the secondary walls of xylem tracheids. The indicator does not naturally exist in the marine environment; its primary source is sewage-related material. This brief summary follows the RSIT from inception through recent studies.

FORMULATION OF HYPOTHESIS

Xylem tracheids are used as indicators in forensic medicine, during stomach content analysis (Bock *et al.*, 1988). The use of xylem tracheids and other terrestrial plant vascular materials in forensic marine ecology commenced 25 years ago, when my laboratory was assigned to the new Nassau County Sewage Treatment Plant (STP), then one of the largest in the world. The Superintendent's first request was to identify the structure in an enlarged photomicrograph of their sludge. It was a typical xylem tracheid, familiar after years of teaching college botany. Samples of sewage and sludge at all stages of treatment were amply available for microscopic analysis. Vascular tissues, both xylem and phloem from food plants and toilet paper were readily observed using a compound microscope, even at low power. Various stains were utilized including methylene blue and a lignin-specific red dye. Phase contrast illumination was preferred to plain light microscopy and polarized light aided the more experienced viewer.

As ocean monitoring increased, especially in the New York Bight 12-mile sewage sludge dump-site area, some samples I was observing for plankton and benthic monitoring also showed the same terrestrial plant vascular structures. Xylem and phloem appeared separately or together. The sites coincided with synoptic samples shown by the other methods to be contaminated (Nassau County Department of Health Report, 1976).

STUDY CONCEPT

It became clear that the presence in the marine environment of xylem tracheids and other vascular tissue from terrestrial higher order plants was indicative of culinary and/or sanitary sewage wastes. Three facts are relevant: 1) that the xylem and phloem cells of the vascular bundles of higher plants, when consumed in fruits and vegetables as dietary fibre, are not digested by the human body and are passed as the roughage component of faecal material, 2) that these cells from food, and toilet paper, are not significantly altered by sewage treatment, and 3) that these cells are not normally found in the ocean.

Because xylem, the water transport tissue, is also the plant's support mechanism, the cells have enduring rigid walls primarily of cellulose, further strengthened by a secondary lignified wall. The lignin deposits appear as spirals, rings, parallel bars, irregular networks, or pits (Fig. 1, Esau, 1965). The cellulose may be partly degraded by the colon micro-flora of man, but lignins are very resistant to colonic degradation and chemical attack. As the food substance passes through the digestive and sewage systems, vascular tissue alteration may occur with the outer primary wall disintegrating, leaving the helical or spiral thickenings of the secondary walls (Fig. 2). These are the prime

XYLEM SECONDARY WALL STRUCTURE

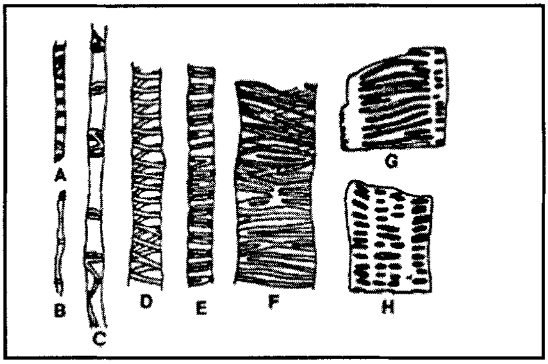

FIGURE 1 A. Annular; B. Annular extended; C. Annular, transitional to helical; D. Helical; E. Helical; F. Reticulate; G. Scalariformly pitted; H. Oppositely pitted. (from: K. Esau, Plant Anatomy (2nd Edition), John Wiley and Sons, Inc. New York, p. 233).

PHOTOMICROGRAPH OF XYLEM WALLS

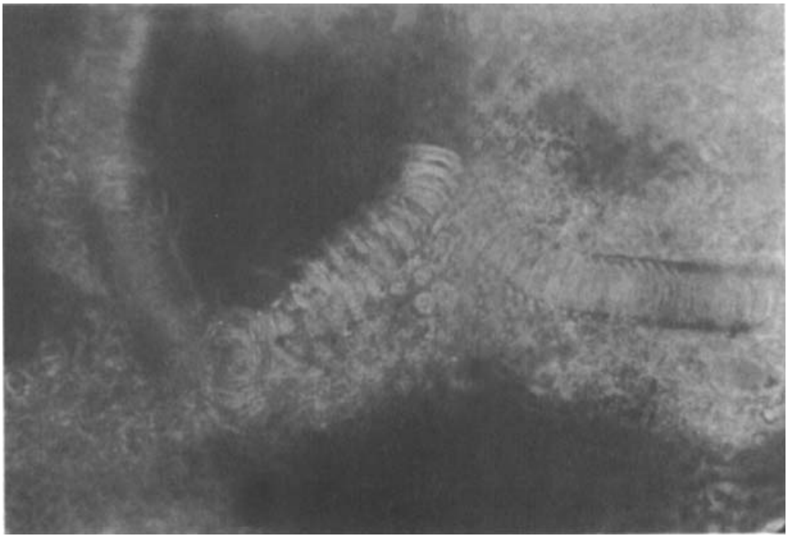

FIGURE 2 Helical/spiral thickenings; typical identifiers in RSIT.

identifiers in the RSIT. With minimal training, all xylem and phloem are readily identified microscopically.

The solids removed from waste-water in both primary and secondary treatment, plus the water removed with them, constitute waste-water sludge. The resistant food and toilet paper fibre settles during primary treatment and remains as part of the sludge, through secondary treatment, until disposal. The helical and spiral remains of the secondary plant cell walls, plus other elements of the conducting tissue of terrestrial vascular plants thus enter the marine environment and can be collected by plankton net, sedimentation and other methods. Though a few vascular plants exist in the marine environment, they do not exhibit the same structural features as terrestrial plants. They are almost totally devoid of any vascular elements with even annular thickenings (Sculthorpe, 1967).

EARLY USE DURING EMERGENCIES

During the mid-1970's, Nassau County experienced real and perceived sewage/sludge emergencies where instant knowledge was necessary to make informed decisions to protect human health and natural resources. The hypothesis, that what had been so often observed in the STP samples would also be found in samples suspected to be contaminated, was pressed into active duty because no other test was readily available to give immediate results. The technique therefore, never passed through an early formal research study phase but went from concept to use.

Suspect sludge washed up on 22 miles of south shore Atlantic Ocean swimming beaches in April, 1975, and fishermen and divers proclaimed as "sludge", extensive areas of black slime in the ocean in 1975 and '76. Negative vascular tissue findings corroborated identification of natural marine organisms as causative agents of each episode. The RSIT helped negate the idea that the leading edge of sludge, "black mayonnaise" from the New York Bight 12-mile dump site, was advancing up the continental shelf on to the ocean beaches in 1974–76. In 1976, sludge erupted to street level, into tidal streams and wetlands from a break in the 13 mile south shore underground transfer pipe. For clean-up, RSIT differentiated sludge from marsh detritus (*Phragmites, Spartina*) and natural soils (Freudenthal, in house reports).

A June 1976 storage tank explosion poured millions of gallons of sludge on to the salt marsh island, tidal flats, adjacent wetlands, and swimming waters of Hempstead Bay, a south shore estuarine embayment. Twenty "blind" samples of bay bottom sediments were evaluated at an improvised field site nearby to delineate the areas of spilled sludge to direct the rapid deployment of expensive vacuum clean-up equipment. It was possible to prioritize four sites as most impacted, four as least impacted and twelve progressively between by distinguishing xylem tracheids, seeds, hair, from natural marsh plant detritus. Four later samples verified effectiveness of clean-up activities (Freudenthal, 1976).

A massive regional electrical "blackout" in summer, 1977, incapacitated eight STP's in adjacent New York City releasing 700 million gallons of by-passed, untreated raw sewage into the East River. The plumes, carried by tides, wind and currents, were tracked by xylem tracheid identification into Long Island Sound and northern Nassau County waters and harbours to determine when and which beaches to be closed (Nassau County Department of Health, 1977). Two weeks later, the plume from a second blackout was traced south out of New York Harbour into the Bight and along the ocean beaches of the County.

Thus, RSIT was increasingly used in the analysis and assessment of hundreds of samples during episodes of real and suspected pollution. In all of these cases the inherent delay in traditional testing was unacceptable. Decisions had to be reached in minimum time with maximum information to protect the health of the public and to avoid economic losses caused by undirected clean-up efforts, unnecessary beach closures and erroneous media reports. In each case, the accuracy of rapidly reached decisions based on RSIT was later confirmed by correlation with traditional tests (Freudenthal, in-house reports).

PILOT PROJECT AND VALIDATION

During 1983–84, 96 "blind" sediment and water column, fresh, frozen and preserved samples were collected by Nassau County or provided by the National Marine Fisheries Service of the National Oceanic and Atmospheric Administration (NOAA) to validate the technique. Uncontaminated and contaminated samples came from the open ocean, near-shore ocean, numerous Mid-Atlantic estuaries and Hempstead Harbour (an arm of Long Island Sound on the north shore of

Nassau County), the 12-mile sewage sludge dump-site in the New York Bight, plus raw and processed sewage from assorted STP's (Freudenthal *et al.*, 1984; Appendix in Commeau *et al.*, 1993). Results presented at conferences (Freudenthal, 1984, 1986, 1987) elicited requests from agencies and municipalities across the USA and around the globe, all eager for new methodology. The United States Environmental Protection Agency (USEPA) sent sediment samples from a New York Bight canyons survey cruise for test examination. The United States Geologic Service (USGS) and EPA received RSIT training at my County laboratory.

RECENT USE FOR MONITORING AND REVALIDATION

Sampling emphasis shifted with closure of the New York Bight 12-mile dump-site. NOAA, USGS, and the USEPA programmed a new round of extensive monitoring with the opening of the 106-mile site. The surveys and studies included use of the RSIT in order to evaluate and assess its sensitivity under new circumstances and in comparison to other concurrently used indicators at the open-ocean deep water dump-site. Other objectives were to improve existing methodology for special collection and analysis.

RESULTS AND DISCUSSION

The results of these recent evaluation studies mainly reaffirm the original conclusions and suggestions for further use. Modifications have been introduced for early handling, calculating numbers of tracheids per sample, and microscopic observation of the sample especially where considerable dilution might take place (Hunt *et al.*, 1992; Hillman *et al.*, 1992). In the field, procedures have been modified to accommodate suspended and bottom sample collection. For example, significant numbers of xylem tracheids were detected in samples collected by tubes or traps that were attached to moorings. However, the sinking of xylem tracheids, some single and others in sheets or bundles, is still not well understood. It may be that the low density of single cells, similar to that of water, results in a different settling velocity than the tracheids in clusters or sheets. The cells may not settle to the bottom very rapidly, or may not settle at all if currents are present (Commeau *et al.*, 1993). Yet, in sediment traps deployed at 100 m, 1000 m, and at 250 m off the sea floor, Hillman *et al.* (1992) identified a large variety of vascular tissue, especially wood tracheids, at all levels, and (Hillman *et al.*, 1996) found substantial numbers in the lower level traps at most sites.

Hunt *et al.* (1992) focused on the fate of the sludge, measured xylem tracheids to determine the feasibility of using them as a sludge tracer at far-field stations, and demonstrated that sludge plumes remain as distinct features in the near-surface waters. Though relatively few tracheids were seen in the far-field samples, the xylem tracheid tracer, plus other indicators (*Clostridium perfringens*, elevated total suspended solids, trace metals concentrations of cadmium, copper, iron and lead) collected at 15, 30 and 75 m, were found up to 40 km from the dump site, the furthest analyzed for the tracheids: Highest observed tracheid concentrations occurred in samples taken within the sludge plumes immediately behind the discharging barges. These showed a great proportion of toilet paper xylem from wood, plus phloem cells with their sieve plates. This was anticipated when the original RSIT report recommended use of terrestrial plant vascular tissue to track the discharge plume. But, as found originally, the long slender cells, pits and sieve plates of phloem can be obscured with sediment and associated cells and often need a very qualified viewer, except when found in great quantity as with the concentrated plume. Quantification may also be more difficult because the toilet paper vascular tissue is more likely to be found in sheets. For this reason the original technique emphasized the more easily detected xylem tracheids of the food plants.

A study by Commeau *et al.* (1993) to assess RSIT in comparison with other indicators at the open-ocean site concluded that the use of terrestrial plant vascular tissues may not be as sensitive at lower levels as some of the more expensive, time-consuming tests commonly used, such as silver and spores of the enteric bacterium, *C. perfringens*. Yet, of these, and other indicators such as the steroid coprostanol and polychlorinated biphenyls, analyses of xylem tracheid cells is probably the fastest and least expensive with respect to equipment used. The method effectively identified high concentrations in suspended sediment from trap samples but only minor concentrations in bottom sediments near the dumpsite, possibly because of the density/settling problem. Using a larger sample, though increasing processing time, may increase sensitivity of the method. Another objective, to improve existing methodology for cell analysis, led to modifications of the procedure for determining the concentration of xylem tracheid cells. The changes enhance the initial sample processing (before proceeding with Step 4 to the end of original RSIT), report data as cells per gram of salt-free dry sediment rather than per volume of wet sediment, and total, rather than average the cells on the three slides. This better accommodates analysis of surficial sediment "fluff" samples from box cores and suspended sediment collected in "tube" and Anderson traps.

Hillman *et al.* (1996) continued to determine the feasibility of using xylem tracheids as a tracer for sewage sludge transport on the premise that when wood tracheids are found in the deep sea near the site, they are probably from sludge, whereas chemical tracers might be from several other sources. Material was collected from sediment traps at three levels at locations expected to receive maximum particle flux. Traps were deployed twice, for 4–9 months each between May, 1990 and June, 1991, first at along- and across-isobath moorings, then at along- and across-shelf moorings. The pattern of tracheid settlement was different in each deployment period, but data using other tracers suggested that conditions affecting sludge transport were also different (Hunt *et al.*, 1993). The closest results in designating the mooring sites with the highest flux measurements occurred during the first deployment, when xylem tracheid results coincided with 11 and 9 of the other tracers (3 isotopes, 4 organics, 7 trace metals) in the mid- and lower depth traps, respectively. In the upper trap, xylem tracheids, coprostanol and the isotope S^{34} designated the same mooring. The modified semi-quantitative technique proved relatively effective and inexpensive as a means to detect sludge up to considerable distances from its initial site of discharge.

In July, 1997, a malfunctioning New York City pump station reportedly released 15 million gallons of untreated raw sewage into western Long Island Sound waters. The Nassau County Health Department used the original RSIT, and beaches were not closed as all results showed no impact to County waters. This was later verified from traditional coliform bacteria test results. Less than one million gallons were actually released. Neighbouring areas closed beaches, assuming impact based on visual observation. They have since requested RSIT training for future emergency use (John Jacobs, personal communication).

CONCLUSIONS

Based on results of modifications and conclusions of the new studies, terrestrial plant vascular tissue, especially xylem tracheids, can be used as a biological tracer in the sea. The modified, or quicker original, RSIT should be used during environmental and health emergencies. On research vessels, a quick scan of each sample as it is brought aboard could result in immediate extra sampling, at other depths or between stations, rather than reaching that decision on land, months later. The RSIT has evolved from emergency situation use only, to become a rapid, inexpensive, and relatively easy to use monitoring tool to:

- be effective as a sludge indicator in water column samples and in suspended sediment trap samples
- replace traditionally used tests under certain circumstances
- trace sewage sludge distribution in sediments in a semi-quantitative manner
- highlight samples, in a tiered test system, on which to perform more quantitative, expensive and time consuming tests
- detect sludge up to considerable distances from initial sites of discharge
- determine extent of influence of the discharge plumes from disposal barges.

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