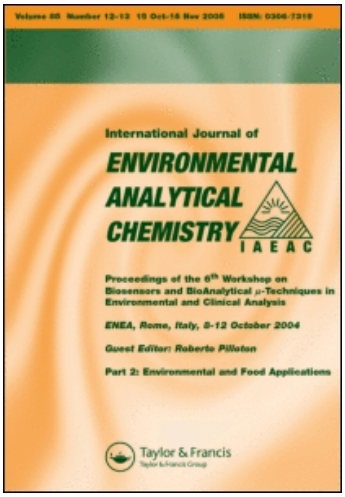


## International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

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Roger Pocklington[a]; Clive D. Macgregor[a]
[a] Department of the Environment. Dartmouth, Bedford Institute of Oceanography, Marine Sciences Directorate, Nova Scotia, Canada

**To cite this Article** Pocklington, Roger and Macgregor, Clive D.(1973) 'The Determination of Lignin in Marine Sediments and Particulate Form in Seawater', International Journal of Environmental Analytical Chemistry, 3: 1, 81 — 93
**To link to this Article: DOI:** 10.1080/03067317308071070
**URL:** http://dx.doi.org/10.1080/03067317308071070

*Intern. J. Environ. Anal. Chem.* 1973, Vol. 3, pp. 81–93

Printed in Great Britain

# The Determination of Lignin in Marine Sediments and Particulate Form in Seawater†

ROGER POCKLINGTON and CLIVE D. MacGREGOR

*Bedford Institute of Oceanography, Marine Sciences Directorate, Department of the Environment, Dartmouth, Nova Scotia, Canada*

(*Received November 30, 1972*)

KEY WORDS: Lignin; marine sediments; particulate forms; seawater.



Alkaline nitrobenzene oxidation was used to produce vanillin and syringaldehyde from the lignin fraction of marine sediments and particulate matter filtered from seawater. Results indicate that this material constitutes a substantial proportion (1–14 %) of the organic matter in the sediments and that a general background level (20–60 mcg/l) of material in the suspended fraction is attributable to lignin. The highest levels were found in the vicinity of forest industry plants.

## INTRODUCTION

Marine sediments and seawater contain low but significant concentrations of organic carbon derived ultimately from the activities of living organisms and covering the whole range of possible cellular, metabolic and decay products.[1] The work described in this paper is part of an attempt to identify those organic compounds which may serve as indicators of the addition of organic material, both naturally and as a consequence of human activity, from the land to the sea. Attention was given to lignin for two main reasons:

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† Presented at the Symposium on Recent Advances in the Analytical Chemistry of Pollutants, Halifax, N.S., August 23–25, 1972.

i) It is a characteristic constituent of higher land plants essentially absent from marine flora,[2] and thus a possible indicator of land-derived material.

ii) It is the major component of the wastes of the pulp and paper industry,[3] and may provide an indication of the impact of these activities on the marine environment.

Lignification is the characteristic of vascular plants (lycopods, ferns, gymnosperms and angiosperms) which distinguishes them from all other organisms. Lignin is not a constituent of the algae except for small amounts in brown algae (Phaecophyceae), macroscopic species of which grow attached in the littoral zone (Laminariales, Fucales) or are free-floating and pelagic (*Sargassum spp.*).[4] The lignin content of conifers (gymnosperms) ranges from 24 to 33% of the dry weight.[5] Spruces (*Picea*), fir (*Abies*), pines (*Pinus*), and larches (*Larix*), all genera characteristic of the Eastern Boreal Forest Region,[6] have lignin contents in the range 24 to 29%. The lignin content of hardwoods (dicotyledon angiosperms), and of grasses (*Graminaceae*) and other monocotyledons is lower, ranging from 16 to 24%.[7]

Lignin is a major component of perennial plants and probably the second most abundant after cellulose[8] of all continuously cycled organic materials on earth. It is resistant to degradation by micro-organisms, especially under anaerobic conditions, as compared to other common biopolymers and has been found histologically intact in fossil wood.[9] Lignin in the wastes of both the acid sulphite and alkaline pulping processes is so modified as to be very resistant to microbial attack.[10] Lignin is considered to contribute substantially to the formation of humic compounds in soils forming beneath higher vegetation where there are found relatively unchanged lignin residues (vanillic, syringic, p-hydroxybenzoic, guaiacyl and syringylpropionic acids). The assumption that these are lignin-derived is supported by their complete absence from humic acid developing below clumps of moss.[11] The humic acids are efficiently natural trace metal chelators important in biological conditioning of seawater for phytoplankton growth.[12]

It is extremely difficult to extract lignin intact from wood. The traditional approach has been to remove the other organic matter leaving a residue, the nature of which is determined by the methodology. Wood or other lignin-containing organic matter (e.g. soil, sediments) is exhaustively extracted with solvents, boiling water and dilute hydrochloric acid, to remove successively fats, waxes, resins, water-soluble polysaccharides and hemicellulose, then treated with concentrated sulphuric acid to hydrolyse cellulose. The residual organic material after the $H_2SO_4$ treatment is termed "lignin-humus"[13] or "Klason lignin".[14,15] Other polyphenolic constituents can remain undissolved in extraction so the formation of an insoluble residue is not a sufficient proof of lignin. A number of u.v. absorption and colorimetric methods

of analysis have been used, both for lignin[5,16] and for lignin sulphonates[17,18] but they are insufficiently sensitive and specific for the analysis of marine samples.

The oxidation of lignin with alkaline nitrobenzene to *p*-hydroxybenzaldehyde (PHB), vanillin (VAN) and syringaldehyde (SYR) is the most effective way of obtaining reproducible yields of readily identifiable degradation products from lignin[7] and this is the method we chose to use. Alkaline nitrobenzene oxidation has been used in other recent investigations of lignin in wood, soils and sediments.[9,19,20] The amounts of each aldehyde obtained relate not only to the total quantity of lignin in the sample but also to its origin. Gymnosperms yield predominantly VAN; dicotyledon angiosperms give a mixture of VAN and SYR, whereas monocotyledons give also some PHB.[7] The alkaline nitrobenzene oxidation does not result in excessive conversion of the aldehydes to the corresponding acids but these, plus 5-formylvanillin, dehydrovanillin, 5-formylvanillic acid, 5-carboxyvanillin, dehydrodivanillic acid and acetoguaiacone, are other characteristic nitrobenzene oxidation products of softwoods.[7] For our present purposes lignin is defined as an aromatic polymer of phenylpropane units yielding one or more of PHB, VAN and/or SYR upon alkaline nitrobenzene oxidation.



## EXPERIMENTAL

### Apparatus

An Ekman grab was used to obtain surface sediment samples. Filters were of two different types: silver, 1.2 micron nominal pore size (Selas Flotronics, Spring House, Pa.; type FM-47); and glass fibre, 1.0 micron mean pore size (Reeve Angel, Clifton, N.J.; Whatman GF/C); both 47 mm diameter, supported in glass-filled polypropylene in-line filter holders (Millipore XX43047). The filters were precombusted (400°C, 2 hr) to reduce further their already low carbon blank. A freeze-drier (Virtis Co., Gardiner, N.Y.; Model USM-15) was used to dry the samples without subjecting them to heat. Derivatizations were carried out in 8-ml screw-cap centrifuge tubes (Corning No. 9826), the screw caps having Teflon liners (Corning 9998), set in an electrically heated duralumin block (Lab-line Instruments, Inc.; Cat. No. 2093). Solvents were removed in an all-glass rotary evaporator (Büchi ROTAVAPOR-R) under vacuum from a water aspirator. Micrometer setting syringes (Hamilton Co., CR700-20) gave repeatable volumes for injection into the gas chromatograph. A quadri-column dual-flame ionization detector gas chromatograph (Bendix 2500) was used. The signal from this was both integrated (Hewlett-Packard 3373B) and displayed on a graphic recorder (Moseley Autograf Model 680).

## Reagents and solvents

Analytical-grade reagents and high-purity gases were used throughout. All aqueous solutions were prepared with water distilled through an all-glass system. This water was also used to rinse all glassware after normal washing. Natural standard materials were Lignum vitae (*Guaiacum officinale*), a highly lignified material, for hardwood, and White Spruce (*Picea glauca*), which constitutes a substantial proportion of the timber in the Boreal and Acadian Forest Regions,[6] for softwood. The siloxane phases OV-1 and OV-7 (manufactured by Ohio Valley Specialty Chemical Co., obtained through Chromatographic Specialties Co.) were protected against ingress of oxygen and moisture during exchange or storage and filter-driers (Chemical Research Services) were installed in line to dry the carrier gas (argon).

## Shipboard sampling

Seawater was collected in 12-l nonmetallic Niskin bottles (rinsed with 10% isopropanol/distilled water before use) and drained by gravity through the filters. Aliquots, (0.5 l) of the filtered water were collected in high-density polyethylene bottles and stored in the deep-freeze for protection against microbial change. The filters were placed in plastic petri dishes and deep-frozen. Sediment samples were obtained by grab, a representative portion was placed in a plastic bag and frozen.



## Preparation of derivatives for gas chromatography

In the laboratory, sediments, filters and seawater samples were freeze-dried. Wood standards and sediments were ground to pass a 40-mesh sieve; filters were processed intact.

Wood (0.2 g), sediment (0.4 g), or whole filter was placed in a stoppered vial with nitrobenzene (1 ml) and sodium hydroxide solution (6 ml, 2N). The vial was placed in a constant temperature block and the reaction was allowed to proceed for 4 hr at 170°C after which time the vials were removed and quenched in cold water. This combination of time and temperature was chosen on the basis of experiments to optimize the yield of VAN. The contents of the vial were then washed into a separatory funnel (250 ml) with an equal volume of 2N NaOH. This solution was extracted with methylene chloride (2 × 5 ml) to remove non-polar products. The aqueous layer was acidified to *ca.* pH 2 with 6N hydrochloric acid (10–15 ml) and the phenolic aldehydes were extracted with methylene chloride (2 × 5 ml). The extract was dried over calcium sulphate (Drierite), then filtered and washed (10 ml of $CH_2Cl_2$) on a Büchner funnel with a 200-mesh stainless steel screen to remove particulate

matter. The filtrate was reduced in volume to 2 ml on the rotary evaporator (45°C) and centrifuged to remove the balance of the particulate matter and to separate the nitrobenzene. Aliquots (2 mcl) of the supernatant $CH_2Cl_2$ layer were injected into the gas chromatograph.

The salts from freeze-dried seawater (8 g) were reacted with correspondingly larger volumes of reagents in a Teflon container (20 ml) placed inside a steel bomb before proceeding as described above.

## Gas chromatography of phenolic aldehydes

The optimum instrumental operating conditions are given in Table I. Glass columns are necessary because stainless steel and aluminium tubing adsorb

TABLE I

Optimum instrumental operating conditions

| | | |
|---|---|---|
| Apparatus | Instrument | Bendix 2500 |
| | Detector type | Flame ionization |
| | Detector mode | Dual differential |
| | Detector setting | $1 \times 10^{-12}$ amps full scale |
| Columns | Length and diameter | 1.83 m × 6 mm o.d. (4 mm i.d.) |
| | Material | Glass U-tube |
| | Packing A column: | OV-7, 3% on Chromosorb W(HP) 80/100 mesh |
| | B column: | OV-1, 5% on Chromosorb W(HP) 100/120 mesh |
| Temperatures | Injection port | 190°C |
| | Detector | 195°C |
| | Column | 185°C |
| Flow rates | Carrier gas (argon) | 60 ml/min |
| | Hydrogen | 45 ml/min |
| | Air | 850 ml/min |
| Sample | Volume injected | 0.2 to 2.0 mcl |

VAN.[21] A number of liquid phases of differing polarity (OV-101, Apiezon L, OV-11, OV-17, OV-22, OV-25) in addition to the two finally chosen, were evaluated. The isothermal operating temperature of 185°C was chosen to minimize the total time of analysis without loss of resolution of the aldehyde peaks of interest from the solvent ($CH_2Cl_2$), reagent ($\phi NO_2$) and reaction product, nitrosobenzene (NOB), as shown in Figure 1. Precision of retention

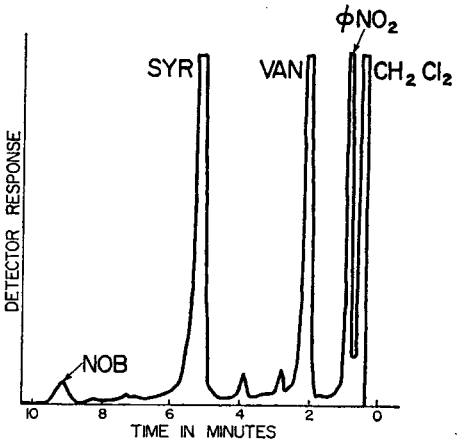


FIGURE 1 Resolution of phenolic aldehyde peaks (spruce wood standard).

times was $\leqq 1\%$ relative standard deviation. By chromatographing on each of two columns with liquid phases of differing polarity, the different retention times of the three aldehydes on each column provide a check on the identification. If doubt remained, samples were spiked with a small quantity of the pure compound and re-run.

Standard solutions (1 mcg/mcl) of the three aldehydes were used to evaluate the precision of the quantitative determination by GC. Duplicate injections

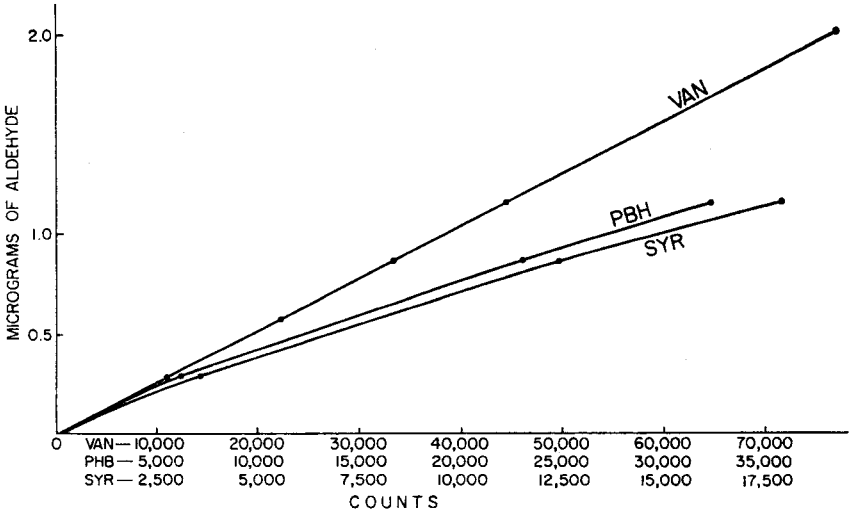


FIGURE 2 Linear response of detector to quantity injected on GC.

agreed with a mean coefficient of variation of $\leqq 3\%$. One microgram VAN injected gives *ca.* 40,000 $\mu$V sec response, and as 100 $\mu$V sec response gives acceptable statistics, the practical lower limit of detectability is *ca.* 0.0025 mcg VAN injected. Linear response of the detector to amount of aldehyde injected was established over the range from 0 to 2.0 mcg (Figure 2). From these quantitative results, the relative response was obtained and these were then used to convert the integrated signals from samples to mass of aldehydes injected. A direct calibration relating normalized detector response to mass of standard dry spruce wood put through the total procedure was also obtained (Figure 3).

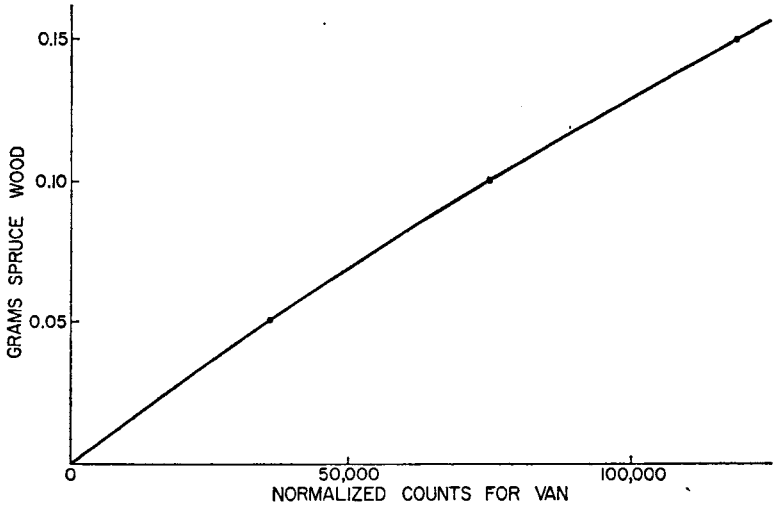


FIGURE 3 Spruce wood standard calibration curve.



## Field samples and blanks

Samples were taken on a recent cruise to the Gulf of St. Lawrence (Figure 4). Paper plants are located in Corner Brook, Nfld. (Station C-1), and at the head of the Saguenay River (Station 056). Results for wood standards and sediment samples are given in Table II and for filters in Table III. No standards or samples showed any PHB, which is essentially absent from hardwood and softwood lignins but substantial in grass lignins, and the one seawater sample processed gave only an indication of VAN which could not be confirmed.

Blanks were run as follows: The contents of the centrifuge tube (i.e. wood, sediment or filter, NaOH, $\phi NO_2$) were extracted with $CH_2Cl_2$. and aliquots of the concentrated extracts analyzed by gas chromatography to determine

TABLE II

Wood standards[a] and sediment[b] samples

| Sample identifier and location | | VAN (mg/g) | SYR (mg/g) | Total alde-hydes (mg/g) | Equiva-lent Spruce wood (ESW) (mg/g) | Lignin (28% of ESW) (mg/g) | Organic matter[c] (OM) (mg/g) | C/N (atoms) | Lignin as % OM | Depth to bottom (m) | T (°C) | S (‰) | O (ml/l) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lignum vitae | | 13.090 | 20.003 | 33.093 | 646 | 181 | 1000 | — | 18.1 | | | | |
| White spruce | | 20.483 | 0.650 | 21.133 | 1010 | 283 | 1000 | 1467 | 28.3 | | | | |
| Corner Brook Harbour | C–1 | 2.323 | — | 2.323 | 125 | 35.0 | 335 | 56.4 | 10.4 | 91 | — | 2.994 | — |
| | | 3.226 | — | 3.226 | 170 | 47.6 | | | 14.2 | | | | |
| | C–2 | 0.097 | — | 0.097 | 5.20 | 1.46 | 41.9 | 11.5 | 3.47 | 88 | — | 19.861 | — |
| Esquiman Channel | 024 | 0.086 | — | 0.086 | 4.63 | 1.30 | 43.4 | 10.3 | 2.99 | 349 | 4.96 | 34.430 | 2.95 |
| | | 0.039 | — | 0.039 | 2.08 | 0.58 | | | 1.34 | | | | |
| Anticosti Island | 034 | 0.096 | 0.059 | 0.155 | 5.13 | 1.44 | 19.7 | 11.5 | 7.29 | 194 | 2.06 | 34.431 | 5.17 |
| | | 0.121 | — | 0.121 | 6.45 | 1.81 | | | 9.17 | | | | |
| St. Lawrence Estuary | 053 | 0.088 | — | 0.088 | 4.70 | 1.32 | 14.0 | 43.0 | 9.42 | 139 | 0.38 | 30.466 | 7.16 |
| | | 0.073 | — | 0.073 | 3.95 | 1.11 | | | 7.90 | | | | |
| Upper Saguenay R. | 056 | 0.584 | 0.022 | 0.606 | 32.3 | 9.04 | 71.3 | 43.0 | 12.7 | 97 | 0.79 | 29.685 | 5.21 |
| Lower Saguenay R. | 060 | 0.129 | — | 0.129 | 6.88 | 1.93 | 22.1 | 22.3 | 8.72 | 230 | 1.49 | — | 5.21 |

[a] Each value is the mean of six replicates; coefficient of variation 3–5%.
[b] Means of duplicate analyses; coefficient of variation 5–15%.
[c] From organic carbon analysis, assuming C to be 53% of the OM.

TABLE III

Filtered water samples

| Station identifier and depth | VAN (mcg/l) | SYR (mcg/l) | Total aldehydes (mcg/l) | Equivalent spruce wood (ESW) (mcg/l) | Lignin (28% of ESW) (mcg/l) | Particulate[a] organic matter (POM) (mcg/l) | Lignin as % POM | T (°C) | S (‰) | $O_2$ (ml/l) |
|---|---|---|---|---|---|---|---|---|---|---|
| C-1 (1 m) | 2.69 | — | 2.69 | 160 | 44.8 | 2500 | 1.79 | — | 2.994 | — |
| 024 (295 m) | 2.51 | — | 2.51 | 130 | 36.4 | — | — | 4.95 | 34.429 | 2.96 |
| 054 (1 m) | 1.86 | 0.44 | 2.30 | 100 | 28.0 | — | — | 13.54 | 2.360 | 6.51 |

[a] From particulate organic carbon analysis, assuming C to be 53% of the POM.

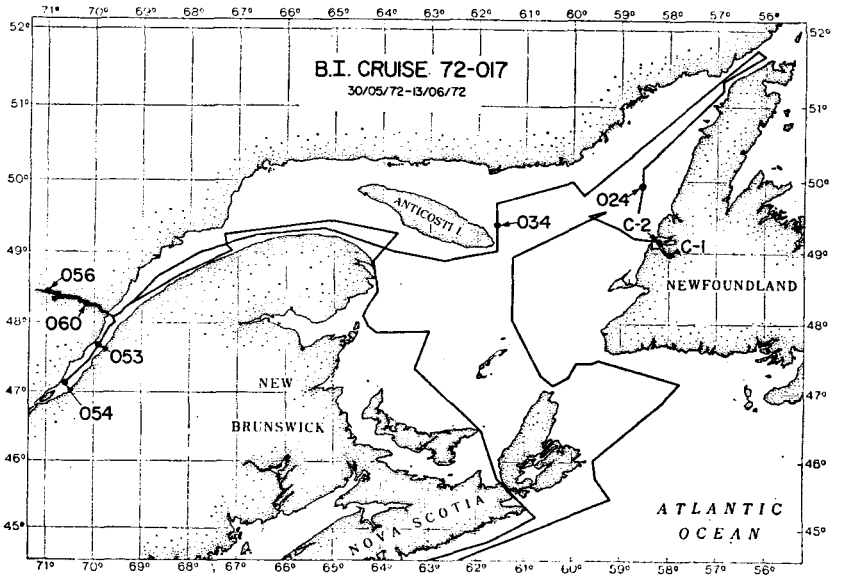


FIGURE 4 Cruise track with station locations.

any pre-existing aldehydes. None was found.[11] NaOH and $\phi NO_2$ were reacted together in a sealed tube for 4 hr at 170°C. The contents were extracted with $CH_2Cl_2$ and analyzed for derived aldehydes by the gas chromatographic method. None was found.

Silver filters, glass-fibre filters and cellulose ester filters (Millipore) were put through the oxidation procedure and analyzed by gas chromatography. Only the cellulose ester filters gave any aldehydes after alkaline nitrobenzene oxidation. Therefore these filters were not used for the collection of samples for lignin analysis.

## RESULTS AND DISCUSSION

The prime problem in interpreting the results lies in relating the amount of phenolic aldehydes produced to the amount of lignin present in the sample. Values, for example, of vanillin content range from 5 to 24% in "lignin" depending upon the various postulated structures.[3,22] We related vanillin produced by alkaline nitrobenzene oxidation to the equivalent quantity of whole dry spruce wood which was then related to lignin content, the literature being in better agreement on this point.[20,23,24] Lignin content was taken as 28% of the weight of air-dried spruce wood. If a direct conversion based on vanillin being obtained from spruce wood lignin in 25% yield is used, then

values for lignin approx. one-quarter of those given here would result. Our results imply a yield of vanillin from lignin of 6–7%. On this basis, whereas white spruce returned 100% as "Equivalent Spruce Wood", our hardwood standard returned only 64.6% ESW. Therefore, this calibration underestimates the contribution of hardwood by one-third. The value of 18.1% for the lignin content of Lignum vitae derived by this method seems reasonable as the range in angiosperms is (as previously mentioned) from 16–24%. The SYR/VAN molar ratio of 1.28 is in the range characteristic of hardwood lignin (0.35–5.2) although the total aldehydes as per cent lignin are approx. half the literature values. This combined with our lower yield from coniferous wood implies a more efficient conversion of syringyl units to SYR than of guaiacyl units to VAN in the oxidation.

The Corner Brook Harbour sediment sample C-1 and its duplicate were taken very close to a paper plant (*ca.* 100 m) in water one-tenth as saline as seawater. The highest concentration of VAN was produced on oxidation of these samples. This is not surprising as the sediment is one-third organic matter of which lignin is calculated to constitute 10–14%. The high C/N ratio implies much non-living material of high carbon and low nitrogen content, for which lignin is a suitable candidate. When compared with the results obtained at other stations where man-made addition of lignin is less likely, the higher organic content, high C/N ratio and high proportion attributable to lignin imply addition of this material to the sediments as a consequence of the nearby industrial activity. Similarity with the sample from the upper Saguenay River where forest industries are located should also be noted.

Station C-2, taken nearer the sea ($S \simeq 20^{o}/_{oo}$), although still high in organic matter (4%) showed a lower percentage (3.5%) attributable to lignin. The C/N ratio is in the range of that of organic matter in marine sediments (5–16).[25]

The duplicate samples taken at Station 024 in the Esquiman Channel illustrate first of all the inhomogeneity of these sediments. Organic matter and C/N ratio are approx. the same as at Station C-2, but the calculated contribution of lignin is from 1–3% in adjacent samples. The oxygen content of the water above the sediment was low, implying an environment of deposition of high BOD and/or poor flushing characteristics, probably the latter.

The two samples run from the station off the southeast coast of Anticosti Island (#034) showed better mutual agreement, lignin being 7.3–9.2% of an organic matter content (2%) characteristic of the general background level in marine sediments affected by influx from the land. The SYR/VAN molar ratio (0.5) in one of these samples is in the range characteristic of angiosperms.

The duplicate samples from #053 agree well. The lignin content is 7.9–9.4% of an organic content of 1.4%, the lowest in all the samples and the C/N ratio is in the range normal for marine sediments.

The two stations in the Saguenay show the same sequence as the two in Corner Brook Harbour and approaches. The sample from the head of the river (#056) where forest industry plants are located shows lignin as 13% of a highly organic sediment (7%) which has a high C/N ratio. These are the same characteristics found for the Corner Brook samples. The sample taken downstream has less lignin (9%) in a less organic (2%) sediment, though the C/N ratio is still moderately high. The SYR/VAN ratio of the sample from the upper river is identical with that of spruce wood (0.03).

No gross differences are shown between Corner Brook Harbour, Esquiman Channel and brackish water in the St. Lawrence Estuary (054) as regards phenolic aldehydes produced on oxidation of filters through which seawater had been passed. The low SYR/VAN ratio of #054 is characteristic of conifers rather than hardwoods. There appears to be a level of material attributable to lignin in all three water bodies of *ca.* 20–60 mcg/l, which is more homogeneous than within sediments from these locations. The inference is that although roughly equivalent quantities of this material are in suspension, its rate of addition to and/or fate within sediments differs considerably.

The general conclusion is that this method does (1) identify lignin through the products of nitrobenzene oxidation, (2) can be calibrated to give a quantitative estimate, and (3), when applied to field samples, gives results for lignin content that are reasonable in terms of total organic matter and C/N ratio. The particular conclusion is that industrial activity in Corner Brook Harbour and the upper Saguenay River contributes organic matter of high C/N ratio, a high proportion of which can be attributed to lignin, to the sediments in the immediate area but the amount of this material contributed to the Gulf of St. Lawrence as a whole does not appear to be excessive.



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