



**DEPARTMENT OF  
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**Technical Report No. 31**

**DETERMINATION AND DISTRIBUTION OF LIGNIN  
IN  
MARINE SEDIMENTS**

**Office of Naval Research**

**Contract N8onr-520/III**

**Project NR 083 012**

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UNIVERSITY OF WASHINGTON DEPARTMENT OF OCEANOGRAPHY  
(Formerly Oceanographic Laboratories)  
Seattle, Washington

DETERMINATION AND DISTRIBUTION OF LIGNIN  
IN MARINE SEDIMENTS

by

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## ABSTRACT

An investigation of the lignin content of marine sediments has been completed on 31 surface samples and 26 samples obtained from 2 cores. It has been found that this lignin is an extremely variable fraction of the sedimentary organics. In view of its great stability and its negligible value as a food source, it is important to consider this fraction when evaluating the nutritional value of marine sedimentary organics for bottom dwelling fauna.

Soil scientists and lignin chemists have shown that the decomposition rate of lignin is very slow and that it accumulates in the soil. On the basis of the two cores examined, the percent loss of non-ligninous carbon is about 6 times the loss of "lignin" carbon. Apparently lignin is extremely stable in the marine environment.

The lignin content of the sediments is important from a geological standpoint. Knowledge of its concentration in sediment cores permits detail and accuracy in paleoecological interpretation which is not possible by use of total organic carbon alone. It is potentially useful as a tool in studies of rates of sedimentation, however we must be able to determine whether it is dependent or independent of the deposition of inorganic particles.

## INTRODUCTION

The presence of lignin in marine sediments was first reported by Trask (1932) and Waksman (1933). The writer (Bader, 1954) studied some surface concentrations of lignin in sediments from the Gulf of Maine. In general very little effort has been expended in studying the distribution of this organic complex in the marine environment, although its characteristics make it an interesting subject for investigation.

Lignin is a very resistant organic complex, only formed by the living plant (Bergenstein, in Brauns, 1952) and never by enzymatic or bacterial decay of other plant products. Waksman and Tenny (1926, 1927, and 1929) and Brauns (1952) as well as other investigators all agree that the anaerobic or aerobic bacterial decomposition of lignin is extremely slow. Waksman and Stevens (1929 and 1929a) found that in soils, under anaerobic conditions, lignin is decomposed in traces or not at all. Under an aerobic environment its breakdown is very slow, making it the most resistant of all common plant materials. Acharya (1935 and 1935a) showed that the decomposition rate of lignin is slow and relatively constant for both aerobic and anaerobic conditions. It is also possible that in the microbiological decomposition of plant materials, lignin may act as an inhibitory factor (Rege, 1927; Waksman and Lyre, 1932; Fuller and Norman, 1943). This is accomplished by the formation of a stable ligno-protein complex.

This investigation was undertaken in view of the apparent presence of lignin in marine sediments and its extreme stability. In general three aspects were examined: (1) its distribution and variation in some

marine sediments, (2) its stability in subsurface samples, and (3) its relationship with the remaining organics.

#### METHOD OF ANALYSIS

Due to the complexity of the lignin molecule and its resistance to solution, the lignin analysis is necessarily accomplished in an indirect manner. The lack of a truly quantitative method of determination, when it is mixed with other organics, requires the removal of the non-ligninous material from the sediments. The presence of other organics interferes with the lignin determination (Waksman, 1933; Norman, 1937; and Brauns, 1952).

In order to remove the non-ligninous organics, an extraction and hydrolysis series was used. Previously dried, crushed and powdered sediment samples were treated with hot acetone in a Soxhlet extraction apparatus for 48 hours. The sediment was then vacuum filtered, washed with hot acetone and transferred to boiling alcohol. Refluxing with 3 changes of solvent was continued for 24 hours. This was followed by vacuum filtering and a thorough washing with hot alcohol. The first hydrolysis was accomplished by the use of boiling 4% HCl. After 24 hours, the sample was vacuum filtered and washed thoroughly with distilled water. The final treatment involved the use of 72% H<sub>2</sub>SO<sub>4</sub>. The sediment sample was placed in the H<sub>2</sub>SO<sub>4</sub> and kept at 8° C for 3 hours. This solution was then diluted to 4% H<sub>2</sub>SO<sub>4</sub> and boiled for 4 hours. After vacuum filtering and washing in distilled water the samples were dried, powdered and thus made ready for the determination of "lignin" carbon. The residual material after the H<sub>2</sub>SO<sub>4</sub> treatment is lignin and its trans-

formation products as well as nitrogenous compounds such as synthesized microbial ligno-protein complexes.

The total organic carbon and the "lignin" carbon were determined by a semi-micro dry combustion method. The samples, dried to constant weight, were combusted in a measured volume of oxygen under controlled pressure. The evolved CO<sub>2</sub> was absorbed, determined gravimetrically and the percent carbon computed. In determining the total organic carbon the sample was first treated with hot, 4% HCl in order to remove carbon from carbonates. The use of such dilute HCl does not cause any loss of carbon by CO<sub>2</sub> evolution from the organic matter. Carbon determinations by this method are reproducible to + 0.03%.

#### RESULTS OF ANALYSIS

The distribution of total organic carbon, "lignin" carbon, and the non-ligninous carbon was calculated for 31 surface samples of sub-aqueous marine sediments off the coast of Maine. The results are shown in Table 1. The total carbon content of these samples ranges from 4.74% to 0.30% with a mean of 1.42%. The "lignin" carbon ranges from 2.76% to 0.05%, having a mean of 0.73%. The standard deviation of this fraction is 0.25 with a coefficient of variation for one standard deviation of 34.2%. The non-ligninous carbon content of the 31 samples varies from 2.26% to 0.17% with a mean of 0.69%. The standard deviation is 0.15 and the coefficient of variation, 22.7%. The percent of "lignin" carbon in the total organic carbon content shows a similarly wide range, from 71.6% to 14.7% with a mean of 43.0%. The standard deviation is 16.8 and the coefficient of variation is 39.0%.

TABLE 1

SURFACE SAMPLE DATA<sup>1</sup>

| Sample No. | Total Carbon (%) | Lignin Carbon (%) | △* Carbon (%) | LC/TC x 100 | Sample No. | Total Carbon (%) | Lignin Carbon (%) | △* Carbon (%) | LC/TC x 100 |
|------------|------------------|-------------------|---------------|-------------|------------|------------------|-------------------|---------------|-------------|
| 1          | 0.88             | 0.38              | 0.50          | 43.2        | 16         | 4.74             | 2.48              | 2.26          | 52.3        |
| 2          | 1.72             | 0.66              | 1.06          | 38.4        | 17         | 4.12             | 2.56              | 1.56          | 62.1        |
| 3          | 0.52             | 0.18              | 0.34          | 34.6        | 24         | 0.34             | 0.09              | 0.25          | 26.5        |
| 4          | 1.23             | 0.85              | 0.38          | 69.1        | 25         | 0.30             | 0.07              | 0.23          | 23.3        |
| 5          | 0.94             | 0.40              | 0.50          | 42.6        | 26         | 1.96             | 1.25              | 0.71          | 63.8        |
| 6          | 0.42             | 0.18              | 0.24          | 42.8        | 27         | 2.13             | 1.07              | 1.06          | 50.2        |
| 7          | 0.45             | 0.20              | 0.25          | 44.4        | 28         | 1.86             | 1.00              | 0.86          | 53.8        |
| 8          | 0.66             | 0.31              | 0.35          | 47.0        | 29         | 2.12             | 1.28              | 0.84          | 60.4        |
| 9          | 1.02             | 0.73              | 0.29          | 71.6        | 30         | 2.34             | 1.44              | 0.90          | 61.5        |
| 10         | 0.46             | 0.21              | 0.25          | 45.7        | 31         | 1.78             | 0.32              | 1.46          | 18.0        |
| 11         | 0.35             | 0.07              | 0.28          | 20.0        | 32         | 0.26             | 0.09              | 0.17          | 34.6        |
| 12         | 1.74             | 0.69              | 1.05          | 39.7        | 33         | 1.38             | 0.48              | 0.90          | 34.8        |
| 13         | 1.15             | 0.38              | 0.77          | 33.0        | 34         | 0.34             | 0.05              | 0.29          | 14.7        |
| 14         | 0.49             | 0.16              | 0.33          | 32.7        | 35         | 0.87             | 0.15              | 0.72          | 17.2        |
| 15         | 4.12             | 2.76              | 1.36          | 67.0        | 36         | 3.17             | 2.11              | 1.06          | 66.6        |
|            |                  |                   |               |             | 37         | 0.37             | 0.08              | 0.29          | 21.6        |

<sup>1</sup>Calculated from data given by Bader (1954).

\*Total carbon - "lignin" carbon = non-ligninuous or △ carbon

TABLE 2

## SUBSURFACE SAMPLE DATA

| Sample No. | Depth (cm) | Total Carbon (%) | Lignin Carbon (%) | △* Carbon (%) | LC/TC x 100 | Sample No. | Depth (cm) | Total Carbon (%) | Lignin Carbon (%) | △* Carbon (%) | LC/TC x 100 |
|------------|------------|------------------|-------------------|---------------|-------------|------------|------------|------------------|-------------------|---------------|-------------|
| 169        | 0          | 1.02             | 0.66              | 0.36          | 64.7        | 221-C      | 0          | 1.96             | 1.16              | 0.80          | 59.2        |
|            | 6          | 0.94             | 0.64              | 0.30          | 68.1        |            | 7          | 1.63             | 1.08              | 0.55          | 66.3        |
|            | 15         | 0.92             | 0.63              | 0.29          | 69.2        |            | 16         | 1.56             | 1.18              | 0.38          | 75.7        |
|            | 30         | 0.37             | --                | --            | --          |            | 32         | 1.93             | 1.11              | 0.82          | 57.6        |
|            | 43         | 0.09             | 0.06              | 0.03          | 66.6        |            | 43         | 1.71             | 0.98              | 0.73          | 57.3        |
|            | 55         | 0.36             | --                | --            | --          |            | 62         | 2.09             | 0.90              | 1.19          | 43.0        |
|            | 65         | 0.79             | 0.64              | 0.15          | 81.0        |            | 74         | 2.38             | 1.50              | 0.88          | 63.0        |
|            | 85         | 0.76             | 0.62              | 0.14          | 81.6        |            | 97         | 1.97             | 1.02              | 0.95          | 51.8        |
|            | 110        | 0.74             | 0.47              | 0.27          | 63.4        |            | 115        | 2.27             | 1.08              | 1.19          | 47.6        |
|            | 115        | 0.74             | 0.69              | 0.05          | 93.2        |            | 125        | 1.78             | 1.49              | 0.29          | 83.7        |
|            | 125        | 0.79             | --                | --            | --          |            | 150        | 2.00             | 1.37              | 0.63          | 68.5        |
|            | 134        | 0.78             | --                | --            | --          |            | 155        | 1.98             | 1.16              | 0.82          | 58.5        |
|            | 140        | 0.77             | 0.60              | 0.17          | 77.9        |            |            |                  |                   |               |             |
|            | 148        | 0.85             | 0.64              | 0.21          | 75.3        |            |            |                  |                   |               |             |

\*Total carbon - "lignin" carbon = non-ligninuous or △ carbon.

The subsurface data obtained from 2 cores of Puget Sound sediments is presented in Table 2. Core No. 169 is primarily composed of inorganic particles of a relatively uniform size in the silt range. A noticeable particle size change occurs between 15 and 65 cm. This particle size change is gradational and is represented by fine sands at 43 cm., the total organic carbon correspondingly decreases in this sand layer with a minimum at 43 cm. The remaining variations in the organic content are not associated with particle size changes. The maximum concentration of total carbon and non-ligninous carbon occur on the surface. With some minor variations the percent "lignin" carbon of total carbon increases with depth.

Core No. 221-C consists entirely of relatively uniform silt sized particles. The fluctuations in organic content are not associated with any particle size variations. The maximum concentration of total organic carbon is at 74 cm. For the immediate surface region, the percent "lignin" carbon of the total carbon increases with depth; however below 16 cm. this characteristic is not evident.

Figure 1 gives a visual presentation of the variations in total carbon, "lignin" carbon and non-ligninous carbon with depth for both cores. When comparing the concentrations of "lignin" and non-ligninous carbon in the immediate surface sediments (0 to 6-7 cm.) it is obvious that in both cores the "lignin" carbon decreases less rapidly with depth than the non-ligninous carbon. Core No. 169 has a lignin carbon loss of 3.0% and a non-lignin carbon loss of 16.6% in the top 6 cm. Core 221-C has a 6.8 lignin carbon loss and non-lignin carbon loss of 52.5% in the top 7 cm. It is not unreasonable to assume that environmental conditions

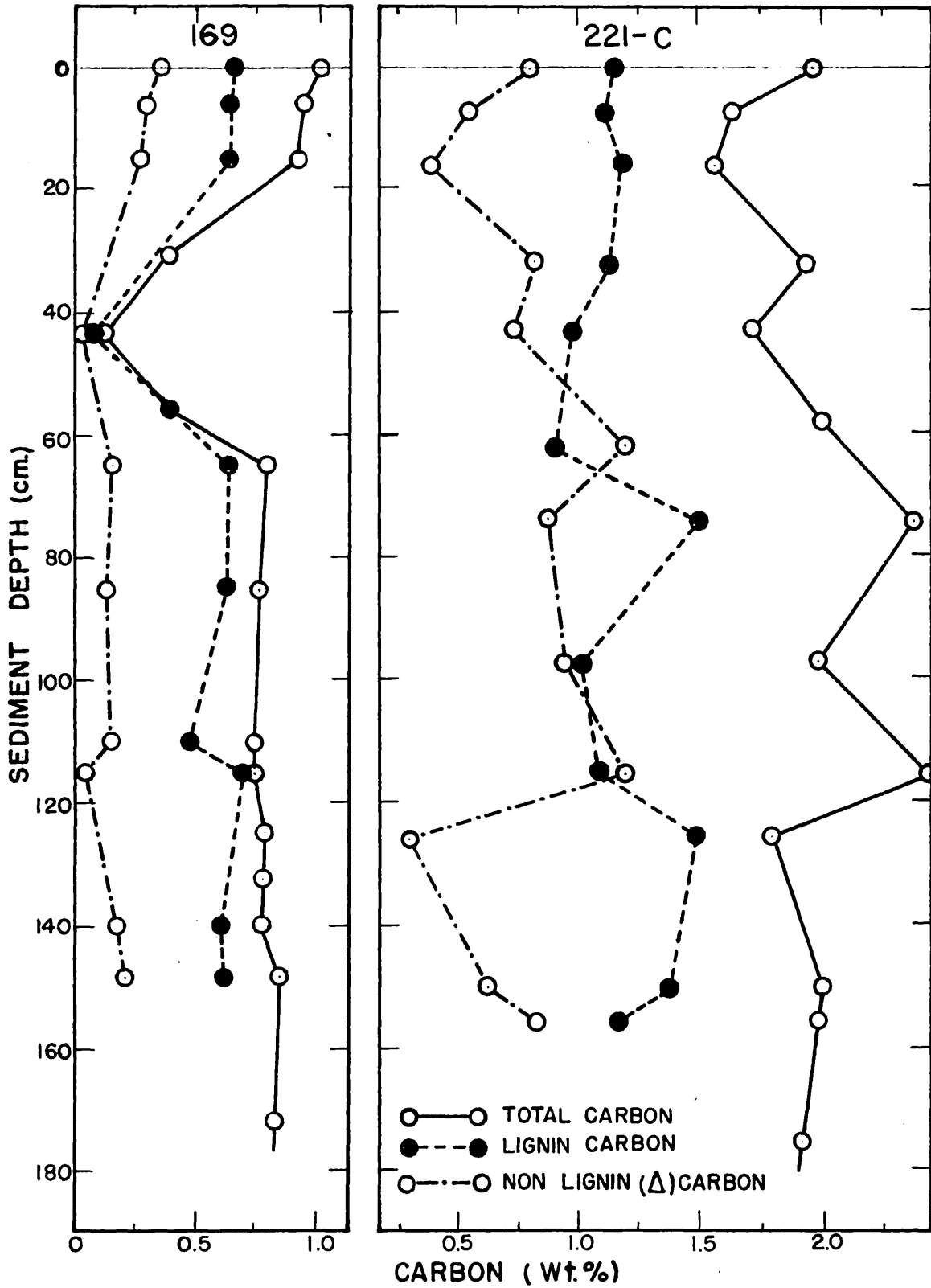


FIGURE 1. Vertical distribution of total organic carbon, "lignin" carbon and non-lignin carbon in two cores of Puget Sound Sediments.

have remained relatively constant during the period of deposition of these 6 to 7 cm. of sediments and that the organic decrease is thus due to decomposition. If such is the case, the percent loss of non-ligninous carbon is 5.5 and 7.7 times the percent loss of "lignin" carbon in the immediate surface zone of the two cores involved.

#### DISCUSSION

The interest in the problem of the lignin content of marine sediments is twofold. It has a biologic and geologic significance, both of which are dependent upon its apparent resistance to decomposition.

The biological importance of this substance is rather obvious. It is not a useful source of nutrition for marine organisms. If the organic debris in the marine sediments is a potential source of food for bottom-living invertebrates, and this is apparently the case for many forms, then we must consider the concentration of various organic fractions and their availability for nutritional utilization. If one sediment consists of 4% organic material, 55% of which is lignin, and another sediment only 2% organics, 10% of which is lignin, the nutritional value for both sediments is equal. If other conditions are similar they should be able to support comparable populations. From the surface samples examined it can be seen that the concentration of lignin carbon to total carbon is extremely variable; the coefficient of variation is 39% for one standard deviation. In view of this, if we consider the total organics as being indicative of potential nutrient source our postulations may well be in error. Secondly, if we have some idea as to the supply of the organic material to the sediments we then have a

substance, lignin, which may be used as a basis for comparing the degree or amount of organic decomposition which has or is occurring in any group of samples. This is surely of importance since microbiological activity on organic material is to be considered an ecologic factor. The basis for this statement rests on the extremely stable character of lignin as shown by soil scientists and lignin chemists. The indication that in the immediate surface zone (0 to 7 cm.) the percent loss of non-ligninous carbon is 5 to 7 times greater than the percent loss of lignin carbon strongly supports the opinion that lignin is stable in the marine environment.

Geologically this substance may be utilized as a tool for assisting in the reconstruction of past environments of modern marine sediments. This may deal with both the biological environment or the depositional environment. If upon the examination of a sediment core it is found that for any specific depth range the total organic content continuously decreases we may draw certain conclusions. It may be that the rate of sedimentation of inorganic particles increased, thus diluting the available organics. A second possibility is that the supply of organics to the sediments continuously decreased with time. A third postulation may be that a continuous increase in the rate of microbiological decomposition with time occurred. Such a situation is shown in Core 221-C between 74 and 43 cm. depths. The total organic carbon decreases steadily upward in the core. From this we can conclude some sort of an environmental situation which varied constantly with time. However, upon examination of the "lignin" and non-ligninous carbon concentrations for

the same depth range such a simple conclusion is not possible. The lignin carbon concentration decreases appreciably from 74 to 62 cm. The non-lignin carbon increases over this depth range. From 62 to 43 cm. the lignin carbon remains essentially the same, increasing very slightly; the non-lignin carbon, however, decreases sharply similar to the total carbon decrease. Similar situations occur between 97 to 74 cm. and 155 to 150 cm.

Core 169 shows a very slightly but continuously increasing total carbon content from 115 to 65 cm. The lignin carbon and thus non-lignin carbon do not show this straight line increase.

From the data obtained for the immediate surface zone (0-7 cm.) a speculative statement, that changes in lignin carbon exceeding approximately 1 to 2 % per cm. are due to supply changes, may be made. We may assume that the previously mentioned changes in lignin content are due to changes in supply; only minor fluctuations may be attributed to variations in decomposition rates.

#### CONCLUSION

1. The "lignin" content of marine sediments is extremely variable. It does not represent a constant proportion of the total sedimentary organics.

2. When considering the nutritional value of marine sedimentary organics to bottom dwelling fauna, the lignin content is an important factor. This is based on its variable proportions in the total organic material and its negligible value as a food source.

3. Apparently, under the marine environment, lignin is an organic compound displaying extreme resistance to microbiological decomposition,

This is based on the work of soil scientists and lignin chemists as well as the very slight decrease in the "lignin" carbon of surface zone sediments on the basis of the two cores examined. The percent loss of non-ligninuous carbon is approximately 6 times greater than the "lignin" carbon loss. Lignin carbon changes which exceed 1 to 2 % per cm. may thus be ascribed to changes in the amount supplied to the sediments. Further work must be done in order to verify this.

4. Data on the concentration of carbon from ligninuous sources in marine sediments gives further information on which to base any paleo-ecologic conclusions. By considering the total carbon alone it is possible to conclude the occurrence of a constant environment with time which an associated lignin carbon determination would refute.

5. The determination of the carbon fraction termed lignin carbon in this report is time-consuming and thus is not practical for general purposes. It may prove useful for specific and critical investigations where some detail is desirable or necessary.

6. Before further conclusions can be drawn it is necessary for continued research on the lignin fraction of marine sediments. The determination must be made more positive; studies concerning the ultra-violet spectra and the concentration of the methoxyl group are planned. Its decomposition, relative to other organics, must be studied as well as the various sources of the substance in the marine environment.

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