# MEASUREMENT OF THE POLYSACCHARIDE CONTENT OF SOILS[1]

C. J. Acton, E. A. Paul and D. A. Rennie[2]

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## ABSTRACT

The polysaccharide content of two soils, measured by precipitating and weighing the microbial gums from the fulvic acid fraction of soil organic matter, and by colorimetric analyses using anthrone, was increased appreciably during the incubation of samples with straw. Applications of nitrogen and phosphorus altered only slightly the amount of polysaccharides present. The polysaccharide content of the amended soils increased rapidly during the first week of incubation and then levelled off.

Peptization of the soil with dilute alkali prior to acid hydrolysis substantially increased the carbohydrates measured with anthrone. The anthrone reagent which measures primarily hexoses indicated a soil carbohydrate content of 10-15 per cent occurring in a range of Saskatchewan soils. Thirty-five per cent more carbohydrate carbon was found using the less specific phenol-sulphuric acid reagent.

The acetone precipitated fraction from the fulvic acids accounted for only 10 per cent of the soil carbohydrates and contained a large proportion of ash and other non-carbohydrate materials. The total acid-soluble base-soluble fraction, fulvic acids, contained 15-25 per cent; the remainder was distributed in the alkali insoluble humin — 55-70 per cent and the humic acid fraction 4-12 per cent.


## INTRODUCTION

The isolation and quantitative measurement of polysaccharides from soils has been reported by several workers using a gravimetric procedure (5, 18, 19). This usually involved precipitation of the microbial gum from an acidified fulvic acid fraction of organic matter by the addition of acetone followed by drying and weighing of the dehydrated gum.

Polysaccharide materials in soils also have been determined quantitatively employing colorimetric techniques. MacLean and DeLong (16) first applied the anthrone reagent (6) to the measurement of carbohydrate material in a soil system. Brink, Dubach and Lynch (4) concluded that a 0.2 per cent solution of anthrone gave a relative measure of the total polysaccharide content present in acid hydrolysates of soil. Lynch and co-workers (11, 14, 22) have used this technique plus carbazole and orcinol reagents for the estimation of total polysaccharides, uronides, and pentoses, respectively in a number of soils. In addition, the soil amino sugars have been quantitatively measured colorimetrically in soils by workers such as Sowden (20), Bremner (3) and Stevenson (21).

Chromatographic separation and identification of the soil polysaccharide constituents by workers such as Bernier (1) and Whistler and Kirby (23) has indicated that these substances contain primarily galactose, glucose, mannose, arabinose, rhamnose, and ribose, plus amino sugars and methylated sugars. Interpretation of the role of polysaccharide substances as constituents of soil organic matter and as aggregating agents is, however, difficult because of variations in methods employed in their estimation by both colorimetric and gravimetric techniques.

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[1]Contribution from the Department of Soil Science, University of Saskatchewan, Saskatoon, Sask. This paper is based in part on a thesis submitted in partial fulfilment of the requirements of the M.Sc. degree, University of Saskatchewan.

[2]Research Officer, Pedology Section (Saskatoon Research Station); and Assistant and Associate Professors, respectively.

The quantitative estimation of soil polysaccharides generally has dealt with the fulvic acid portion of soil organic matter (10, 11, 18, 23). Polysaccharide materials have, however, also been reported in the acid insoluble portion, humic acids (13, 14), and in the alkali-insoluble humin (9). The relative distribution of polysaccharides in these fractions of soil organic matter in amended and non-amended soils is, however, unknown. This study was therefore initiated to measure the effect of organic amendments on carbohydrate constituents in the soil and in various fractions thereof, by both gravimetric and colorimetric techniques.

## MATERIALS AND METHODS

### *Soils*

Soil samples were obtained from the Aa horizon of the Orthic Black sub-group profile of the Oxbow and Melfort associations, the Calcareous Black profile of the Yorkton association, the Orthic Dark Brown profile of the Elstow association, and the Dark Brown Solodized Solonetz profile of the Trossachs association. A sample also was taken from the eluviated Ae horizon of an uncultivated Low Humic Eluviated Gleysol (bluff podzol) profile belonging to the Oxbow association. These soil associations are further described in the Saskatchewan Soil Survey Report No. 12 (17).

### *Measurement of Microbial Gum*

Wheat straw at a rate equivalent to 20 tons per acre, and $NH_4NO_3$ at rates equivalent to 100, 300 and 600 pounds N per acre were mixed with Trossachs clay loam, sieved to 2 millimeters in size. The soil samples were incubated in the dark in 50 × 100-millimeter crystallization dishes in a constant temperature growth chamber at 63 ± 2°F., and the moisture content maintained at field capacity. Glass wool was placed over the surface of the soil to prevent crusting on watering and to reduce evaporation.

The method used for the extraction and gravimetric measurement of microbial gum is essentially the procedure described by Rennie *et al.* (18). The nitrogen content of the microbial gum was determined by the micro-Kjeldahl method. Total ash was determined by heating a dried sample of the gum in a muffle furnace for 60 minutes at 550°C.

The carbohydrate content of the microbial gum was measured by adding 5 milliliters of 0.5 *N* NaOH to 0.05 gram of dried gum ground to 0.1 millimeter in size. The suspension was allowed to stand for 1 hour and then titrated with 3 *N* $H_2SO_4$ to the equivalence point, and an excess of 5 milliliters 3 *N* $H_2SO_4$ added. The solution was hydrolyzed for 24 hours at 85°C. and carbohydrates determined by the anthrone method.

### *Colorimetric Measurement of the Carbohydrate Content of Soil*

Ten grams of soil were dispersed in 40 milliliters of 0.5 *N* NaOH for 10 minutes in a Waring blendor, transferred to Erlenmeyer flasks by means of an additional 60-milliliter NaOH, and shaken for 3 hours. The suspension was then adjusted with concentrated HCl to a pH value of 2-3 and the fulvic acids separated from the humic acids + soil colloids by centrifuging. The humic acids + soil colloids remaining in the centrifuge tube were washed with acidified water (10 milliliters concentrated HCl in 400 milliliters $H_2O$), the centrifugation repeated and the supernatant added to the

TABLE 1. — EFFECT OF SOIL TREATMENT AND PERIOD OF INCUBATION ON THE GUM CONTENT OF TROSSACHS SOIL

(gm./100 gm. soil)

| Soil amendment | C:N ratio of straw | Time in weeks | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 |
| None | | 0.20 | 0.17 | 0.18 | 0.21 | 0.17 | 0.21 |
| Straw | 60:1 | 0.31 | 0.24 | 0.21 | 0.21 | 0.21 | 0.26 |
| Straw + $P_2O_5$ | 60:1 | 0.28 | 0.19 | 0.24 | 0.25 | 0.21 | 0.24 |
| Straw + $P_2O_5$ + low N | 45:1 | 0.28 | 0.20 | 0.21 | 0.22 | 0.22 | 0.23 |
| Straw + $P_2O_5$ + medium N | 30:1 | 0.29 | 0.21 | 0.21 | 0.23 | 0.20 | 0.23 |
| Straw + $P_2O_5$ + high N | 20:1 | 0.27 | 0.22 | 0.21 | 0.25 | 0.20 | 0.22 |

previous extract. The supernatant (fulvic acids) was diluted to volume and two volumes of acetone were added to one volume of an aliquot of the solution. The supernatant solution, after centrifuging, comprised the acetone soluble fraction. The acetone was removed by evaporation to dryness on a steam bath, and the residue dissolved in water. The flocculent precipitate designated as microbial gum was re-dissolved in 0.5 *N* NaOH and also adjusted to volume.

Organic matter fractions were hydrolyzed using sufficient $H_2SO_4$ to give a solution equivalent to 100 milliliters 3 *N* $H_2SO_4$. Polysaccharide constituents were then estimated with anthrone (4). The carbohydrate-carbon content of hydrolyzed fractions of humin, humic acids, and fulvic acids were also determined using the phenol-sulphuric acid method outlined by Dubois *et al.* (7).

The sugars in the hydrolyzed humin fraction were purified by removing the $H_2SO_4$ with $Ba(OH)_2$ and desalted by passing the hydrolysate through an anion exchange resin, Dowex 2-x8 (acetate cycle) then the cationic resin Amberlite 1R-120 (H+ cycle). Paper chromatograms were developed with 1-butanol : acetic acid : water = 40:10:22 v/v and 1-butanol : pyridine : water = 125:40:125 v/v. The spots were detected with aniline phthalate (2).

## RESULTS

Amendment of the soil with wheat straw, and incubation resulted in substantial accumulations of microbial gum (Table 1). The gum concentrations were at a maximum level after 1 week of incubation regardless of the soil treatment, with the gum content varying randomly with time after the first week. A similar trend was reported by Rennie *et al.* (18) where the gum content of a Spencer silt loam soil increased during the first 6 days of in-

TABLE 2. — COMPARISON OF THE MICROBIAL GUM AND POLYSACCHARIDE CONTENT OF SOILS AND THE EFFECT OF SODIUM HYDROXIDE PRE-TREATMENT ON THE POLYSACCHARIDES EXTRACTED FROM SOME SASKATCHEWAN SOILS

(Carbohydrates as glucose — gm./100 gm. soil)

| Soil | Percentage organic matter | Percentage of microbial gum in the soil | Percentage of polysaccharides in the soil Ratio-soil: $3N$ $H_2SO_4$ | | | |
|---|---|---|---|---|---|---|
| | | | 1:10 | | 1:20 | |
| | | | Peptized | Not peptized | Peptized | Not peptized |
| Yorkton | 5.6 | 0.78 | 0.87 | 0.78 | 0.84 | 0.74 |
| Oxbow | 4.4 | 0.14 | 0.55 | 0.49 | 0.57 | 0.49 |
| Melfort | 9.8 | 0.48 | 1.07 | 0.86 | 1.03 | 0.98 |
| Trossachs | 4.4 | 0.21 | 0.72 | 0.56 | 0.67 | 0.61 |

cubation after addition of organic materials, and then declined for the remainder of the experiment. Addition of inorganic fertilizer to the straw slightly decreased the microbial gum content of the soil from that of the straw amendment alone.

The acetone precipitation of materials designated as microbial gums from a NaOH or water extract of soil, although it has been used by a fairly large number of investigators (5, 18, 19), has the disadvantages that it is time-consuming and does not lend itself to routine analyses. The precipitate varies in chemical composition and does not lend itself to chromatographic identification of the sugar constituents. The results obtained by this technique were, therefore, compared to some of the colorimetric data obtained in investigating the effect of NaOH peptization of soils before hydrolysis (Table 2).

The concentration of polysaccharides in the Yorkton soil was similar when measured gravimetrically and colorimetrically. This was, however, not true for the Melfort, Trossachs and Oxbow soils. This suggests that the amount of non-carbohydrate material present in the microbial gum precipitate varied with the soil type. The microbial gums were found to contain an average of 67.5 per cent ash. The flocculent precipitate obtained in this determination was not purified, which possibly accounts for this exceptionally high value. Halstead (12) purified the flocculent precipitate by washing with distilled water, reflocculating with acetone and centrifuging until the precipitate was free of chlorides. The gum isolated from a Wisconsin soil contained up to 24 per cent ash even after this purification.

Nitrogenous compounds were also present in the microbial gum, as the average of four determinations of the total nitrogen content was 1.24 per cent. The polysaccharide content of the precipitate, measured using the colorimetric anthrone technique, was found to be only 18.8 per cent.

*Colorimetric Determination of the Carbohydrate Content of the Complete Soil and Fractions of the Organic Matter*

Peptization of the soil samples prior to hydrolysis substantially increased the quantity of polysaccharide extracted from each of the four soils at the 1:10 and 1:20 soil : acid ratios (Table 2). This increase was attributed to improved separation of the soil and organic materials such that the polysaccharides are more likely to be hydrolyzed on addition of the acid. The procedure of Brink *et al.* (4) was, therefore, modified for succeeding determinations of the carbohydrate content of complete soil hydrolysates. Ten grams of soil were dispersed in 40 milliliters of 0.5 *N* NaOH for 10 minutes in a Waring blendor, transferred to Erlenmeyer flasks and hydrolysed with ten parts of 3 *N* $H_2SO_4$ per gram of soil.

An experiment was designed to study the effects of soil amendments and incubation on the polysaccharide concentration of the complete soil and on fractions of the organic matter of the two soils. One soil was a surface sample from the Dark Brown Solonetz sub-group profile of the Trossachs catena, and the other an eluviated Ae horizon from the Low Humic Eluviated Gleysol sub-group profile of the Oxbow catena. Finely ground wheat straw and solutions of ammonium nitrate and potassium di-hydrogen phosphate were incorporated with portions of the soil at rates equivalent to 20 tons straw, 200 pounds $P_2O_5$, and 400 pounds N per acre.

Substantial increases in the polysaccharide content of the "complete soil" occurred due to the straw amendment (Table 3). The soils amended with straw + inorganic nitrogen and phosphorus contained slightly higher concentrations of polysaccharides than the soils amended with straw alone. Slightly higher polysaccharide concentrations were observed in the soils amended with nitrogen and phosphorus compared to the unamended soils.

There was a slight decline in the polysaccharide content of the unfertilized Trossachs soils in the latter period of the experiments. This trend was not evident in the Trossachs soils amended with inorganic nitrogen and phosphorus; hence, it appears that inorganic fertilizers had a stabilizing effect on polysaccharides produced in the soil.

In the fractionation technique utilized, the complete soil was divided into two fractions, the humic acids + soil colloids fraction and the fulvic acid fraction. The fulvic acid fraction was again separated into the acetone soluble and precipitated gum fractions. Approximately 75-85 per cent of the total carbohydrate content of these soils was found to be present in the humic acid + non-dispersed soil colloids fraction. This may indicate incomplete separation of the constituents on dispersion of the soil sample prior to hydrolysis.

It was reported by Forsyth (9) that significant portions of the sodium hydroxide insoluble fraction may be composed of carbohydrate materials containing glucose, xylose and glucosamine residues. The carbohydrate constituents in this fraction are probably associated with non-sugar components and the name polysaccharides must be applied with reservation to the carbohydrate carbon measured in the hydrolysates.

TABLE 3. — EFFECT OF SOIL AMENDMENT AND PERIOD OF INCUBATION ON THE POLYSACCHARIDE CONTENT OF VARIOUS FRACTIONS OF THE SOIL.
(Carbohydrates as glucose — gm./100 gm. soil)

| | Incubation period | Surface horizon of Dark Brown Solodized Solonetz (Trossachs) | | | | Eluviated horizon of Low Humic Eluviated Gleysol (Oxbow) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Check | Inorganic $N + P_2O_5$ | Straw | Straw + inorganic $N + P_2O_5$ | Check | Inorganic $N + P_2O_5$ | Straw | Straw + inorganic $N + P_2O_5$ |
| Complete soil | 10 days | 0.69 | 0.68 | 0.77 | 0.79 | 0.23 | 0.22 | 0.35 | 0.37 |
| | 42 days | 0.71 | 0.72 | 0.74 | 0.77 | 0.23 | 0.27 | 0.37 | 0.39 |
| | 84 days | 0.62 | 0.67 | 0.73 | 0.80 | 0.23 | 0.24 | 0.38 | 0.39 |
| Humic acids + soil colloids | 10 days | 0.59 | 0.57 | 0.61 | 0.68 | 0.19 | 0.17 | 0.33 | 0.32 |
| | 42 days | 0.58 | 0.57 | 0.76 | 0.71 | 0.19 | 0.20 | 0.30 | 0.28 |
| | 84 days | 0.53 | 0.56 | 0.57 | 0.66 | 0.19 | 0.19 | 0.33 | 0.31 |
| Fulvic acids | 10 days | 0.15 | 0.17 | 0.20 | 0.21 | 0.06 | 0.05 | 0.10 | 0.12 |
| | 42 days | 0.19 | 0.21 | 0.24 | 0.26 | 0.06 | 0.07 | 0.11 | 0.13 |
| | 84 days | 0.20 | 0.21 | 0.25 | 0.26 | 0.06 | 0.07 | 0.10 | 0.11 |
| Acetone soluble | 10 days | 0.11 | 0.14 | 0.15 | 0.17 | 0.05 | 0.03 | 0.08 | 0.08 |
| | 42 days | 0.12 | 0.12 | 0.15 | 0.15 | 0.04 | 0.06 | 0.07 | 0.09 |
| | 84 days | 0.11 | 0.11 | 0.13 | 0.14 | 0.05 | 0.04 | 0.08 | 0.08 |
| Precipitated gum | 10 days | 0.04 | 0.04 | 0.07 | 0.06 | 0.01 | 0.02 | 0.02 | 0.03 |
| | 42 days | 0.07 | 0.06 | 0.08 | 0.07 | 0.02 | 0.02 | 0.03 | 0.03 |
| | 84 days | 0.06 | 0.06 | 0.08 | 0.07 | 0.01 | 0.01 | 0.03 | 0.03 |

The fulvic acid fraction contained 15-25 per cent of the total soil carbohydrates. Fractionation of the fulvic acid on the basis of the solubility of its components in acetone indicated that the acetone soluble portion contained greater concentrations of polysaccharides than the precipitated gum portion; the latter accounted for less than 10 per cent of the total soil carbohydrates.

Maximum production of carbohydrate carbon in all the soil fractions was associated with an abundant energy source and an adequate nutrient level for the soil micro-organisms. One exception was apparent in the precipitated gum fraction of the Trossachs soil (Table 3) where the straw plus fertilizer treatment contained less carbohydrate carbon than the straw only treatment. This was also observed when the polysaccharides in this fraction were measured gravimetrically (Table 1).

Due to the unexpected distribution of the polysaccharides in the various fractions of the soil, the organic matter of four soils was fractionated into the humin, humic acid and fulvic acid fractions. Adequate washing and reprecipitation of the fractions ensured that contaminating agents were at a minimum. After hydrolysis the polysaccharide concentration of each of these fractions was determined colorimetrically with anthrone and phenol-sulphuric reagents.

The results (Table 4) complement the previous observations that the NaOH insoluble (humin) fraction of the organic matter contained considerable quantities of polysaccharide substances. A range of 55-70 per cent of the total soil polysaccharide, measured with the anthrone reagent, was found in this fraction of the organic matter. When the phenol-sulphuric reagent was used, the per cent of the total soil polysaccharides found in the humin increased to 68-83 per cent. In most cases, the fulvic acid fraction comprised approximately 15-25 per cent of the total soil polysaccharide content, as determined with both anthrone and phenol-sulphuric acid reagents. Similar percentages were reported in the previous fractionation study. The contribution of the humic acid fraction to the total polysaccharide content of the soil was the lowest of the three fractions. It ranged from 4 to 12 per cent for both methods.

Measuring polysaccharides by the phenol-sulphuric acid method resulted in slightly higher values for the fulvic and humic acid fractions, and substantially higher values for the humin portion of organic matter. The higher values are attributed to the difference in the specificity of the two reagents. The phenol-sulphuric acid reagent in addition to hexoses, measures pentoses, methyl pentoses and β-keto acids, aldehydes and ketones which are not measured by the anthrone reagent at the wavelength used, and thus some non-carbohydrate constituents are probably included in this estimation.

Lynch and co-workers (11, 14) found that interfering substances did not invalidate the soil polysaccharide measurements obtained with the anthrone reagent. However, because of the high values obtained for the humin fraction with both reagents, and the possibility that this may be due to non-carbohydrate materials, a portion of the hydrolysed humin from the Elstow Aa and Oxbow Ae soil samples was purified by passage through

TABLE 4. — COMPARISON OF THE ANTHRONE AND THE PHENOL-SULPHURIC ACID METHODS FOR DETERMINING THE POLYSACCHARIDE CONTENT OF VARIOUS FRACTIONS OF THE SOIL

(Carbohydrates as glucose)

| Soil | Soil fraction | Anthrone | | Phenol-Sulphuric | |
|---|---|---|---|---|---|
| | | gm./100 gm. soil | Per cent of total polysaccharides | gm./100 gm. soil | Per cent of total polysaccharides |
| Elstow Aa horizon | Fulvic acid | 0.13 | 27.1 | 0.15 | 13.6 |
| | Humic acid | 0.02 | 4.2 | 0.04 | 3.6 |
| | Humin | 0.33 | 68.7 | 0.92 | 82.8 |
| | Humin de-salted | 0.34 | | 0.42 | |
| Melfort Aa horizon | Fulvic acid | 0.22 | 25.0 | 0.23 | 17.7 |
| | Humic acid | 0.10 | 11.4 | 0.16 | 12.3 |
| | Humin | 0.56 | 63.6 | 0.91 | 70.0 |
| Oxbow Aa horizon | Fulvic acid | 0.08 | 36.4 | 0.09 | 20.4 |
| | Humic acid | 0.02 | 9.1 | 0.05 | 11.4 |
| | Humin | 0.12 | 54.5 | 0.30 | 68.2 |
| Oxbow Ae horizon | Fulvic acid | 0.06 | 22.2 | 0.09 | 14.8 |
| | Humic acid | 0.02 | 7.4 | 0.03 | 4.9 |
| | Humin | 0.19 | 70.4 | 0.49 | 80.3 |
| | Humin de-salted | 0.16 | | 0.27 | |

anion and cation exchange resins, and the carbohydrate content again determined. This would remove any ionized non-carbohydrate substances that affected the intensity of color developed with either the anthrone or phenol-sulphuric reagents. There was no reduction of the carbohydrate carbon content of the de-salted humin from the Elstow soil, and only a small loss from the Oxbow Ae soil when measured with anthrone reagent (see Table 4). A 50 per cent reduction of the compounds reacting with phenol-sulphuric acid was, however, noted after de-salting.

Portions of the de-salted humin were spotted on paper chromatograms to identify the sugars present in this fraction of the organic matter. Using butanol-pyridine, butanol-acetic acid and phenol-water systems as solvents, glucose, galactose, xylose, arabinose and mannose were identified. The humin fraction thus had a composition not essentially different from that found in the water soluble or dilute alkali soluble fraction of organic matter (8, 23).

## DISCUSSION AND CONCLUSIONS

The use of an acetone precipitated fraction from the fulvic acids (designated as microbial gum) has been shown to have serious limitations as an indication of the polysaccharide content of Chernozemic soils. It has a high non-carbohydrate content and measures only a fraction of the total carbohydrate constituents.

The colorimetric anthrone method, if preceded by peptization of the soil with NaOH in a Waring blendor, was found suitable for measuring the polysaccharides of the soil as well as the distribution of carbohydrate carbon in fractions of the soil organic matter. All the fractions of the organic matter contained carbohydrates; however, 75-85 per cent of the measured soil carbohydrate carbon was present in the humic acids + soil colloids fraction. Upon further fractionation approximately 55-70 per cent of the carbohydrate carbon was found to be in the alkali-insoluble humin portion, 15-25 per cent in the acid-soluble fulvic acid fraction, and 4-12 per cent in the acid-insoluble humic acid fraction of organic matter.

In the studies reported, the organic matter insoluble in repeated extractions with 0.5 *N* NaOH was designated as the humin fraction. However, this widely used fractionation, since the conclusion of the above studies, has been shown to have limitations when used on the Black Chernozemic soils such as the Melfort. Campbell* has demonstrated that repeated extractions with dilute alkali remove only one-third of the soil organic matter, the other two-thirds remaining in the humin fraction. Preliminary treatment with 0.1 *N* HCl followed by 0.5 *N* NaOH results in lowering of this figure so that only one-third of the organic matter is not removed by dilute alkali. This type of extraction procedure could significantly alter the reported distribution of carbohydrates.

The colorimetric techniques are fairly rapid and have been found in this study to give an accurate estimation of carbohydrates present in soil organic matter. The anthrone technique has previously been applied to soil analyses (4, 11, 16). This technique was found to measure from 45

*Campbell, C. A., University of Saskatchewan. *Private communication.*

to 65 per cent of the carbohydrate carbon determined with the phenol-sulphuric acid reagent. This agrees with the theoretical distribution of the hexose carbon by workers such as Lynch *et al.* (15) and by Whistler and Kirby (23).

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