

CHARACTERIZATION OF SOIL POLYSACCHARIDES

SAÍZ-JIMÉNEZ, C. and GÓMEZ-ALARCÓN, G.

Agency of Environment, Junta de Andalucía
Seville, Spain

INTRODUCTION

Polysaccharides are found in all living organisms. In plants about 75% of the dry weight is polysaccharide, with cellulose, the most abundant in all naturally occurring organic compounds, constituting at least 10% of all vegetable matter (Cheshire, 1979). When living cells die and are returned to the soil, most of the polysaccharide constituents are decomposed by microorganisms and single sugars and partial degradation products are utilized for synthesis of microbial polysaccharides.

Polysaccharides probably constitute by quantity one of the most important fractions in soil organic matter and because many different organic and inorganic molecules are present, chemical interaction of carbohydrate with these occur. In fact, polysaccharides are distributed in all humic fractions, being particularly important in fulvic acids and humins. Polysaccharides might be covalently linked to or adsorbed on to humic substances. Covalent linkages might include phenolic glycoside structures as suggested for a fulvic acid fraction (Saíz-Jiménez and de Leeuw, 1985). Soil polysaccharides also appear to be chemically linked in proteinaceous complexes or to the humic matrix by cation bridges, therefore can be contaminated by proteins or peptides, humic materials, polyphenols and inorganic constituents. Removal of these materials is a major problem in the purification process and the isolation of "pure" polysaccharide samples is difficult to be accomplished. Most polysaccharide fractions isolated from soils contain very high ash percentages.



Polyclar AT (polyvinyl pyrrolidone) appears to be one of the most useful methods for recovering polysaccharides from soil extracts. This polymer preferentially retains polyphenols, tannins and protein-polyphenol complexes. Thus, brown humic materials that have been coextracted with polysaccharides from soils can be efficiently separated.

The aim of the present investigation is to examine the polysaccharides isolated from different soils by using Polyclar AT.

MATERIAL AND METHODS

Details of the soils used are given in Table 1.
Table 1.

Soil ^x	Description of soils			
	pH(H ₂ O)	C	N	vegetation
Aracena	6.5	5.8	0.5	<u>Quercus ilex</u> , gramineous
Olalla	5.6	3.5	0.4	<u>Medicago</u> , <u>Trifolium</u> , gramineous
Higuera	7.7	3.3	0.3	<u>Cistus albidus</u> , <u>C. ladaniferus</u>
Arahal	7.7	0.6	0.06	<u>Chamaerops humilis</u> , gramineous

^xAracena, Ruptic-Alfic-Dystric Eutrochrept; Olalla, Typic Xerochrept; Higuera, Typic Rendoll; Arahal, Typic Chromoxerert

The polysaccharide fractions were isolated as described by Saíz-Jiménez et al. (1979). A polysaccharide from the Olalla soil was previously studied (Saíz-Jiménez et al., 1979; Saíz-Jiménez and de Leeuw, 1984, 1985). The sample reported in this work belongs to a different batch.

Analysis of carbon, hydrogen, nitrogen, sulphur and ash values was made by Organic Chemistry Institute, Madrid.

For identification of sugars, 20 mg of polysaccharide was hydrolysed with 2N H₂SO₄ for 16 h in sealed evacuated tubes in an oven at 105 °C. After neutralization with barium hydroxide the sugars were converted in alditol acetates and their amount calculated by gas-liquid chromatography. Analytical conditions were described elsewhere (Ruperez et al., 1983).

Infrared spectra were obtained in KBr discs using a Perkin Elmer 377 spectrophotometer.

The pyrolysis mass spectrometry method was described by van der Kaaden et al. (1983). The procedure to pyrolyse soil polysaccharides was described by Saíz-Jiménez et al. (1979).

RESULTS

The soil polysaccharide fractions are a creamy-yellow, light and fluffy powder. Composition from each of the four samples is shown in Table 2.

Table 2. Elemental and sugar composition of soil polysaccharides

%	Aracena	Olalla	Higuera	Arahal
C	33.0	34.7	33.0	37.8
H	5.8	5.1	5.0	6.2
N	3.0	2.5	2.2	2.7
S	9.1	3.7	6.3	9.8
O	49.1	54.0	53.5	43.5
ash	35.8	17.0	23.4	37.2
glucose	2.2	3.5	4.6	2.7
galactose	2.0	1.3	2.3	2.9
mannose	2.6	1.7	2.5	3.8
arabinose	0.4	0.6	0.3	1.0
xylose	trace	0.2	trace	trace
rhamnose	1.1	0.8	0.8	trace
fucose	trace	0.4	trace	0.7

The polysaccharides contain relatively high percentages of ashes. Carbon, hydrogen and nitrogen values are in the range of these types of materials (Cheshire et al., 1974; Cheshire, 1979). However, sulphur values are higher than in any of the other previously reported soil polysaccharides.

Monomer composition shows that glucose, galactose and mannose, as usual, are the major neutral sugars, although the pentoses arabinose and xylose, and the deoxyhexoses rhamnose and fucose are present in lower amounts. Variations in the composition of

soil polysaccharides are widely reported in the literature (Lowe, 1978) and it depends on the isolation method, soil fraction, horizon, parent plant material, etc.

Infrared spectrum of a representative soil polysaccharide (Fig.1) shows the presence of CH_2 groups at 2930 cm^{-1} , C=O of carboxyl groups at 1720 cm^{-1} , C=O of acetamido groups at 1645 cm^{-1} , NH of acetamido groups at 1540 cm^{-1} and S=O of sulphate ester groups at 1230 and 810 cm^{-1} . Absorption of OH from bonded hydroxyl groups are found at 3400 cm^{-1} and C-O and OH absorption bands of alcohols are evident in the region 1410 - 1260 and 1150 - 1050 cm^{-1} , although in the region 1200 - 400 cm^{-1} contribution of inorganic materials (silicates, phosphates, etc.) cannot be discarded due to the high ash content of all the samples.

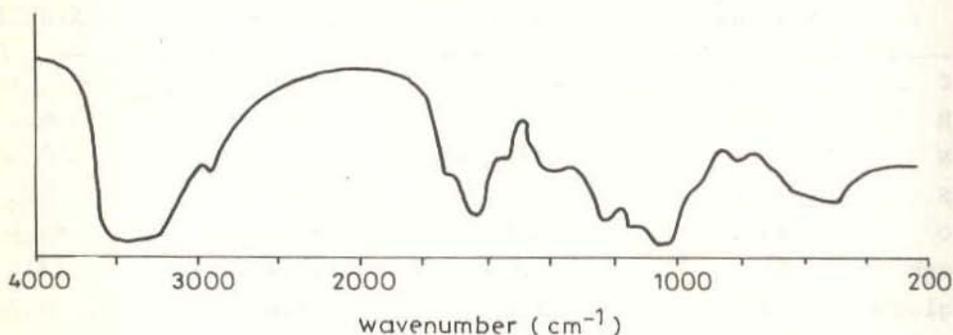


Fig.1 Infrared spectrum of the Arahal polysaccharide

The pyrolysis mass spectra of the four polysaccharides are quite similar, with only intensity differences in some of the fragments. In Fig.2 is represented the spectrum of the Arahal polysaccharide.

Major peaks are consistent with the presence of carbon monoxide (m/z 28), ketene and/or propene (m/z 42), carbon dioxide and/or ethanal (m/z 44), sulfur dioxide (m/z 64), furan (m/z 68), butenone, dihydrofuran and/or crotonaldehyde (m/z 70), methylfuran (m/z 82), dihydromethylfuran, methylcyclobutanone and/or crotonolactone (m/z 84), dimethylfuran and/or furaldehyde (m/z

96), furfuryl alcohol and/or angelicalactone (m/z 98), methylfuraldehyde (m/z 110), hydroxydihydropyranone (m/z 114), hydroxymethylfurfural and/or hydroxymethylpyranone (m/z 126) and anhydrohexose and/or dianhydroglucopyranose (m/z 144). All these compounds have been identified upon pyrolysis of plant and soil polysaccharides (van der Kaaden *et al.*, 1983; Saíz-Jiménez and de Leeuw, 1984, 1985).

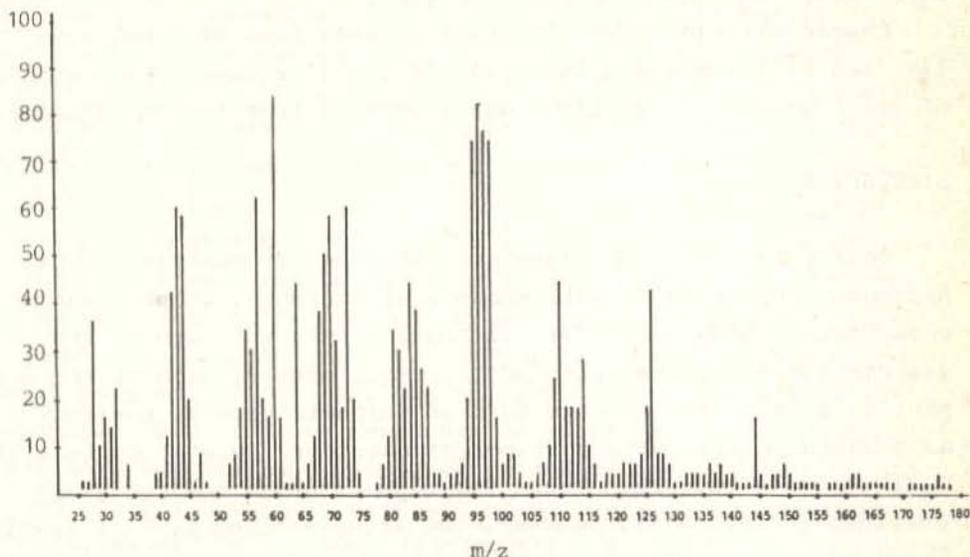


Fig.2 Pyrolysis mass spectrum of the Arahal polysaccharide

Particularly interesting is the group of peaks at m/z 95, 96, 97 and 98, which point to the presence of furfural (major fragments at m/z 95 and 96) and levoglucosenone (very characteristic triplet at m/z 96, 97 and 98), although other compounds can also contribute. Furfural and levoglucosenone are well-known and major pyrolysis products of polysaccharides.

Peaks and intensities at m/z 57, 60 and 73 agree well with those of levoglucosan mass spectrum (van der Kaaden *et al.*, 1983), which is the most important primary pyrolysis product of polysaccharides.

Peaks at m/z 109, 11 and 125, which are also major peaks in pyrolysis of chitin, can be observed, suggesting that acetylglucosamine moieties are present in the polysaccharide. In addition, the preparation contains residues of polyvinyl pyrrolidone, evidenced by the peak at m/z 85 (pyrrolidone). It is remarkable that traces of Polyclar have also been recognized in fulvic acids isolated with this polymer (Saíz-Jiménez *et al.*, 1979), indicating that some materials are released and incorporated in to the soil organic fractions.

Characteristic peaks of alkyl phenols (m/z 94, 108, 122, 136) and methoxyphenols (m/z 124, 138, 150) suggest the presence of small amounts of guaiacyl units derived from lignin residues.

DISCUSSION

Soil polysaccharides have been reported to contain carbon, hydrogen, oxygen, and small amounts of nitrogen, sulphur and phosphorus (Cheshire, 1979). Although little is known of the association of sulphur with soil organic matter, much of the sulphur is likely to be in the form of sulphate esters, possibly associated in part with soil polysaccharides. Sulphur content of 0.06 to 0.41% have been found for nine Canadian soil polysaccharides (Lowe, 1978). However, the amounts found for the Spanish samples are considerably higher. Saíz-Jiménez and de Leeuw (1985) identified among the pyrolysis products of the Olalla polysaccharide high amounts of sulphur dioxide, suggesting that this compound might originate from sulphated polysaccharides. Also, intensity of the sulphur dioxide peak observed in the pyrolysis mass spectra agree well with sulphur values. Further, absorptions at 1230 and 810 cm^{-1} in the infrared spectra point to the existence of sulphate ester groups.

Analysis of hydrolysates indicates the presence of at least seven sugars. In addition, uronic acids, amino acids, amino sugars, phenols, fatty acids, etc. have been reported (Cheshire, 1979, Saíz-Jiménez and de Leeuw, 1984, 1985). Because information on uronic acids has proved hard to obtain due to serious losses during hydrolysis and to the shortcomings of analytical methods (Lowe, 1978), attempts to study these compounds have not

been accomplished. However, evidence of carboxylic groups have been found in the infrared spectra, and identification of glucuronic and galacturonic acids in hydrolysates of soil polysaccharides is well documented (Cheshire, 1979).

Protein-like material has been detected in soil polysaccharides and amino acids have been observed in hydrolysates of various polysaccharide preparations (Cheshire, 1979). In the studied samples evidence of amino acids is very faint, suggested by the minor fragments at m/z 67 (pyrrole), 79 (pyridine), 117 (indole) and its partly methyl derivatives, which have been demonstrated to be protein and peptide pyrolysis products (Saíz-Jiménez *et al.*, 1979). Further, in a preparation of the Olalla polysaccharide, Saíz-Jiménez and de Leeuw (1985) found no significant evidence of amino acid and/or protein pyrolysis products.

It is most likely that amino sugars found in soils originate from microorganisms, where these sugars have a major structural role. Glucosamine is present in the cell walls of fungi as chitin whereas galactosamine probably derives from bacterial cell wall polysaccharides. The presence of chitin moieties in the samples under study is corroborated by the infrared and pyrolysis mass spectra, and Saíz-Jiménez and de Leeuw (1985) identified typical pyrolysis products of chitin such as acetamide, 3-acetoxypyridine, trianhydro-2-acetamido-2-deoxyglucose, dianhydro-2-acetamido-2-deoxyglucose, etc. in the pyrolysate of the Olalla polysaccharide.

According to Finch *et al.* (1971) brown humic and/or fulvic acid materials have been coextracted with polysaccharides from soil in the cases of all of the solvents so far used. From the data obtained in this and previous studies (Saíz-Jiménez and de Leeuw, 1984, 1985) it appears that polysaccharides contain lignin-derived phenols, guaiacol, ethylguaiacol, vinylguaiacol, eugenol and vanillic acid being the most conspicuous. They probably originate from biodegraded lignin residues linked to the polysaccharide. It may be interesting to note that Clark and Tan (1969) considered hymatomelanic acid as a naturally occurring ester compound composed of humic substances in ester linkages with polysaccharides. Very recently, Saíz-Jiménez and de Leeuw (1985) demonstrated that hymatomelanic acid consists almost en-

tirely of lipids, including alkanes, alkenes, acyclic isoprenoid hydrocarbons, fatty acids and aliphatic dicarboxylic acids. Lignin and possible carbohydrate pyrolysis products represented a very minor percentage (less than 5%) of total pyrolysis products.

Soil polysaccharides have diverse origin including residual plant components resistant to degradation and microbial polysaccharides. The presence of seven or more monosaccharides in the hydrolysates point to a microbial origin, in contrast to polysaccharides of higher plants which are commonly composed of only one to three monomeric constituents. This microbial origin is further supported by the identification of acetylglucosamine moieties and sulphate ester groups. Therefore, it appears clear that a great variety of polysaccharides are present in soils. The stability of these materials is related to inaccessibility caused by chemical combination, complexing or insolubility as a result of its relationship with other soil components (Cheshire *et al.*, 1974).

SUMMARY

Fractionation of fulvic extracts from four Spanish soils using adsorption on insoluble polyvinyl pyrrolidone (Polyclar AT) permitted the separation of polysaccharide fractions. No significant differences between fractions were found with respect to elemental analysis, sugar composition, infrared and pyrolysis mass spectra. The most striking feature was the high sulphur content, varying from 3.7 to 9.8%. In addition to monosaccharides (neutral sugars) the isolates consisted of acetylglucosamine moieties and sulphate ester groups, suggesting that a complex mixture of different polysaccharides and origin were extracted from soils.

REFERENCES

- Clark, F.E. and Tan, K.H. (1969): Soil Biol. Biochem. 1, 75-81.
- Cheshire, M.V. (1979): *Nature and Origin of Carbohydrates in Soils.* Academic Press, London.
- Cheshire, M.V., Greaves, M.P. and Mundie, C.M. (1974): J. Soil Sci., 25, 483-498.
- Finch, P., Hayes, M.H.B. and Stacey, M. (1971): The biochemistry of soil polysaccharides. In: *Soil Biochemistry*, 2, McLaren, A.D. and Skujins, J. (eds) Marcel Dekker, New York, 257-319.
- Kaaden, A. van der, Haverkamp, J., Boon, J.J. and Leeuw, J.W.de (1983): J. Anal. Appl. Pyrol. 5, 199-220.
- Lowe, L.E. (1978): Carbohydrates in soil. In: *Soil Organic Matter.* Schnitzer, M. and Khan, S.U. (eds) Elsevier, New York, 65-93.
- Ruperez, P., Gómez-Miranda, B. and Leal, J.A. (1983): Trans. Br. Mycol. Soc. 80, 313-318.
- Saíz-Jiménez, C. and Leeuw, J.W. de (1984): Org. Geochem. (in press)
- Saíz-Jiménez, C. and Leeuw, J.W.de (1985): J. Anal. Appl. Pyrol. (in press)
- Saíz-Jiménez, C., Haider, K. and Meuzelaar, H.L.C. (1979): Geoderma 22, 25-37.