Organic Matter in Soils

Our greatest but unappreciated resource

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Organic C in Soils

- It is estimated that there are ca 2.35 x 10^{15} tonnes of Organic Carbon (OC) in the top 3 m of the world's soils. (That is ca 4 x the amount of C in all living matter on the earth's surface.)
- Humin can compose up to 50% of that C. and up to 70% of that in lithified sediments
- ***Note:*** ***We no longer consider HUMIN to be a component of HSs, as will emerge in the presentation***

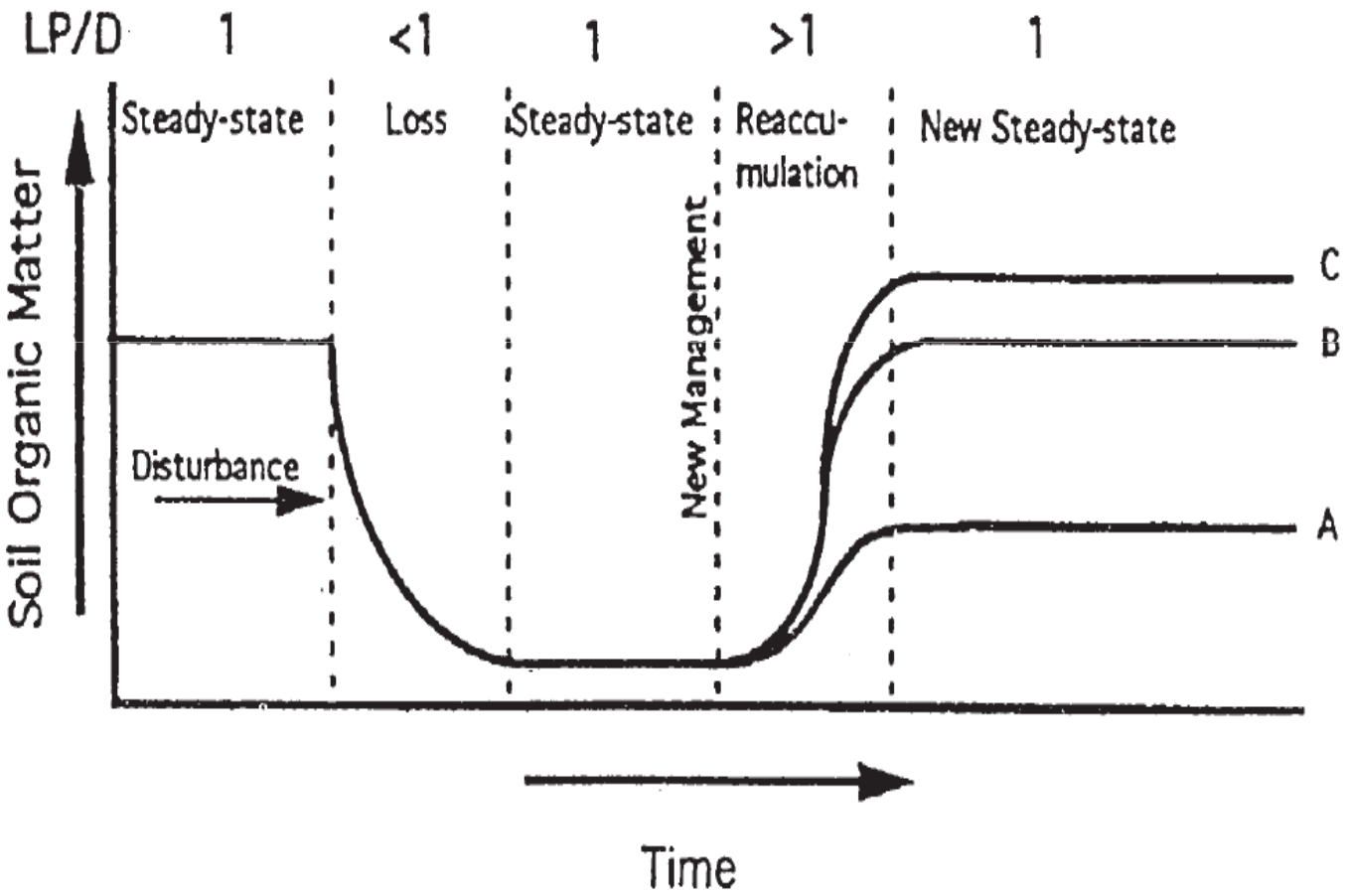
Soil Organic Matter (SOM)

- **In classical definitions (Hayes and Swift 1978, The Chemistry of Soil Organic Colloids *In* DJ Greenland and MHB Hayes, eds, *The Chemistry of Soil Constituents*, Wiley , Chichester) SOM is separated into two major groups (based largely on proposals by Kononova :**
- **(I) Unaltered materials that include fresh debris and transformed older debris;**
- **(II) Transformed products or HUMUS** with no morphological resemblances to their substances of origin . These are subdivided
- (IIa) **Amorphous brown Humic Substances (HSs)** differentiated into Humic **Acids (HAs),** Fulvic Acids **(FAs)** and **Humins;** and
- **(IIb) Compounds in recognisable classes, e.g. polysaccharides, peptides, altered lignins, etc. that can be synthesized by microorganisms or arise from alterations of similar products in soil.**
- **3**

SOM Degradation

- The content of SOM in any soil will depend on such factors as the organic residues, the soil mineralogy, especially the inorganic colloids, soil moisture, soil temperature, the soil microbial population, the soil pH, etc.
- The SOM will reach a steady state regardless of the management system that applies
- Johnson, M.G. (1995). The role of soil management in sequestration of soil carbon. p. 351-364. *In* R. Lal et al. (eds.) *Soil management and the greenhouse effect.* Lewis Publ., Boca Raton.
- has graphically depicted the effects of long-term cultivation on the SOM content in soil.

Effects of Long-Term Cultivation (Johnson, 1995)



Topics for Discussion

- Concepts of SOM in the first half of the 20th century
- Some advances during the 1950-1960 period
- Approaches during the 1960-1980 period
- The formation of the IHSS and unifying approaches to studies by Soil, Water and Coal humic scientists
- The Carbolea procedure for the isolation and fractionation of soil humic substances and humin
- Modern procedures for studying humin (considered the most intractable component of SOM, but now it is relatively easy to categorise based on applications of modern NMR procedures)
- Humeomics; contribution to awareness of HSs compositions
- Uses of heterogeneous bioimetic catalysis to stabilise humic suprastructures
- Summary and Ways Forward
- Acknowledgements

Origins of the Humic Sciences

- **Dokuchaev (1883) had a definitive influence when he regarded soil as a natural body formed through the combined action of natural factors, and in particular to the biological factors contributing to soil formation, such as vegetation cover and the activities of living organisms. Thus, humus was seen to have an important role in soil formation and soil fertility**

Concepts in the First Half of the 20th Century

- Based on a series of studies commencing in 1902, Williams (1939) produceda set of perceptive conclusions (as listed by Kononova, 1966):
- (1) that humus substances exist in soil as a natural body;
- (2) that various plant materials which undergo complex biochemical transformations serve as sources of humus substances;
- (3) that plant materials decompose to more simple products from which the complex humic substances are synthesized; and
- (4) that microbial enzymatic processes are involved in the decomposition and in the synthesis processes

Concepts in the First Half of the 20th Century (continued)

- **Deherain (1902) considered HSs to be products of interactions between proteins and "encrusting substances" (mainly lignin). This concept preceded the ligno-protein theory attributed to Waksman (1936,(Humus, Williams and Wilkins) and Waksman and Iyer (1932, 1933, Soil Sci. 36:69-82.) that subsequently dominated concepts of the origins and compositions of HSs for a generation. Their work indicated that oxidized lignin when reacted with protein gave a product similar to HAs, and based on their work the concept took hold of HAs as complexes of oxidized lignin with protein.**

Changes in the Second Half (5th decade) of the 20th Century

When Frank Stevenson was a graduate student at the Ohio State University he introduced moving boundary electrophoresis to studies of HSs sorbed on clays. These reflected the polyelectrolyte nature of the colloidal components, and ~~the~~ frictional ratio data from ultracentrifugation suggested that the colloidal components were spherical in nature.

- Michael Hayes followed FS at OSU, and he used Paper Curtin Electrophoresis to fractionate organic matter extracts from a biologically oxidised organic (muck) soil that had been extensively cultivated for vegetable crop growing.
- This technique was able to give a fractionation of HSs components that had not been achieved previously.

Paper Curtin Electrophoresis of LMW Water Soluble Peat

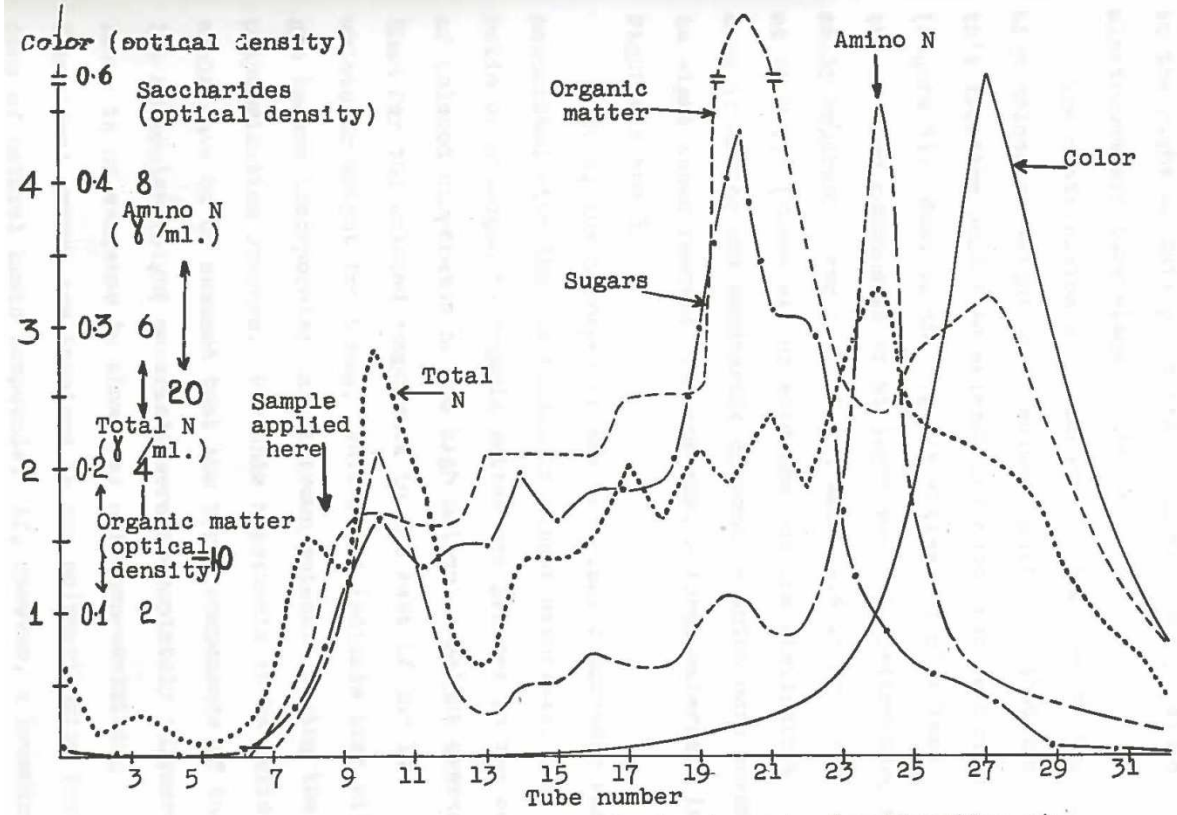


Figure 3. Continuous flow paper electrophoresis fractionation at 650 volts of low molecular weight materials from total water soluble peat.

Electrophoretic Fractionation of Non-Dialysable Water-Soluble Peat

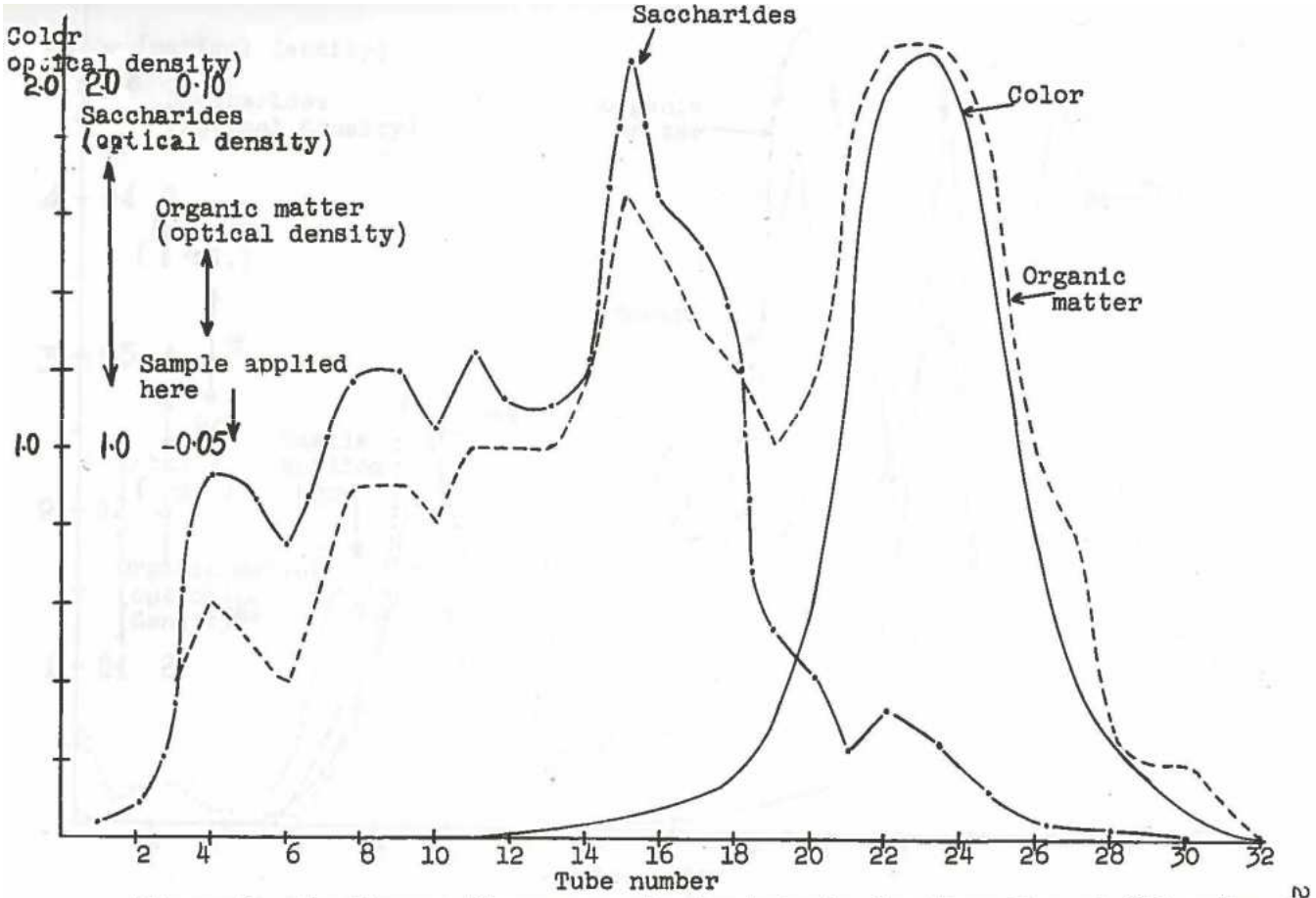


Figure 2. - Continuous flow paper electrophoresis fractionation at 650 volts of the total water soluble peat in borate buffer.

253

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Conclusions from Continuous Flow Paper Electrophoresis and Applications of Column Electrophoresis

Continuous flow paper curtin electrophoresis achieved almost complete separation of the amino N containing and the saccharide components in the case of the low molecular weight (dialysable), and of the saccharide components from the higher molecular weight (retained in Visking dialysis tubing) components of the soxhlet water extract from an organic soil. The soxhlet extraction process may not give products characteristic of soil components because of the heating involved. Nevertheless the technique indicates the potential value of such qualitative electrophoresis techniques. Quantitative separation could be achieved using powdered cellulose (or other) packing, and a variety of buffers for column electrophoresis fractionation based on charge density differences. Hayes used the process and showed in preliminary experiments (under the supervision of JE Dawson) that this could be achieved. C.E Clapp, a fellow grad student with Hayes at Cornell, was able (PhD thesis, 1956) to isolate a microbial polysaccharide and identify the component sugars from a water soluble (soxhlet extracted) peat fraction.

Challenging the Ligno-Protein Concept

- **In the second half of the 5th decade of the 20th century Hayes formed an oxidized lignin complexes with casein using the Waksman and Iyer (1932, 1933) process. That complex had titration and other properties described by Waksman, but Differential Thermal Analysis (DTA) data indicated that this complex was very different from HAs.**
- **That caused Hayes to investigate claims that Browning Reaction products, formed from reactions between reducing sugars and methylglyoxal or pyruvaldehyde (CH_3 C(O) CHO)) and glycine (representing amino acids and peptides) gave products similar to Humic Acids (HAs) from SOM.**

DTA of the Complex of Alkali Lignin with Casein

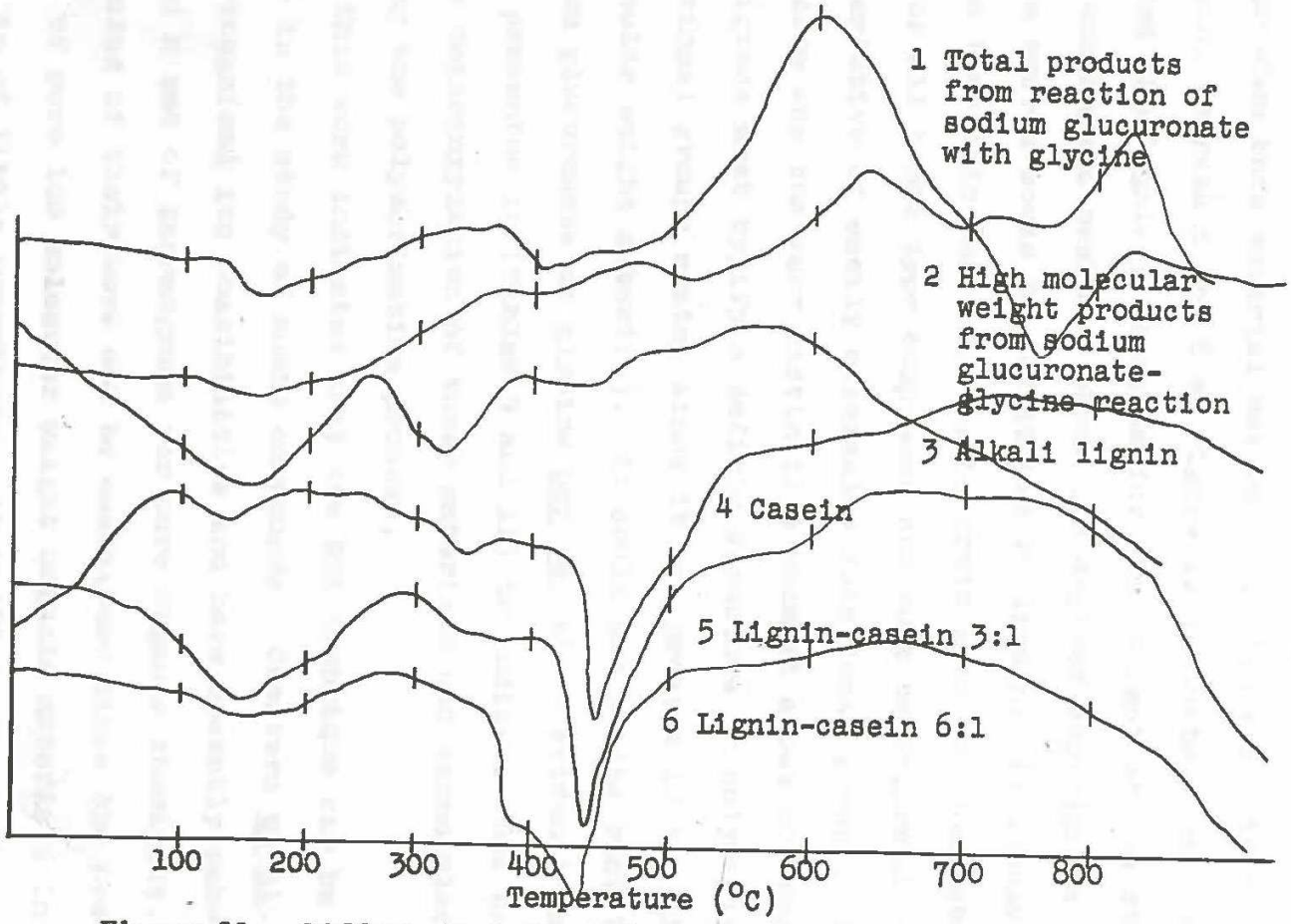

Figure 11. Differential thermal analysis for some synthetic humic

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Browning Reaction Products with Similarities to HSs

- **Maillard (1912, 1916, 1917) showed that Browning products from the reaction of glucose with glycine had resemblances to natural humic materials from soil. Consequently, considerable emphasis was placed in the middle of the 20th century on what is called the Browning reaction.**

Maillard (1917) showed that the products of the glucose–glycine reaction resembled in many respects those of soil humic acids.

Enders (1943b) showed that methylglyoxal (CH_3 C(O) CHO)) could be released by soil microorganisms under conditions unfavourable for microbial growth (lack of substrate, low or high temperature, etc.) and then polymerize rapidly in the presence of amino acids. Later, Enders and Sigurdsson (1947) showed the presence of methylglyoxal in 10 of the 16 soils tested, and Enders et al. (1948) showed that the products of this reaction had many properties similar to those of soil HSs.

References in Hayes et al (Adv. In Agronomy 2017, 143 , 48-138)

Set Up for Synthesis of Browning Reaction products

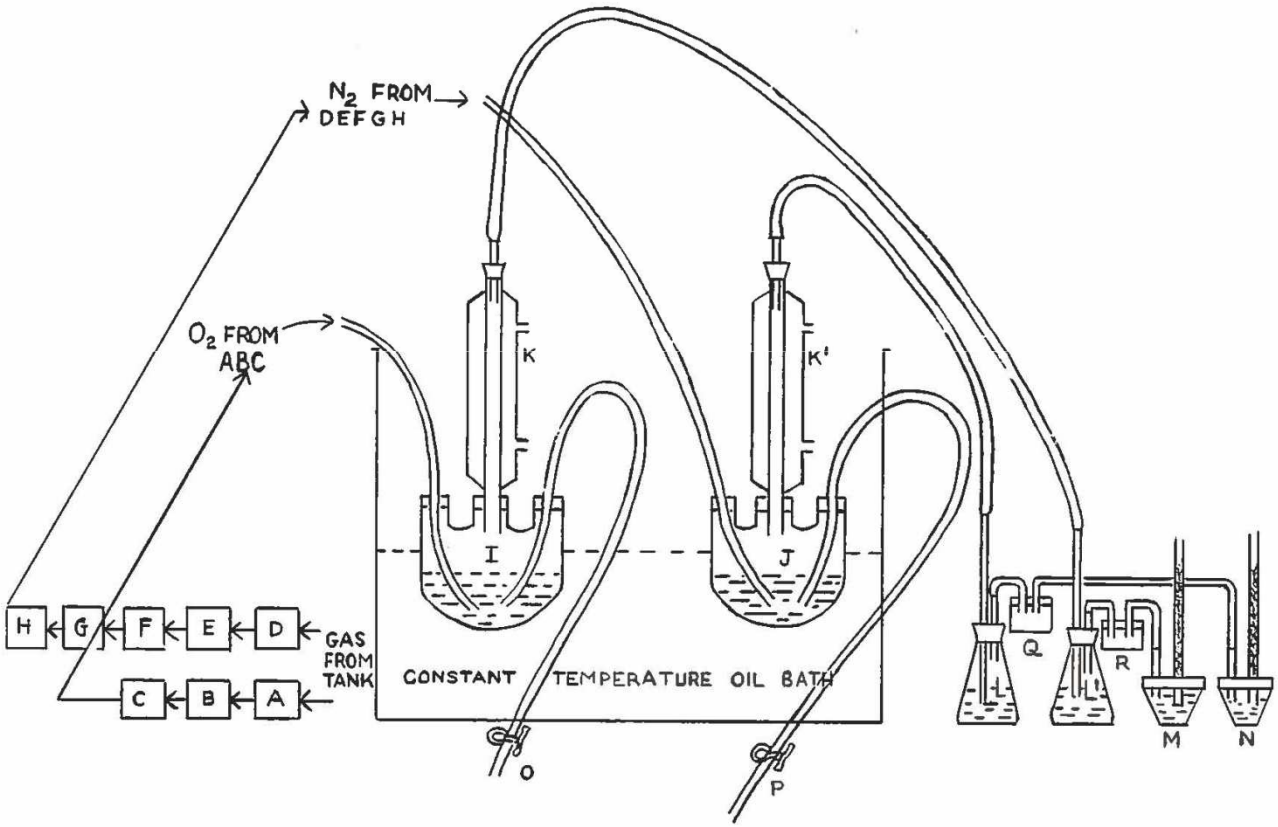

Figure 1. Apparatus for the synthesis of Browning reaction polymers.

Cumulative Evolution of CO2 during Browning reactions

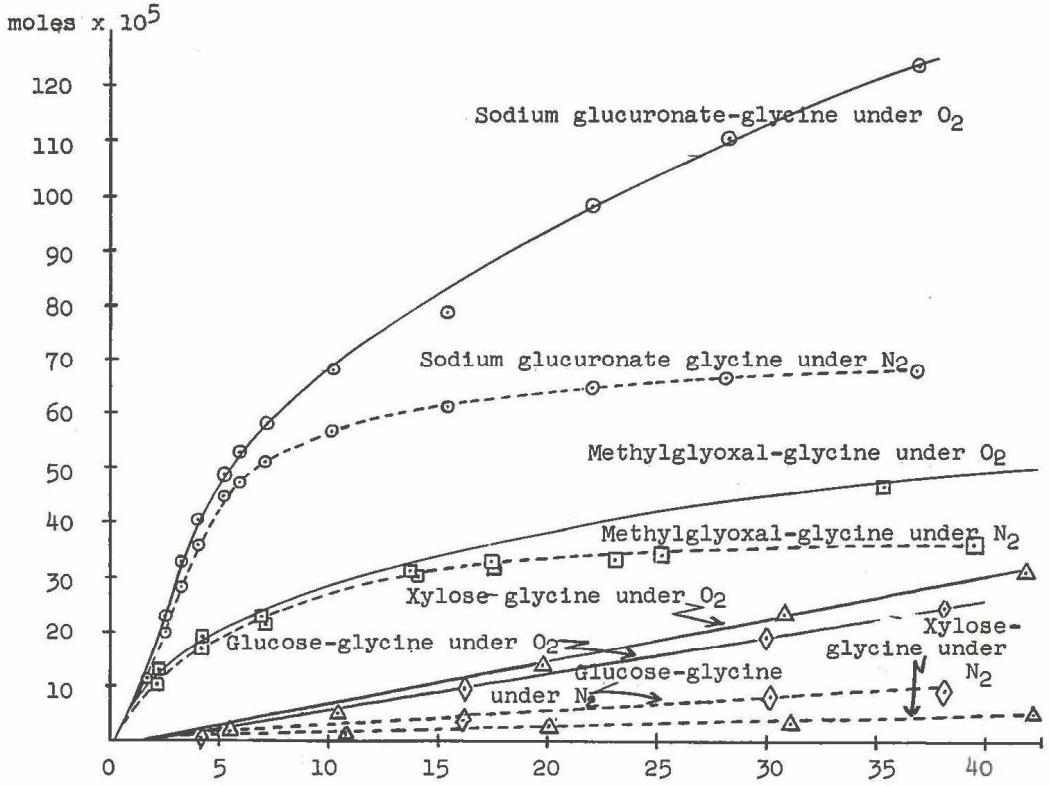

Figure 8. Cumulative evolution of carbon dioxide from various Browning reaction monomers reacted in atmospheres of oxygen and nitrogen.

Thermograms of HAs and of Browning Reaction Products

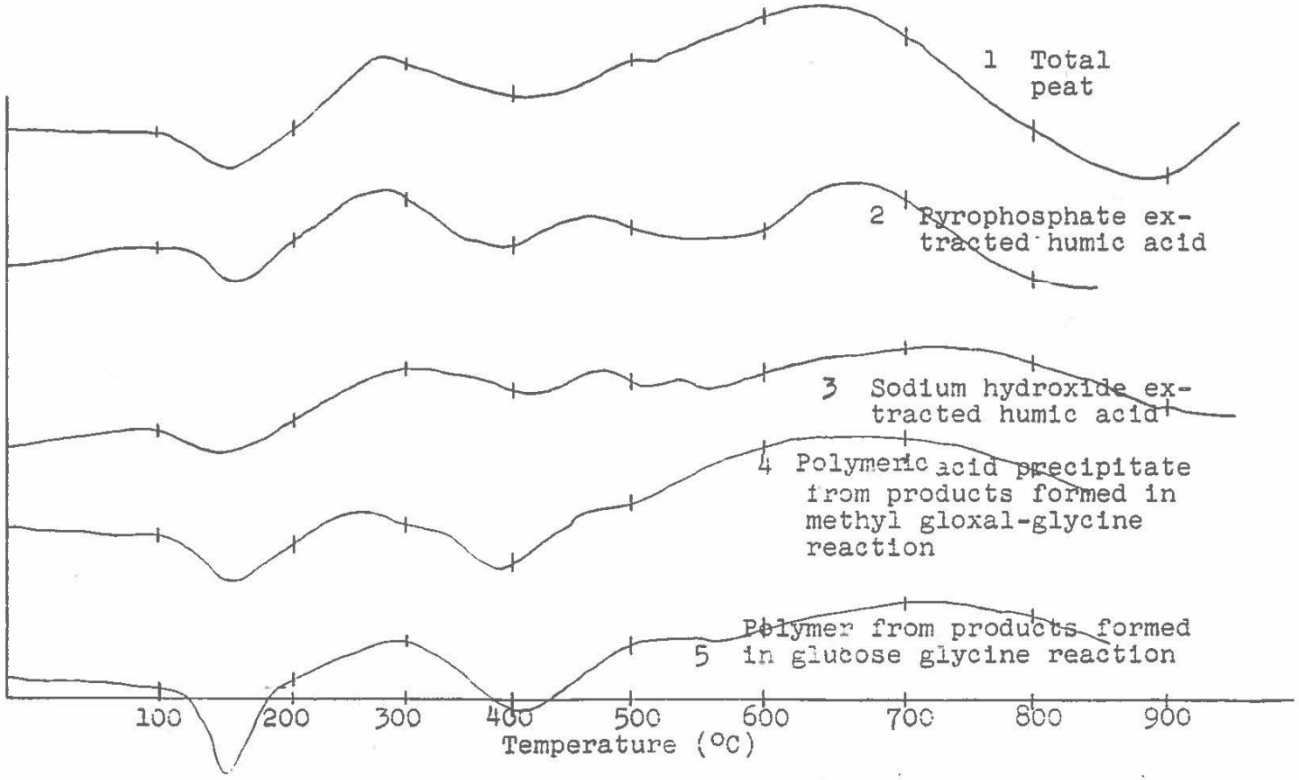

Figure 10. Differential thermal analysis thermograms for natural and synthetic humic polymers.

Should We Dismiss Browning Reactions as Sources of Humic Products?

Many arguments have been put forward to dismiss possible origins for HSs in Browning Reaction processes. These include considerations of the low concentrations of reactants in the soil solution. However, account has not been taken of possibilities of interactions in the hydrophilic areas of decaying organic matter where microorganisms abound and where there is an abundance, but not an excess of water. Also we should take note of the accumulation (observed in the work of Haider and of Martin, and referenced in Hayes and Swift, 1978) of Browning-type products inside the mycelia of some fungi.

Sixty years ago, when seeking an alternative to the ligno-protein theory, Hayes, inspired by the work of Schuffelen and Bolt (1950), produced methylglyoxal/glycine reaction products, and these, like those of Schuffelen and Bolt, had many properties similar to HAs. That applied also to reactions of many reducing sugars with glycine (see slides 15, 19.) We now have the technologies that will allow us to explore possibilities in these areas.

The 1960-1980 Period

- **The availability of gcms allowed the facile identification of the digest products formed when various oxidation and reduction reagents were reacted with HSs fractions.**
- **Morris Schnitzer of Agriculture Canada was a major contributor to this approach.**
- **Some excellent work was carried out by Haider, Munich and Martin, UC Riverside, who greatly advanced the awareness of the contribution by microbes to the synthesis of humic-type substances.**

For references see Hayes and Swift (1978) The chemistry of soil organic colloids, In Greenland and Hayes (eds), *The Chemistry of Soil Constituents ,pp 179-320, Wiley, Chichester; and* Clapp, Hayes, Simpson, Kingery (2005) The chemistry of soil organic matter. In A. Tabatabai and D.L. Sparks (eds), *Chemical Processes in Soils*. Pp. 1-150. SSSA Book Series No. 8 Madison, WI.,

Oxidative Degradation Reactions

- The major oxidative degradation processes involved reactions of derivatised (methylated) HSs preparations with KMnO4 (slide 23) and with alkaline cupric oxide (slide 24). It was considered that the digest products identified were the ‘building blocks’ of the humic molecules. Hayes and Swift (1978) and Clapp et al., (2005) (see slide 21 for references) have outlined the reaction mechanisms for the different oxidative degradations, and these indicate that the products identified could have arisen from a variety of precursors that were greatly altered during the oxidation processes. Hence the products listed in the next two slides are, at best, ‘distant relations’ of the humic component structures.

Permanganate Degradations of HAs and Fas

(From Hayes and Swift, 1978)

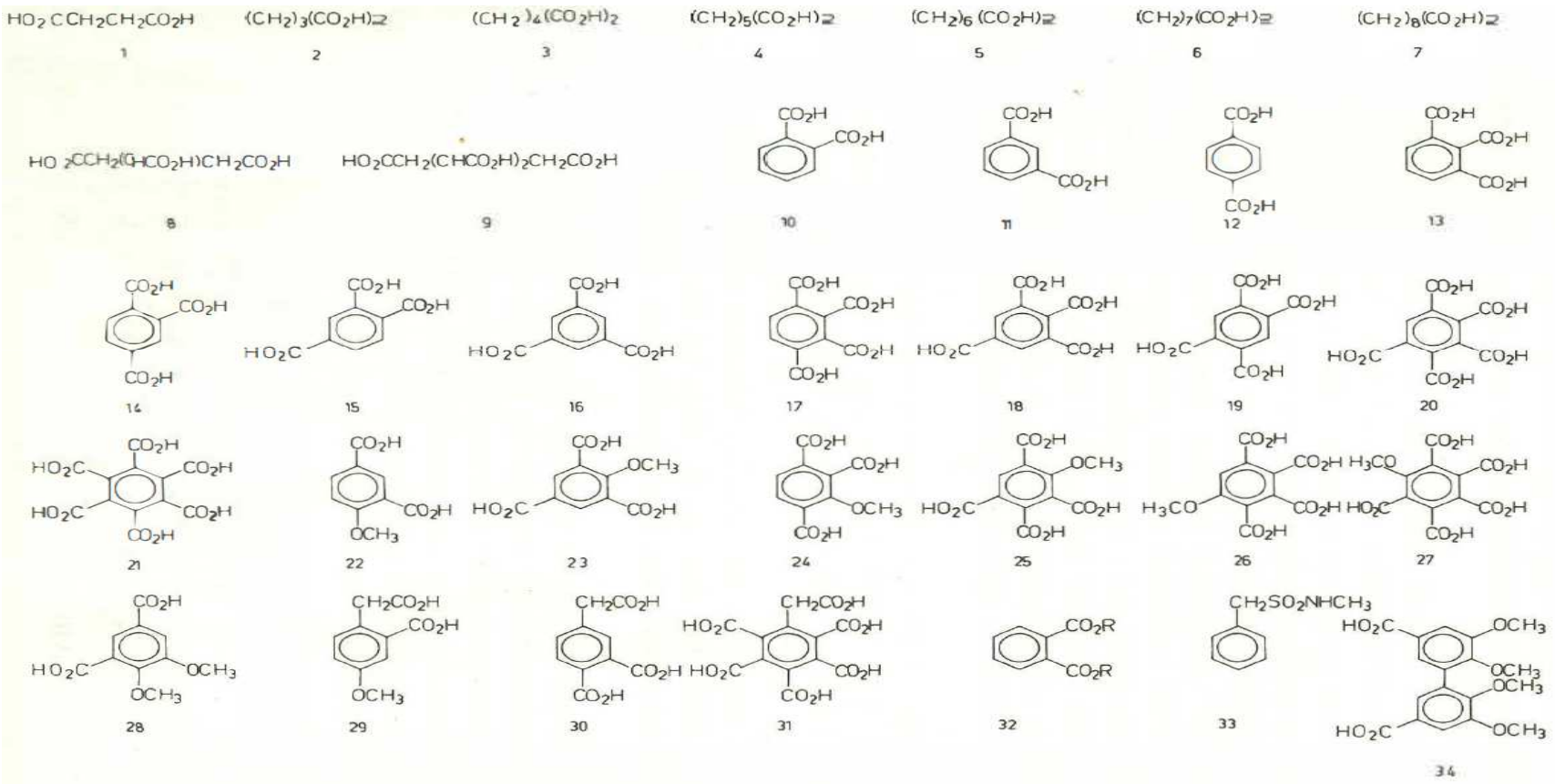

Figure 3.4 Products identified in the digests from permanganate oxidations of humic and fulvic acids

Alkaline Cupric Oxide Degradations of HAs and Fas

(From Hayes and Swift, 1978)

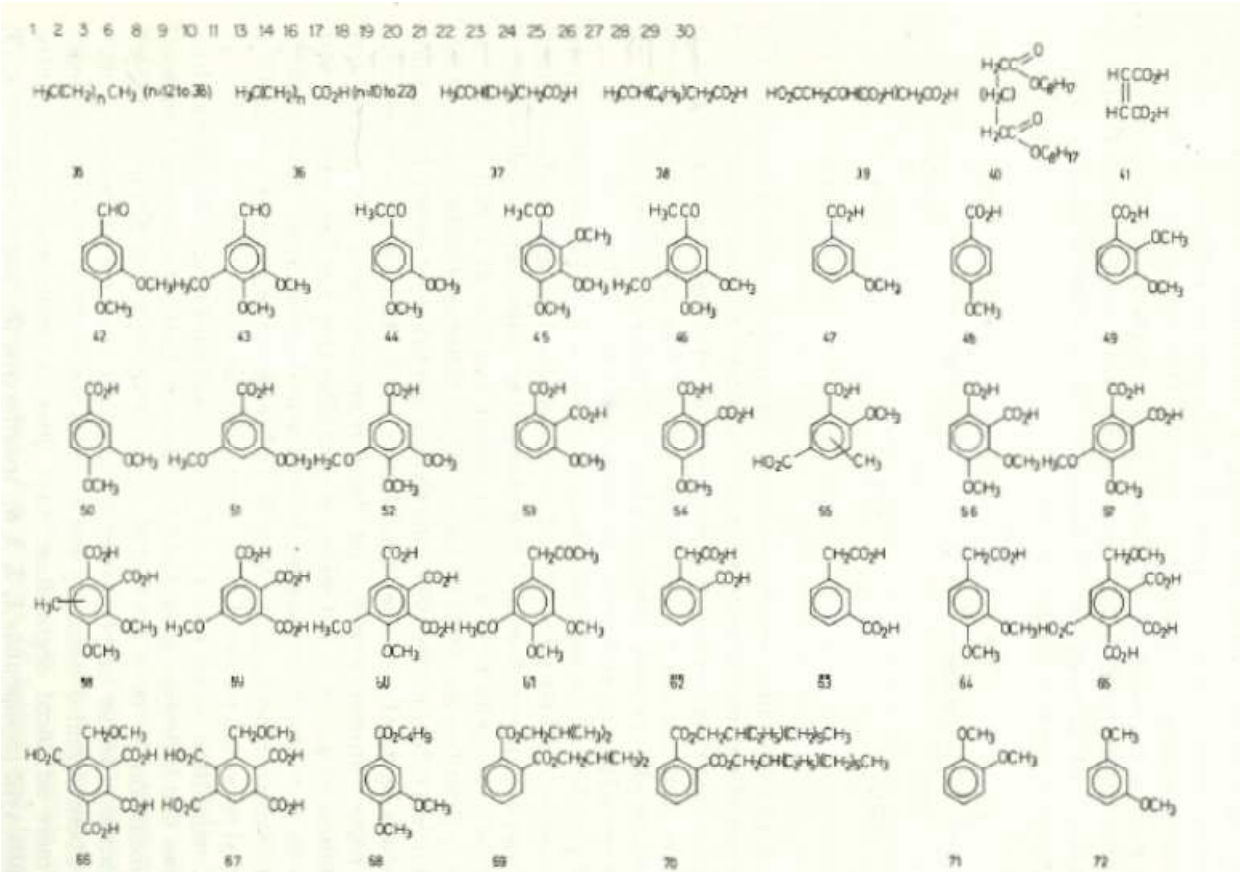

Figure 3.5 Products identified in the digests from alkaline cupric oxide degradations of humic and fulvic acids

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Reductive and Other Degradation Procedures

- **The sodium amalgam degradation (slide 26) is a relatively mild procedure and is worthy of renewed interest. Hayes and Swift (1978) and Clapp et al. (2005) (refs in Slide 21) have listed the products identified, and outlined what was then known about the degradation mechanisms. The products clearly indicate origins for some in lignin, or in lignin alteration products.**
- **Degradation by Na_2S in base (based on the Kraft delignification process) at elevated temperatue (250 ^oC) and pressures (autoclave conditions) has given high yields of identifiable products (slide 27), initiated by quinone methide mechanisms, and again suggestive of origins in linin-type precursors.**
- **The Zn dust distillation and fusion processes give rise to extensive fused aromatic artefacts (slide 28) and the structure prototype of Haworth (slide 29) is unrealistic.**

Sodium Amalgam (*Reductive*) Degradation of HSs

From Clapp et al., 2005)

Compounds identified by various workers in sodium amalgam digests in alkaline conditions.[1]

XLVII	$R_1 = R_2 = OH$; $R_3 = R_4 = R_5 = R_6 = H$
XXXII	$R_1 = R_2 = OH$; $R_3 = R_4 = R_5 = R_6 = H$
XLVIII	$R_1 = R_2 = R_6 = OH$; $R_3 = R_4 = R_5 = H$
XXXI	$R_1 = R_3 = R_5 = OH$; $R_2 = R_4 = R_6 = H$
XLIX	$R_1 = CH_3$; $R_3 = OH$; $R_2 = R_4 = R_5 = R_6 = H$
L	$R_1 = CH_3$; $R_4 = OH$; $R_2 = R_3 = R_5 = R_6 = H$
LI	$R_1 = CH_3$; $R_2 = R_6 = OH$; $R_3 = R_5 = R_6 = H$
LII	$R_1 = CH_3$; $R_3 = R_5 = OH$; $R_2 = R_4 = R_6 = H$
LIII	$R_1 = CH_3$; $R_2 = R_6 = OH$; $R_3 = R_4 = R_5 = H$
LIV	$R_1 = CH_3$; $R_2 = R_6 = OH$; $R_3 = R_4 = R_5 = H$
LV	$R_1 = CH_3$; $R_3 = R_4 = R_6 = OH$; $R_2 = R_5 = H$
LVI	$R_1 = R_3 = OH$; $2(CH_3)$ unassigned; $2(H)$ unassigned
LVII	$R_1 = COOH$; $R_3 = OH$; $R_2 = R_4 = R_5 = R_6 = H$
LVIII	$R_1 = COOH$; $R_6 = OH$; $R_2 = R_3 = R_4 = R_5 = H$
LIX	$R_1 = COOH$; $R_2 = R_6 = OH$; $R_3 = R_4 = R_5 = H$
LX	$R_1 = COOH$; $R_2 = R_6 = OH$; $R_3 = R_4 = R_5 = H$
LXI	$R_1 = COOH$; $R_3 = R_4 = OH$; $R_2 = R_5 = R_6 = H$
LXII	$R_1 = COOH$; $R_3 = R_5 = OH$; $R_2 = R_4 = R_6 = H$
LXIII	$R_1 = COOH$; $R_3 = R_4 = R_5 = OH$; $R_2 = R_6 = H$
LXIV	$R_1 = COOH$; $R_3 = OCH_3$; $R_4 = OH$; $R_2 = R_5 = R_6 = H$
LXV	$R_1 = COOH$; $R_3 = R_5 = OCH_3$; $R_4 = OH$; $R_2 = R_6 = H$
LXVI[‡]	$R_1 = COOH$; $R_3 = R_6 = OCH_3$; $R_2 = R_5 = R_6 = H$
LXVII[‡]	$R_1 = COOH$; $R_3 = R_5 = OCH_3$; $R_2 = R_4 = R_6 = H$
LXVIII[‡]	$R_1 = COOH$; $R_3 = R_4 = R_5 = OCH_3$; $R_2 = R_6 = H$
LXIX[‡]	$R_1 = CH(O)$; $R_3 = R_4 = OCH_3$; $R_2 = R_5 = R_6 = H$
LXX[‡]	$R_1 = CH(O)$; $R_3 = R_4 = R_5 = OCH_3$; $R_2 = R_6 = H$
LXXI[‡]	$R_1 = H_2C(COO)$; $R_3 = R_6 = OCH_3$; $R_2 = R_5 = R_6 = H$
LXXII[‡]	$R_1 = H_2C(O)$; $R_3 = R_4 = R_5 = OCH_3$; $R_2 = R_6 = H$
LXXIII[‡]	$R_1 = H_2C(COO)CH_3$; $R_3 = R_4 = R_5 = OCH_3$; $R_2 = R_6 = H$
LXXIV[‡]	$R_1 = H_2CCH_2COOH$; $R_3 = OCH_3$; $R_6 = OH$; $R_2 = R_5 = R_6 = H$
LXXV	$R_1 = CH_2CH_2COOH$; $R_3 = R_5 = OCH_3$; $R_4 = OH$; $R_2 = R_6 = H$
LXXVI	$R_1 = HC = CHCOOH$; $R_6 = OH$; $R_2 = R_3 = R_4 = R_5 = H$
LXXVII	$R_1 = HC = CHCOOH$; $R_3 = OCH_3$; $R_6 = OH$; $R_2 = R_4 = R_5 = H$

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Sodium Sulphide Degradation of HSs

From Hayes and O'Callaghan, *Humic Substances II. In Search of Structure.* (1989) Hayes MacCarthy, Malcolm, swift (eds) Wiley, Chichester, pp. 143-180..

Compounds identified in digests after humic acids were degraded in saturated aqueous sodium sulphide solutions at 250°C, under autoclave conditions.†

$CH_3(CH_2)_xOH$ ($x = 1 - 4$) (XCVII)	CH_3COOH (XCVIII)	$CH_3(CH_2)_xCOOH$ ($x = 1 - 4$) (XCIX)
$H_2CCH(OH)COOH$ (C)	$H_2C(O)(CH_2)_2COOH$ (CI)	$(COOH)_2$ (CII)
$HOOC(CH_2)_4COOH$ (CIII)		$HOOCCH(CH_3)COOH$ (CIV)
$HOOCCH(CH_3)CH(CH_3)CH_2COOH$ (CV)		$CH_3C(O)OC(CH_3)_2CH_2COOH$ (CVI)

CVII	$R_1 = HC = CHCH_2CH_3$; $R_2 = R_3 = R_4 = R_5 = R_6 = H$
CVIII	$R_1 = R_2 = COOH$; $R_3 = R_4 = R_5 = R_6 = H$
CIX	$R_1 = OCH_3$; $R_2 = R_3 = R_4 = R_5 = R_6 = H$
CX	$R_1 = CH_3$; $R_2 = OCH_3$; $R_3 = R_4 = R_5 = R_6 = H$
CXI	$R_1 = H_2CCH_3$; $R_4 = OCH_3$; $R_2 = R_3 = R_5 = R_6 = H$
CXII	$R_1 = CH_2OH$; $R_4 = OCH_3$; $R_2 = R_3 = R_5 = R_6 = H$
CXIII	$R_1 = H_2CCH_2COOH$; $R_4 = OCH_3$; $R_2 = R_3 = R_5 = R_6 = H$
CXIV	$R_1 = H_2CCH_2COOH$; 1 alkyl, 1 OCH_3 in ring; 3 R_2 as H
CXV	$R_1 = H_2CC(O)CH_3$; $R_4 = OCH_3$; $R_2 = R_3 = R_5 = R_6 = H$
CXVI	$R_1 = C(O)CH_3$; $R_4 = OCH_3$; $R_2 = R_3 = R_5 = R_6 = H$
CXVII	$R_1 = COOH$; $R_3 = CH_3$; R_5 = Alkyl; $R_2 = R_4 = R_6 = H$
CXVIII	$R_1 = COOH$; $R_3 = CH_3$; $R_4 = OCH_3$; $R_2 = R_5 = R_6 = H$
CXIX	$R_1 = OCH_3$; $R_2 = COOH$; $R_6 = CH_3$; $R_3 = R_4 = R_5 = H$
CXX	$R_1 = R_2 = OCH_3$; $R_3 = R_4 = R_5 = R_6 = H$
CXXI	$R_1 = R_2 = OCH_3$; $R_4 = CH_3$; $R_3 = R_5 = R_6 = H$
CXXII	$R_1 = H_2CCH_3$; $R_2 = R_3 = OCH_3$; $R_4 = R_5 = R_6 = H$
CXXIII	$R_1 = H_2CCH_2COOH$; $R_2 = OCH_3$; $R_4 = C(O)CH_3$; $R_3 = R_5 = R_6 = H$
CXXIV	$R_1 = H_2CCH_2COOH$; $R_2 = R_6 = OCH_3$; $R_3 = CH_3$; $R_4 = R_5 = H$
CXXV	$R_1 = H_2CCH_2COOH$; $R_3 = R_6 = OCH_3$; $R_4 = CH_3$; $R_2 = R_5 = H$
CXXVI	$R_1 = H_2CCOOH$; $R_3 = R_4 = OCH_3$; $R_2 = H_2CCH_3$; $R_5 = R_6 = H$
CXXVII	$R_1 = COOH$; $R_3 = R_4 = OCH_3$; $R_2 = CH_3$; $R_5 = R_6 = H$

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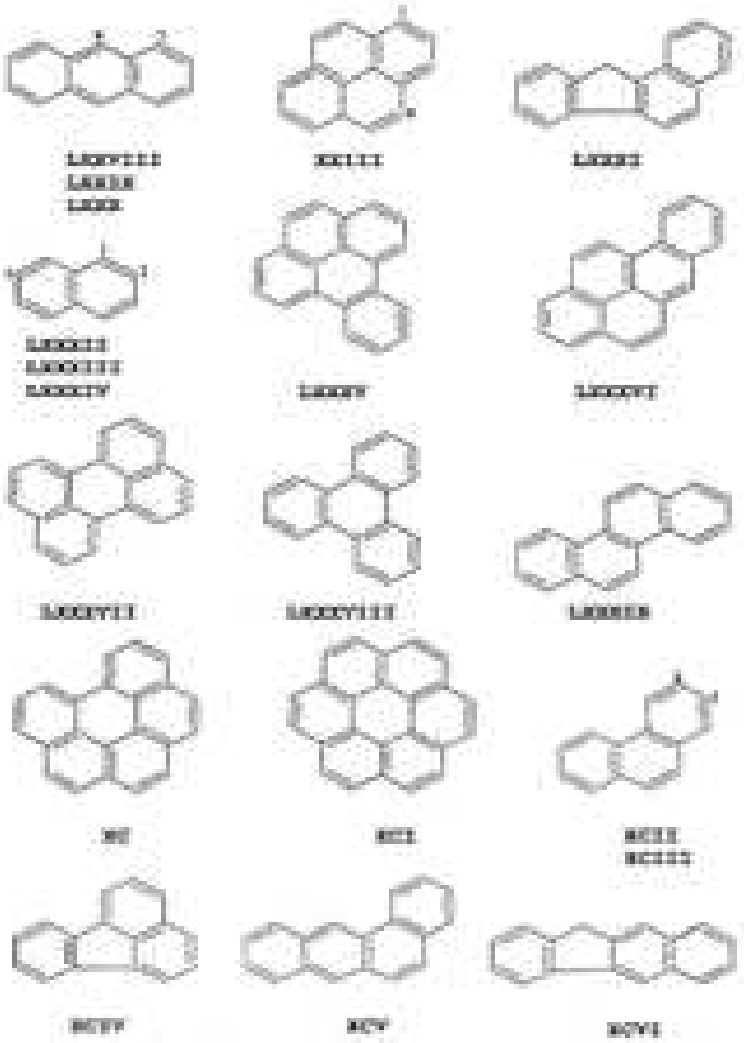
Zinc Dust Distillation and Fusion of HSs

(from Hayes and Swift, 1978; Clapp et al (2005)



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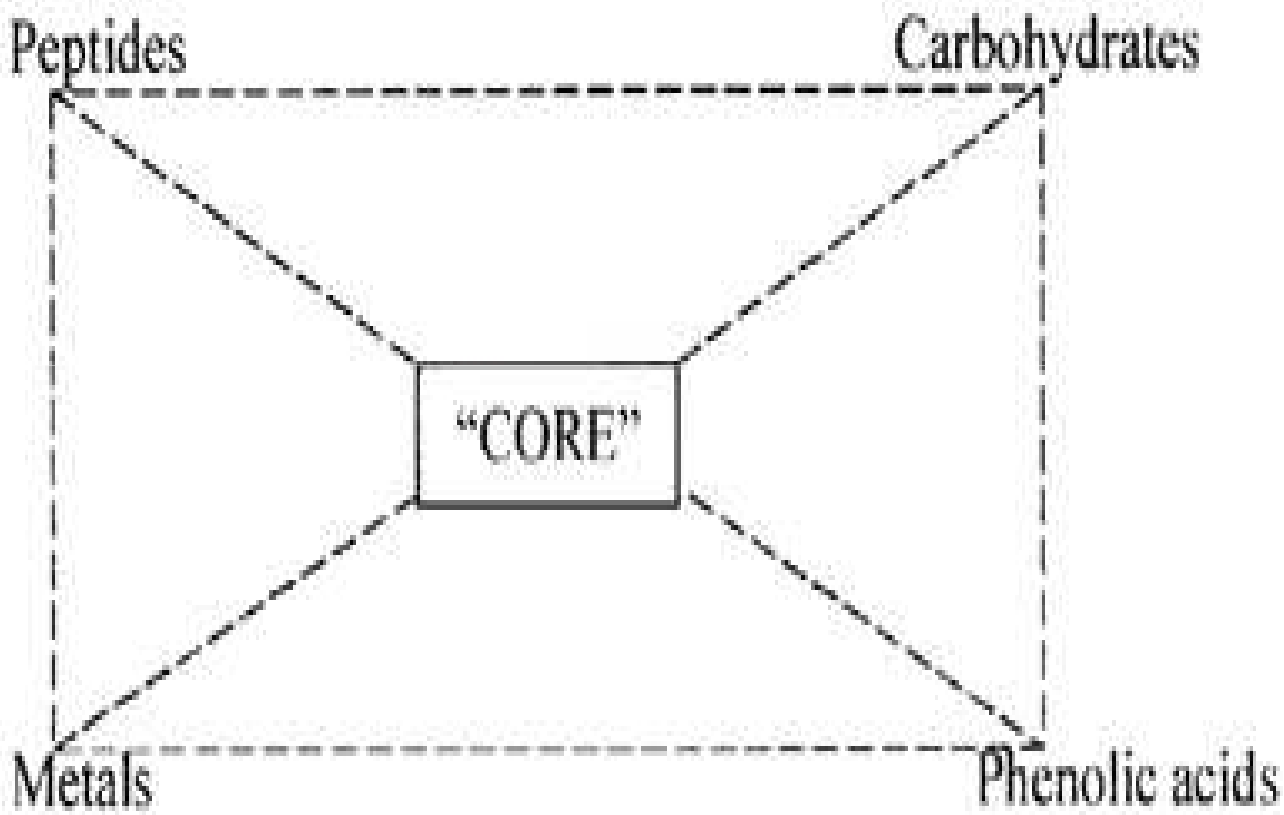
Humic Acid Composition (According to Haworth **(Haworth.RD, (1971*) Soil Sci, 111,* 71-79)**



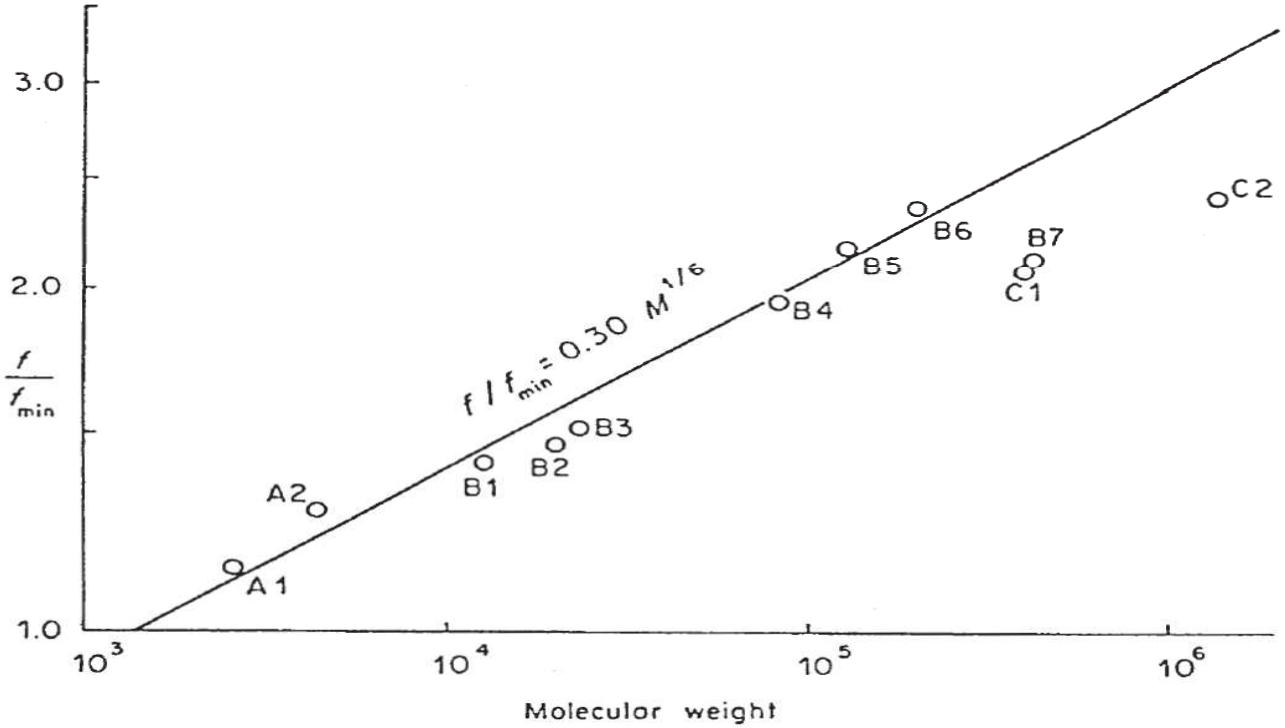
A diagrammatic representation of humic acid. (After Haworth, 1971.)

Sizes and Shapes of HSs

- **In a classic contribution, led by RS Swift, Cameron et al. (*J. Soil Sci. (1972) 23, 394-408)* carried out an extensive gel chromatography fractionation of a HA and subjected the fractions to MW detrminations by ultracentrifugation. The plot of MW versus frictional ratio gave a relationship indicating that the molecules had random coil conformations in solution. The gel fractionation was exhaustive and there was every reason to consider that the fractions were size homogeneous. A major fraction had a MW value of 2000 Da, but the size range extended to ca 1.5 million.**
- **The work of the Piccolo Group, more than a generation later, using HPSEC fractionation, gives evidence suggesting that HSs are supramolecular associations of heterogeneous, relatively small (<1000 Da) molecules held together in seemingly large sizes by weak linkages such as H and hydrophobic bonds.**

Relationship between Frictional ratio(f/fmin) and Molecular weight for HA Fractions

(Cameron et al. (1972), Soil Sci. 23, 342-349) See also Hayes and Swift, 1978 and Clapp et al., 2005) See comments in Slide 30



From 1980 till the Present

- **This period has seen great progress in awareness of aspects of compositions of HSs, and a more realistic awareness of the nature of humic molecules and of their origins and structural components. This has required a more systematic approach to their isolation and fractionation. Progress has been facilitated by the availability of highly sophisticated instrumentation.**

The Formation of the IHSS

- At a meeting in Denver Colorado the IHSS was formed on September 11th 1981, with Dr. Ronald L Malcom of USGS WRD as President, Prof. Roger S Swift of Lincoln University, NZ, as Vice President, and Dr. Patrick MacCarthy of Colorado School of Mines as Secretary/Treasurer.
- The IHSS has had a profound influence in the advancement of the Humic Sciences.
- The 18th International meeting of the Society was held in Kanazawa, Japan in Sept 2016
- The 19th Meeting will be held in the Albena resort, Bulgaria, in Sept. 2018

IHSS Texts

- At the first meeting of the IHSS, at Estes Park, Colorado, Aug, 1983, invited presentations formed chapters for: 'Humic Substances in Soil, Sediment, and Water' (Geochemistry, Isolation and Characterization), (1985)
- G.R. Aiken, D.M. McKnight, R.L Wershaw, P. MacCarthy (eds), Wiley, NY.
- At the second meeting at the University of Birmingham (England) in July, 1984, invited presentations formed chapters for:
- 'Humic Substances II. In Search of Structure'
- M.H.B. Hayes, P. MacCarthy, R.L. Malcolm, R.S. Swift (eds)
- Wiley, Chichester.

Isolation OF SOM Fractions

- Studies of compositions and structures begin with isolation and fractionation. Systems used for Isolation are detailed by Hayes (1985) (in Aiken et al., slide 34) and by Clapp et al (2005) (slide 21) for Isolation and fractionation. (The IHSS process is covered by Swift (1956) Methods of Soil Analysis Part 3. Book series 5 pp 1011-1069, Soil sci Soc Am) Solvents with absorbance values >18 (in Slide 35) are considered good solvents. These have an Electrostatic value (EF) >140 (i.e the Product of Relative Permittivity or dielectric constant x Dipole Moment), and pK_{Hb} (a measure of the relative strength of an acceptor when a H-bond complex is formed) >2. (For a detailed discussion of solvents see Hayes 1985 and Clapp et al. (2005)

Dipolar Aprotic (DA) solvents have dielectric constant values > 15 and are incapable of donating H atoms to form strong H bonds. Good DA solvents in slide 18 have Absorbance values of 18 & >.

Absorbance values for HA solutions in different Solvents*

Values for 0.2% w/w HA in aqueous and organic solvents

Solvent Absorbance value(i.e Absobance at 400 nm X dilution)

- **Water 1.0**
- **Dioxane 0.0**
- **Acetonitrile 0.0**
- **Ethanol 1.0**
- **Formic acid (90%) 4.0**
- **Pyridine 5.0**
- ***N*,*N*-Dimethylformamide (DMF) 18.0**
- **Formamide 19.0**
- **Dimethyl sulfoxide (DMSO) 21.0**
- **0.5 *M* NaOH, pH 9.2 23.0**
- **0.5 *M* NaOH 24.0**
- ***From Hayes (1985).**

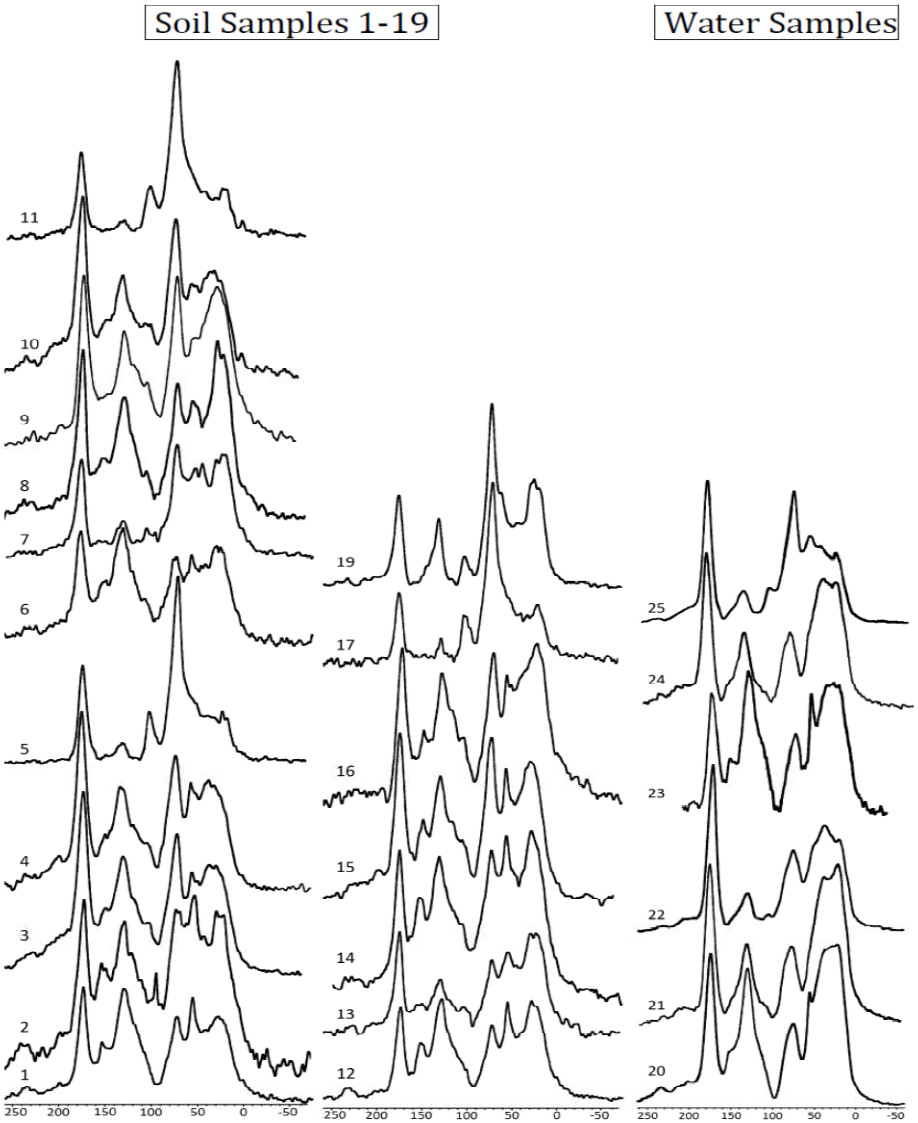
Isolation and Fractionation of HSs
('Carbolea Process')

- The procedure used by the Carbolea Research Group of UL involves:
- Exhaustively extracting H^+ exchanged soils (using Na^+ as the exchangeable cation) at pH 7, 10.6 and 12.6. This removes all of the classical HAs and FAs.
- Then further exhaustive extraction is with 0.1 M NaOH solution made 6 M wrt Urea. The urea breaks H-bonds. (This fraction would be HUMIN in the classical definitions, but it will be shown that it is very similar to the extract at pH 12.6).
- Urea is removed by dialysis and/or washing, and the residual material is dried.

Fractionation Using Resins

- The IHSS procedure uses XAD- resin ((poly)methylmethacrylate) to sorb the more hydrophobic components of FAs. The polar components are adsorbed by XAD-4 resin (styrenedivinyl benzene) (in tandem with XAD-8). Malcolm and MacCarthy (1992) *(Environ. Internat. 18:597-607)* adapted the XAD-8 and XAD-4 resin in tandem procedure to studies of soil HAs, and the Carbolea Group adapted this procedure to further fractionate the HAs isolated from soil at pH 7, pH10.6 and pH12.6. The HAs are dissolved, diluted to < 50 ppm, pH adjusted to 2, and applied to the resins. The fractions isolated from XAD-4 resin are called XAD-4 acids, or hydrophilic acids.
- Comparisons of the NMR spectra of samples isolated by the IHSS procedure (I) and by the resin in tandem procedure (R) can be seen in Slide 39
- **38**

^{13}C NMR Spectra of Fractions (pH 7, pH 10.6, pH 12.6 from the Denchworth Soil



1)SR HA pH 7.0; (2) SI HA pH 7.0; (4) SI FA pH 7.0; (5) SR XAD pH 7.0; (6) SR HA pH10.6; (9) SR FA pH 10.6; (10) SI FA pH 10.6; (11) SR XAD pH 10.6; (12) SR HA pH 12.6; (13) SR $HA_{>2}$ pH 12.6; (17) SR XAD pH 12.6; (19) SR XAD4N. drainage (20) W1 HA; (22) W1 XAD; (25) W2 XAD.

Further details in next slide.

Spectra of base/urea isolates are not included

Reference TM Hayes et al (2012) Organic Geochemistry 52, 13-22.

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Legend for CPMAS ^{13}C NMR Spectra of Fractions from the Denchworth Soil and Drainage Water

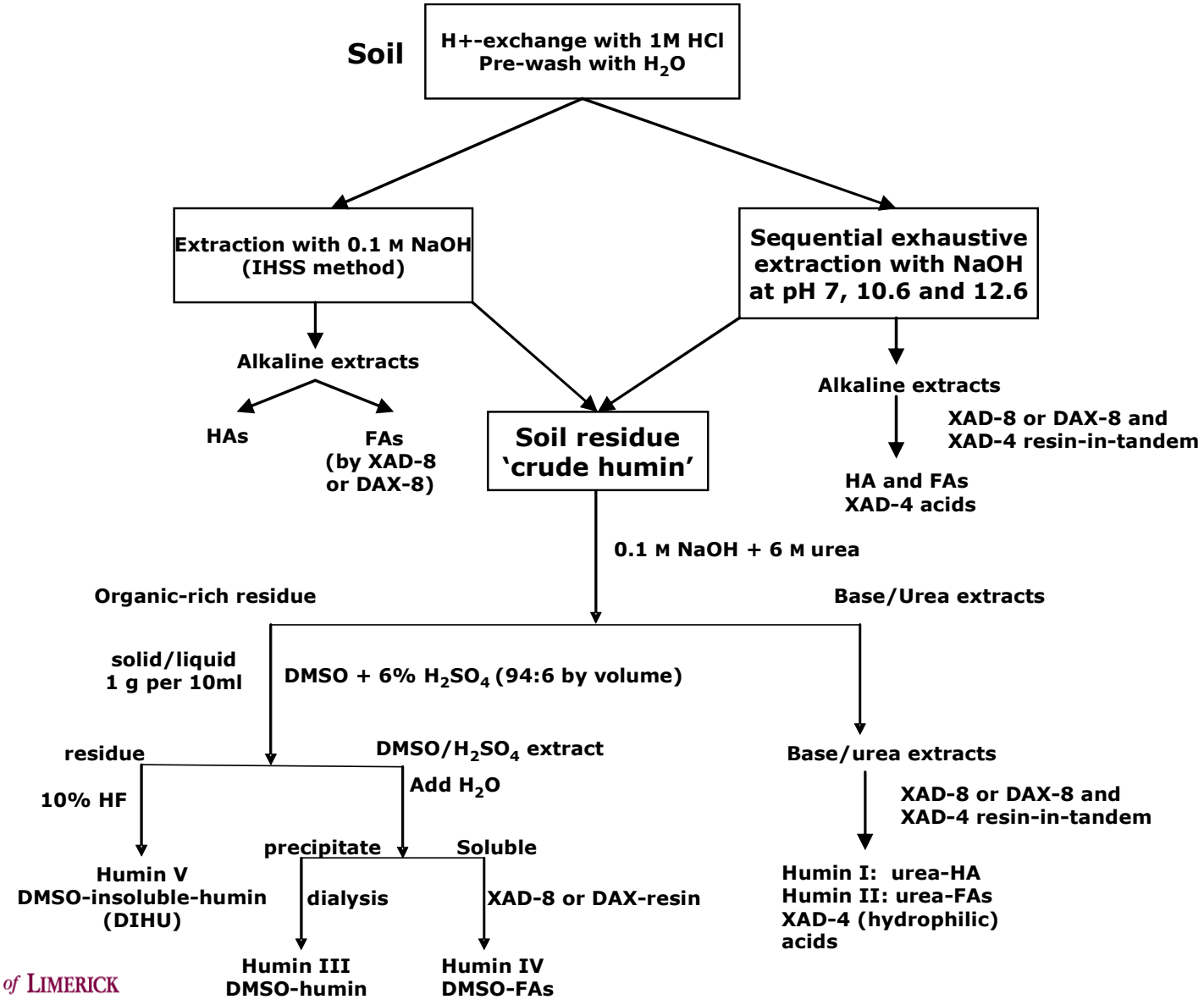
- **Fractions were obtained by the IHSS (I) and Resin in Tandem (R).**
- **Denchworth; a pelo-stagnogley of swelling clay passing to clay or mudstone with topsoil (0-20 cm), 44.2% clay (< 2µm), 16.3% silt (2-63 µm), 7.4% fine sand (63-212 µm) 2.7% coarse sand 3.3% organic C**
- HA = Humic acids; FA= Fulvic acids; XAD = acids recovered from XAD-4 resin; and XAD-4N= neutrals from XAD4, all isolated from the Denchworth soil (S) at pH 7.0, pH 10.6, and pH 12.6 using the XAD-8 and XAD-4 resin-in tandem procedure (R) or the IHSS process (I).
- Spectra are: (1), SR HA pH 7.0; (2), SI HA pH 7.0; (3), SR FA pH 7.0; (4), SI FA pH 7.0; (5), SR XAD pH 7.0; (6), SR HA pH10.6; (7), SR $HA_{>2}$ pH 10.6 (ie. Ppt formed at pH>2); (8), SI HA pH 10.6; (9), SR FA pH 10.6; (10), SI FA pH 10.6; (11), SR XAD pH 10.6; (12), SR HA pH 12.6; (13), SR $HA_{>2}$ pH 12.6; (14), SI HA pH 12.6; (15), SR FA pH 12.6; (16), SI FA pH 12.6; (17), SR XAD pH 12.6; (19), SR XAD4N. **Spectra for the drainage waters are:** (20), W1 HA; (21), W1 FA; (22), W1 XAD; (23), W2 HA; (24), W2 FA; (25), W2 XAD. **(Sample W1 was taken in April and W2 in January)**.

Further Extractions (Non-Aqueous)

- **After the exhaustive extraction with 0.1 M NaOH + 6 M Urea, the residual material, after washing and drying, is exhaustively extracted using dimethylsulphoxide (DMSO) + 6% H_2SO_4 . (In this system the H_2SO_4 behaves as a solvent in a non-aqueous medium rather than as a strong acid and it enhances the solvation properties of DMSO. The DMSO + 6% H_2SO_4 extract is poured into a copious quantity of water; ~95% of the extracted material precipitates.**
- **This precipitate satisfies the classical and IHSS definition of Humin**
- ***But is it Humic material?***

Summary of Extraction and Fractionation of SOM

From Song et al. (2011) *Naturwissenschaften 98, 7-13.*



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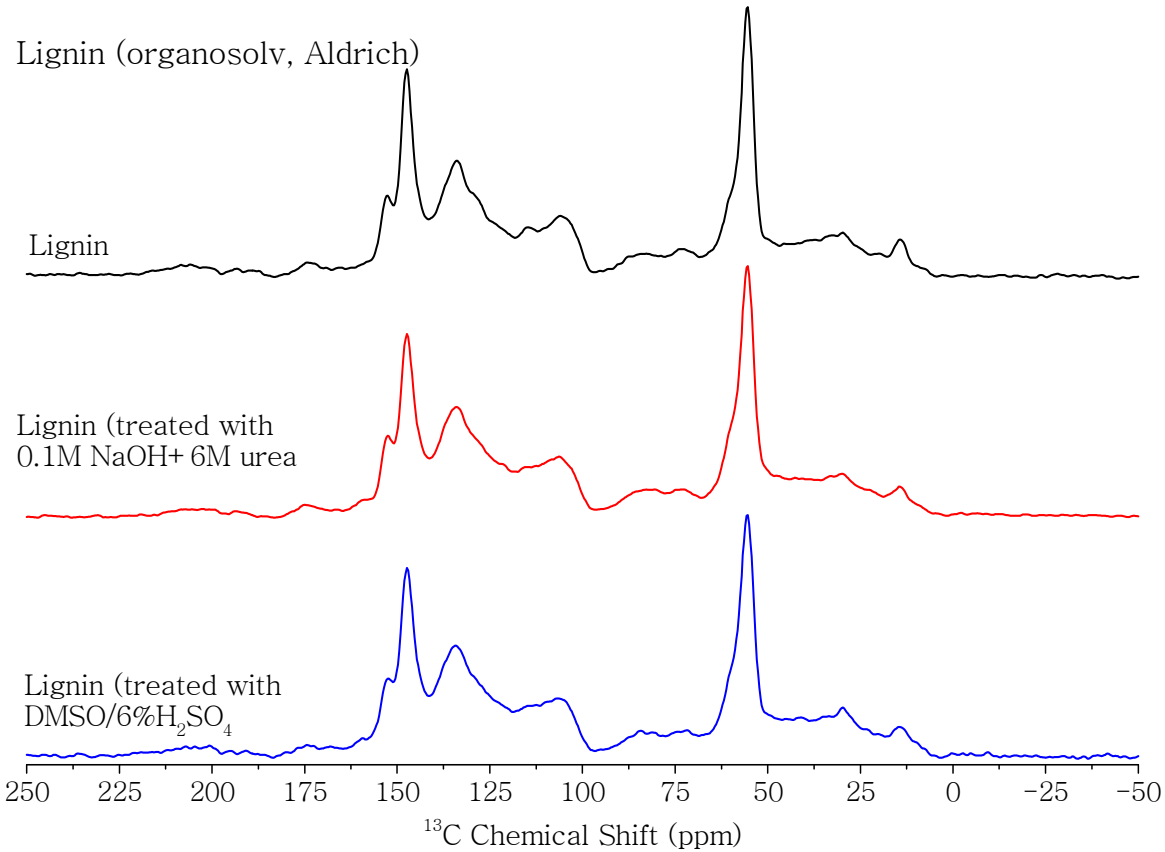
Does DMSO/ H_2SO_4 Cause Damage?

NMR and FTIR evidence indicates No



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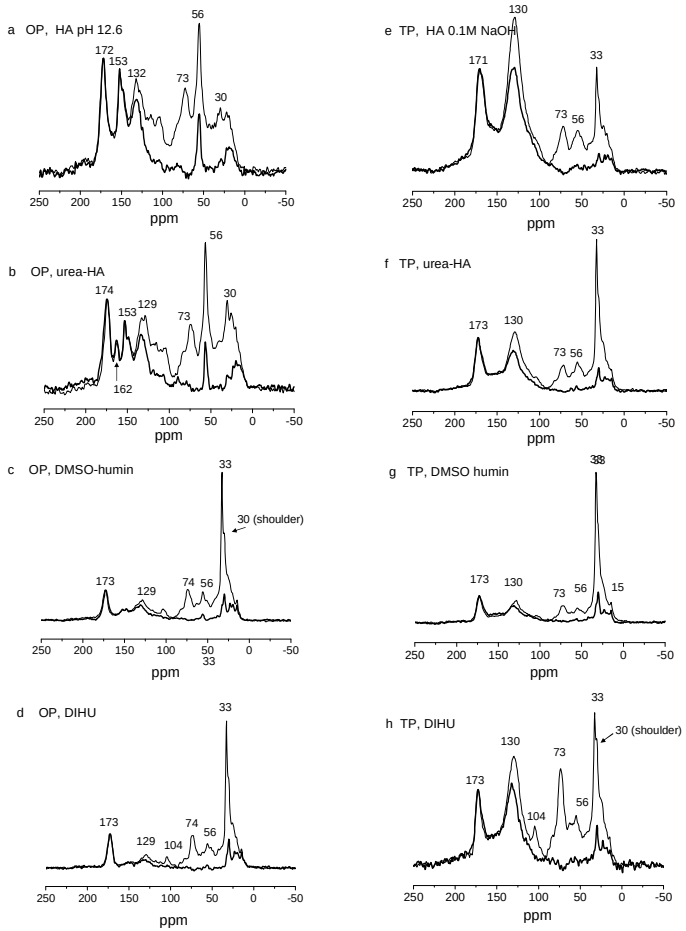
Yields of Extracts

- In general, aqueous base isolates 20-30% of the humified OM in surface mineral soils and up to 40% from organic soils.
- The base + urea extraction system isolated an additional 15-20% material
- Therefore 50-65% of the SOM remains
- The DMSO + 6% H_2SO_4 system isolates a further 70-95% of these residual materials

Illustration of Similarities Between Humic Acids Isolated at pH 12.6 and in 0.1 M NaOH + Urea to 6 M

The spectral similarities indicate. that the/base/urea extract is Humic and not Humin. Note the differences between spectra for HAs and Humin



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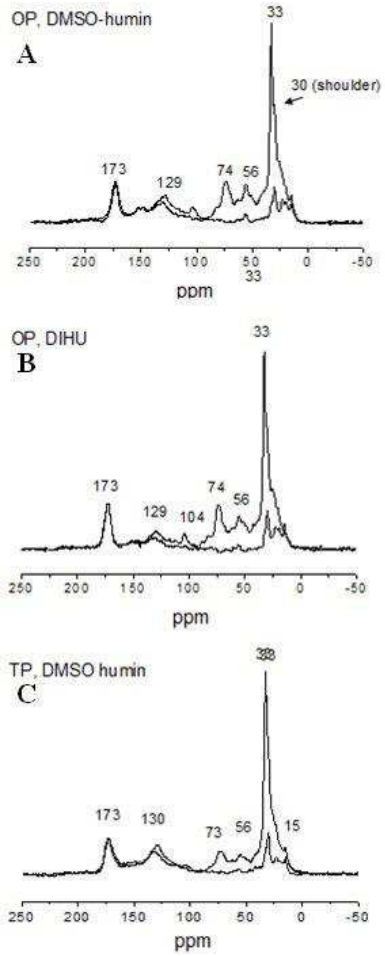
CPMAS ^{13}C NMR and Dipolar Dephasing spectra (lower and darker line) for: (A), the Brown Earth (OP) HUMIN (HU) isolated in the DMSO-acid system; (B) the HU from the demineralised clay (Using HCl/HF); and (C) the HU from the Terra Preta soil.



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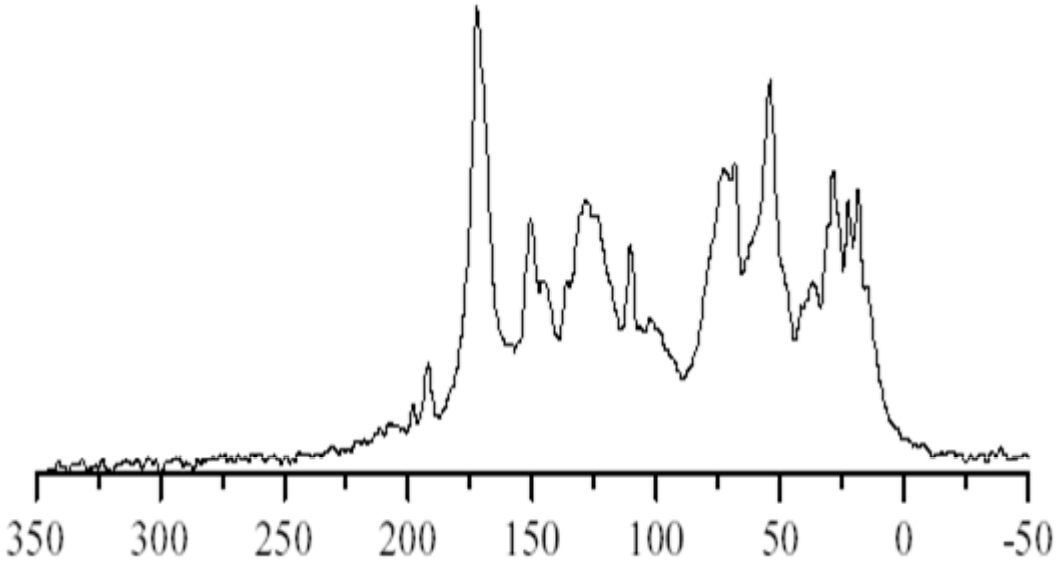
Dipolar Dephasing (DD) Spectra

- In DD experiments signals from any nucleus directly bonded to H are removed.

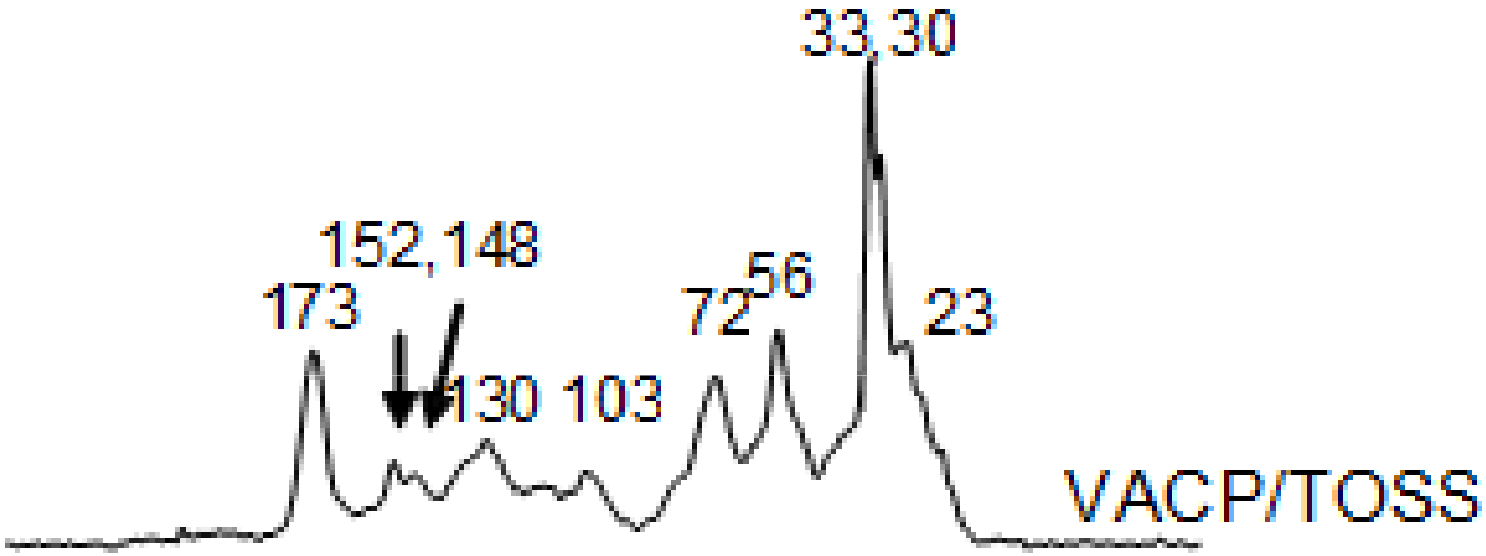
- Thus it gives indications of the extents of replacement of H substituents in the aromatic nucleus or of the extents of fusions of aromatic structures.

^{13}C NMR Spectrum of a Humic acid from a Grey Brown Podzol



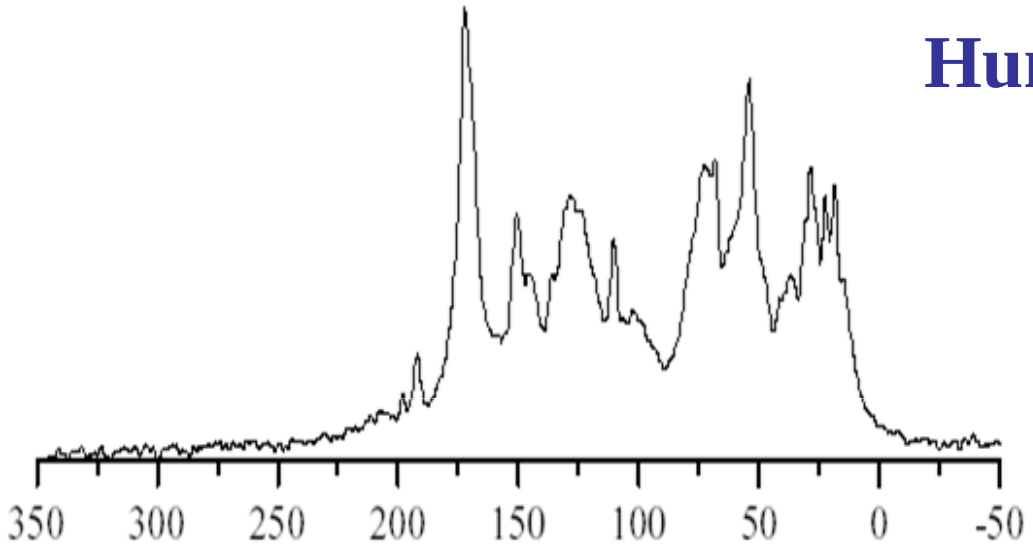
Notes: Relatively strong carboxyl, (160-180 ppm); aromatic (120-140 ppjm). lignin (140-150 and 55 ppm) and carbohydrate (70-90 and 105 ppm) contributions, significant aliphatic C (15-40 ppm).
The spectrum indicates that humification is incomplete.

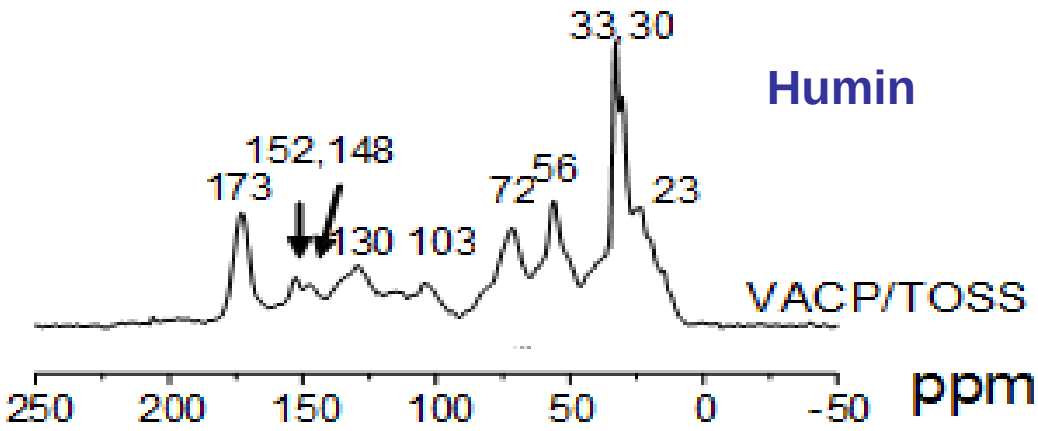
^{13}C NMR spectrum of humin extract from a Grey Brown Podzol



Note: Strong aliphatic-C23, Me; 30 amorphous methylene; 33 crystalline methylene; , moderate amino (56 ppm also methoxyl), carbohydrate (72, 103; and carboxyl/ester/amide(173) and low aromatic (130) & lignin (148, 152 signal)

Contrasting the Spectra of HAs and of Humin from Grey Brown Podzol





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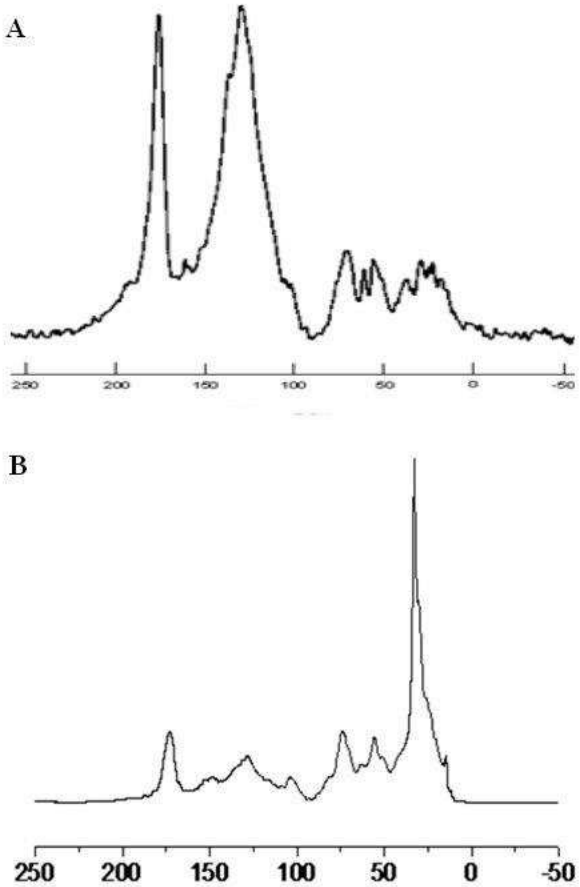
CPMAS Spectra of A, Mollisol HA, and B Mollisol HU solated in the DMSO-acid



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- **IT MUST BE EMPHASIZED THAT THERE ARE SIGNIFICANT DIFFERENCES BETWEEN HAs FROM DIFFERENT SOURCES BUT THERE ARE DISTINCT SIMILARITIES BETWEEN ALL HUMINS ISOLATED BY THE CARBOLEA PROCEDURE.**

- **It is clear that HSs are the products of the biomass source and of the biological transformation processes that operate in any particular environment whereas the HU materials are the most resistant biomass components, and/or those that are protected (e.g. by sorption) by the mineral colloids**

Why has Emphasis on Humin Lagged behind that of Humic Acid?

The main reasons are that:

- **When studied *in situ* in whole soil, accurate interpretation of data on humin composition is very difficult because of the presence of numerous other organic and mineral components**
- **Also, until recently it has not been possible to isolate humin from soil. Thus it has been difficult to characterise humin using chemical and/or spectroscopic techniques**

Why is Humin important?

- In most soils studied, the alkali insoluble humin is the major component of soil organic matter accounting for 50-70% of the total SOM (cf 15-35% Humic Substances).
- Due to its chemical composition humin is recalcitrant and breaks down very slowly in soil.
- Hence it plays an important role in carbon sequestration and in the period of retention by soil.
- Manipulation of this fraction may provide an opportunity to increase soil carbon sequestration.

What do We know About the Compositions of Humin?

- **Humin was regarded as the intractable component of SOM, largely because of the difficultes in isolating it in solution.**
- **The DMSO/H_2SO_4 solvent system now allows it to be recovered as a solid virtually free from mineral contamination and can be studied by solid and liquid states NMR.**
- **It will be shown in slides that follow that the major components are aliphatic, some with long chain hydrocarbon moieties characteristic of long chain fatty acids/esters, waxes, and include plant components that are difficult to degrade, such as the cuticular materials cutin, cutan, suberin, suberan. It will be seen in the slides that follow that all of the humins that we have isolated include small amounts of saccharides and peptidic materials, including lipoprotein from microbial cell walls, and possibly ordered or 'crystalline' cellulose, but very little lignins. These materials may be protected by sorption on the inorganic colloids, or entrained in the resistant hydrocarbons**

How have we advanced the study of Humin?

1. *By the removal of other components*

- **Removal of all the aqueous extractable humic components by exhaustive extraction with 0.1 M NaOH (IHSS Method) then with added urea to 6 M to remove strongly H-bonded components**
- **Removal of mineral components such as silicates and oxides**
- **The residual humin can then be studied with less interference from other organic and mineral components**

How have we advanced the study of Humin?
2. *By identifying a 'solvent' that dissolves humin*

- **Previously a wide of range polar and non-polar solvents have been used in attempts to extract humin but with limited success.**
- **DMSO + 6% H_2SO_4 has been found to be a very effective at solubilising humin which can then be recovered without discernible adverse effects.**
- **In this non-aqueous system the H_2SO_4 behaves as a solvent rather than a strong acid**
- **For evaluation of relevant properties of organic solvents see Hayes (1985) Extraction of humic substances from soil. In G.R. Aiken, D.M. McKnight, R.L. Wershaw, and P. MacCarthy (eds), *Humic Substances in Soil, Sediment, and Water.* Wiley, New York, pp. 329-362; And Clapp et al. (2005) (Ref in Slide 20.**

How have we advanced the study of Humin?

3. By Applications of Modern NMR Procedures

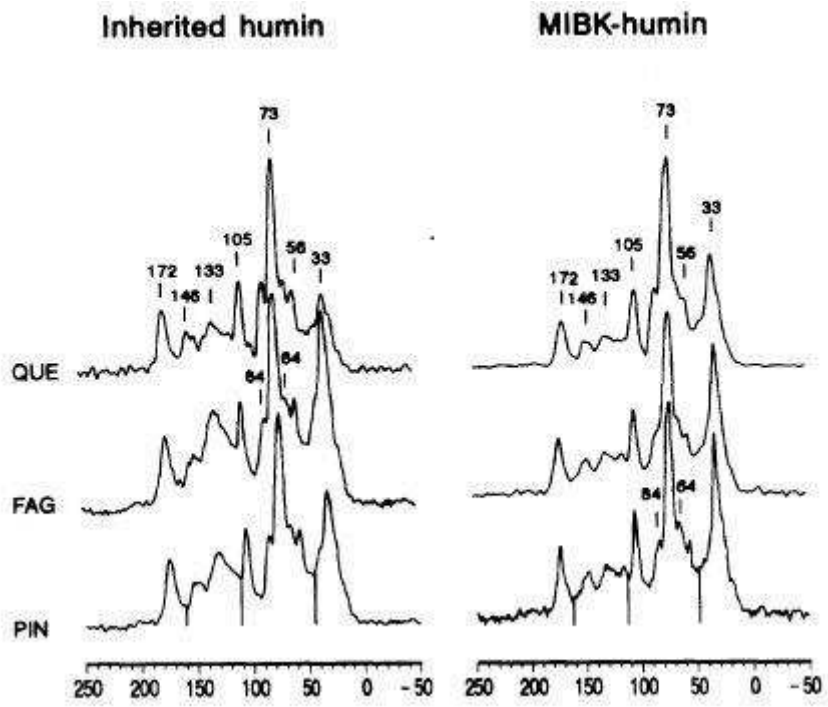
- **We focus initially on CPMAS NMR results that provide spectra of humins from processes that involve degradation of the associated silicates and on those isolated by a partitioning process**
- **Then we show Comprehensive Multiphase ^{13}C NMR spectra**
- **Then Proton NMR showing contributions of peptide-type structures**
- **Then Diffusion edited ^{1}H NMR**
- **2-D (^{1}H-^{1} H) Total correlation spectroscopy (TOCSY)**
- **2-D Heteronuclear (^{1}H-^{13} C) Multiple Quantum Coherence (HMQC)** spectroscopy

CPMAS ^{13}C NMR of Inherited and MIBK Humins

From Almendros *et al*. *Org. Geochem*. **24**, 651-659 (1996)

Inherited from HCl/HF treatment; MIBK from methylisobutylketone /base partitioning. The HCl/HF procedure was significant, but all of the Humics were not pre-isolated. The MIBK was the first application of organic solvent systems.

Note the unusually high carbohydrate and significant aromatic and O-aromatic contents



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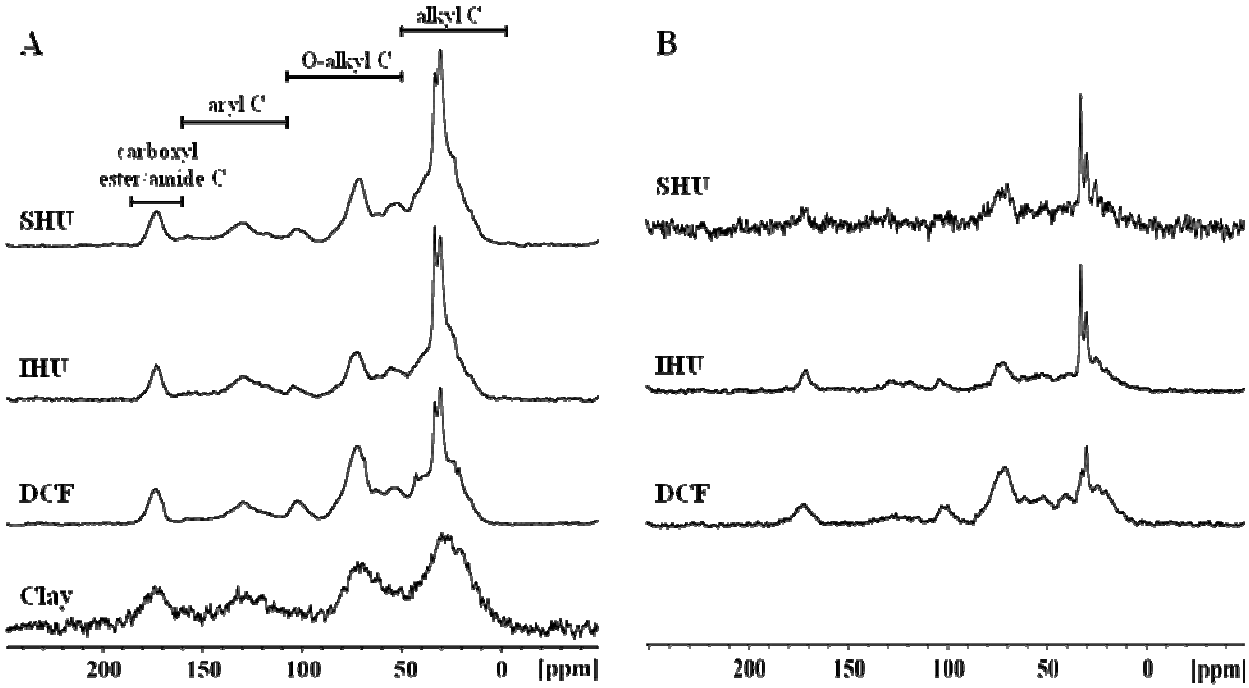
A, CPMAS solid-state ^{13}C NMR spectroscopy; and B, Comprehensive Multiphase (CMP) ^{13}C NMR spectroscopy of samples from the top 1 m of an estuarine core. SHU = DMSO/acid-soluble humin; IHU = DMSO/acid-insoluble humin; DCF = Demineralised Clay Fraction ; and Clay = untreated isolated clay fraction. (See Slide 54 for explanations).



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Comprehensive Multiphase (CMP) ^{13}C NMR

(See Courtier-Murias et al. J. Magn Reson. (2012) 217, 61-74

- **The CMP probe can allow the full range of solution-, gel-, and solid-state experiments to be carried out. This technology provides opportunities to study both structures and interactions independently in each phase as well as interactions between phases, within a heterogeneous sample.**
- **Note, the resonances in A are better defined than those in B (swollen in DMSO). However, observe the relative abundances of resonances at 30 (amorphous methylene) and 33 (ordered or 'crystalline' methylene. That of the 33 ppm resonance illustrates the difficulty of DMSO solvation of the ordered structure.**

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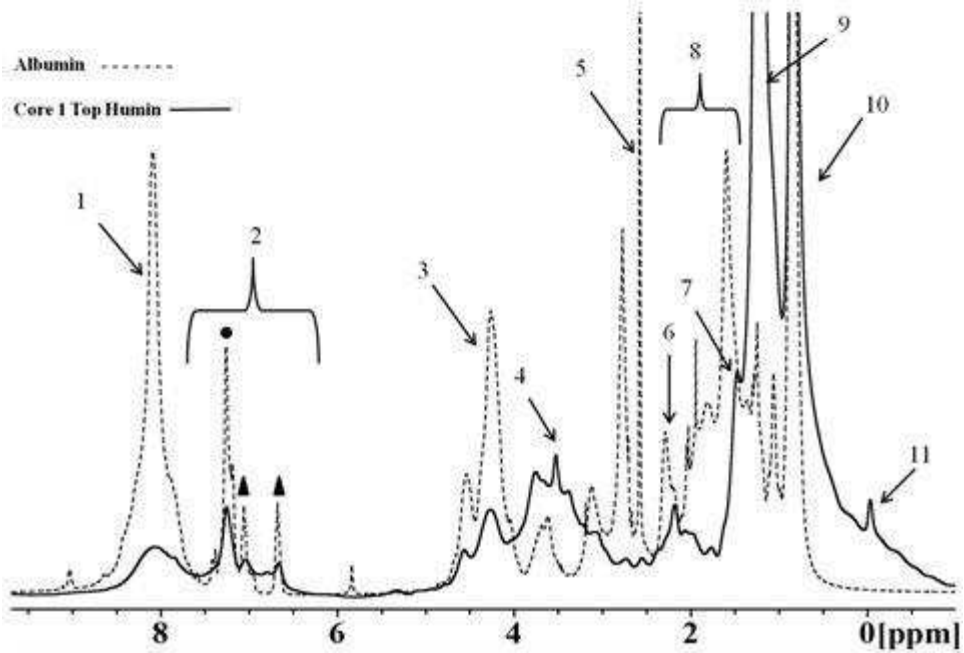
Proton NMR of Albumin and of DMSO/acid Humin from an Estuarine Core

1, Amide in peptides; 2, aromatic amino acids; 3, alpha protons (peptides); 4, O-aromatics (methoxyl); 5 Solvent (DMSO); 6, methylene adjacent to carbonyl; 7, aliphatic methylene gamma to acid/ester; 8, amino acid sidechains; 9, aliphatic methylene; 10, methyl; 11, silicate.

See Mylotte et al. (2016) Org.Geochem 98, 155-165.

63



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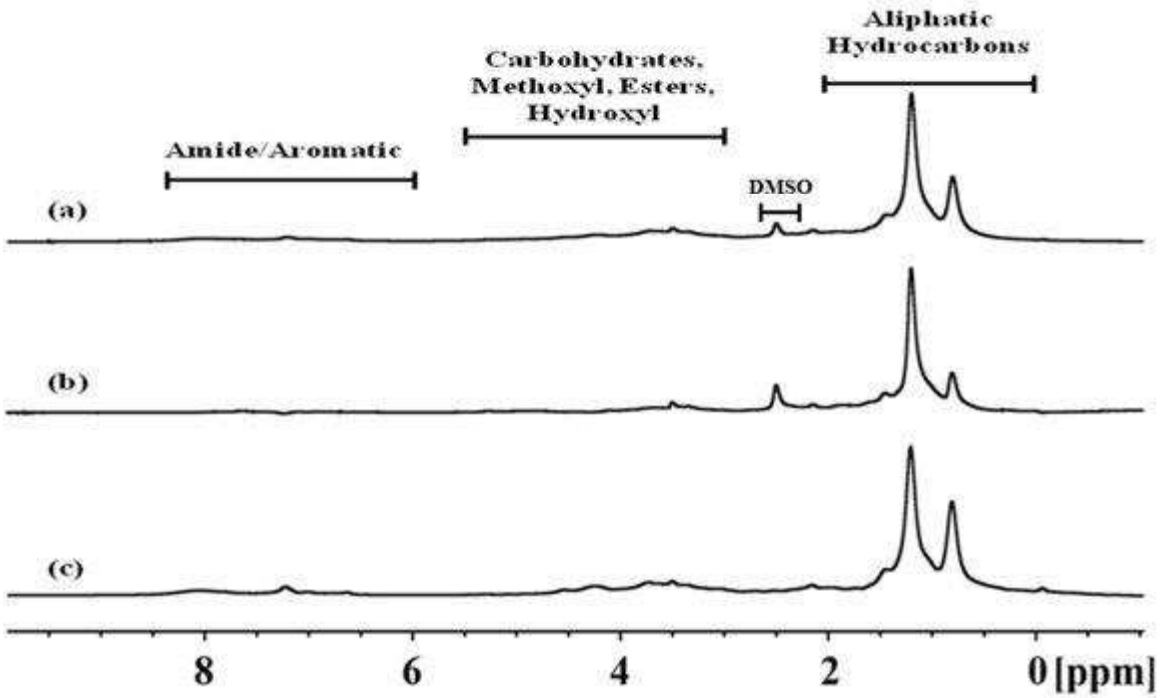
Diffusion Edited ^{1}H NMR spectra of the acidified DMSO humin extract from the 0-1 m depth of an estuarine core. (a),. Conventional 1H NMR spectrum; (b) Inverse diffusion edited spectrum (showing fast-diffusing molecules; and (c) Diffusion edited spectrum showing slow diffusing (solid/gel) molecules.



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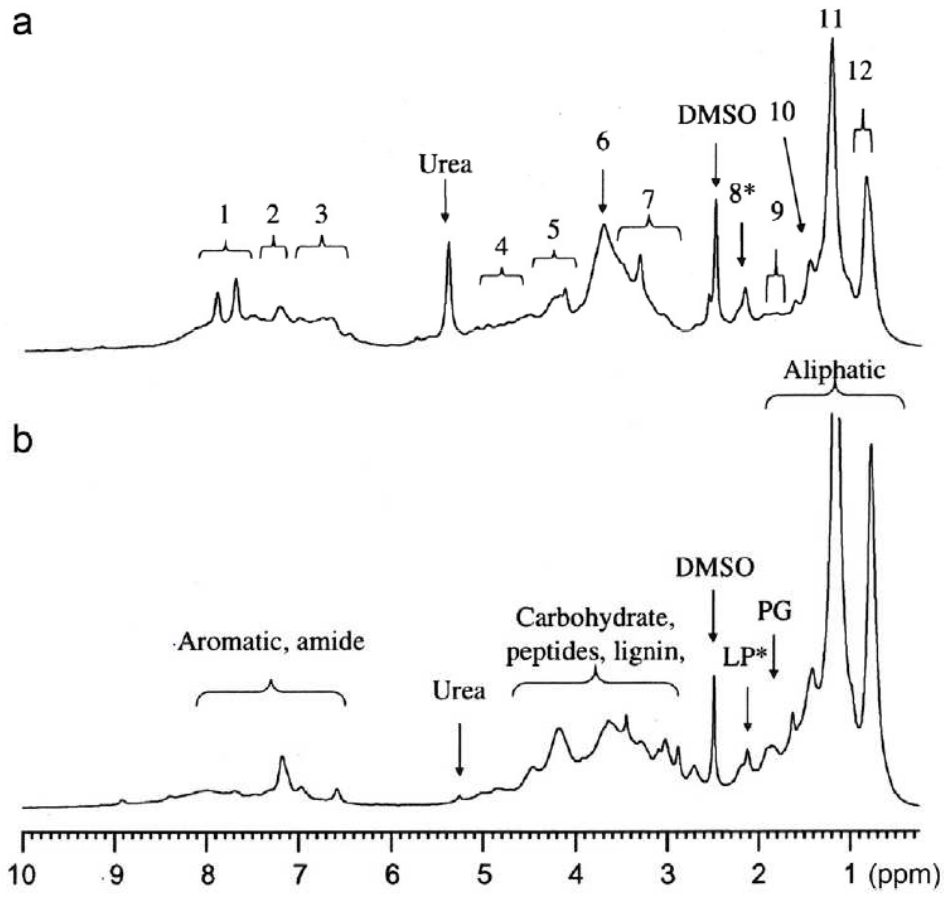
Diffusion Editing (DE) Relevant to Proton NMR

- In DE molecules are “spatially encoded”at the start of the experiment and “refocussed” at the end of the experiment. Species that exhibit high motion are not refocussed and “gated”from the final spectrum. The spectrum recorded will contain only signals with little or no diffusion. Hence the structures identified will be macromolecular or in rigid domains

Diffusion-edited spectra of (a), Base/urea extract of Mollisol following extractions at pH 7, 10.6, 12.6; and (b), DMSO/acid ext following base/urea extract. (See Legend Slide 56)



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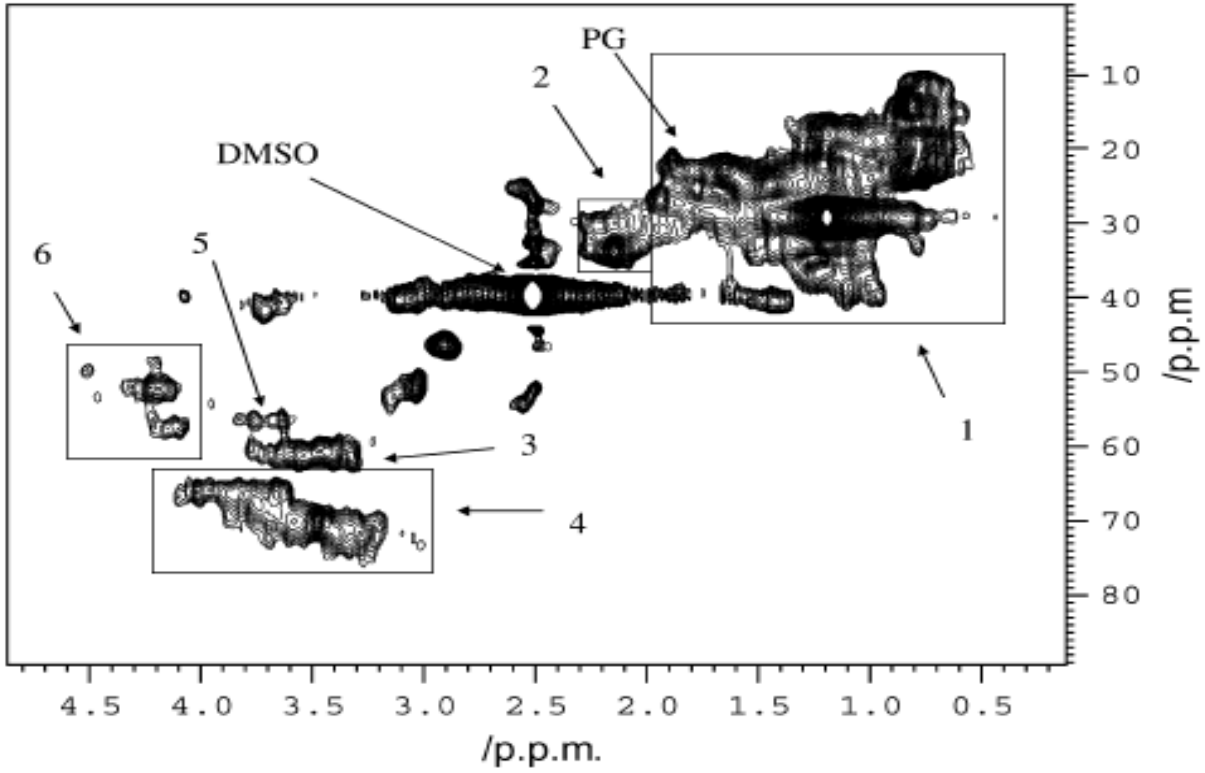
Assignments for DE Spectra

- Assignments in (a) refer to: 1, amide; 2, phenylalanine; 3, aromatics in lignin; 4, anomeric protons in carbohydrates; 5, α-protons in proteins and peptides; 6, methoxyl in lignin; 7, other carbohydrate protons; 8*, P–OCO–R methylene units adjacent to the carbonyl in lipoproteins; 9, N-acetyl group in peptidoglycan; 10, methylene units in an aliphatic chain β to an acid or ester; 11, methylene (CH2)n in aliphatic chains; 12, CH3. (Note when this peak is large relative to 11, it often indicates strong contributions from proteins, as in these examples).
- 65

Heteronuclear Multiple Quantum Coherence (HMQC) spectrum of the humin fraction, Mollisol (IHSS). Assignments are: (1) Aliphatic; (2) R-OCO-CH_2 – R methylene unit adjacent to the carbonyl in lipids and cutins; (3) CH_2 carbohydrate; (4) CH carbohydrate; (5) methoxyl; (6) α H-C in peptides/protein



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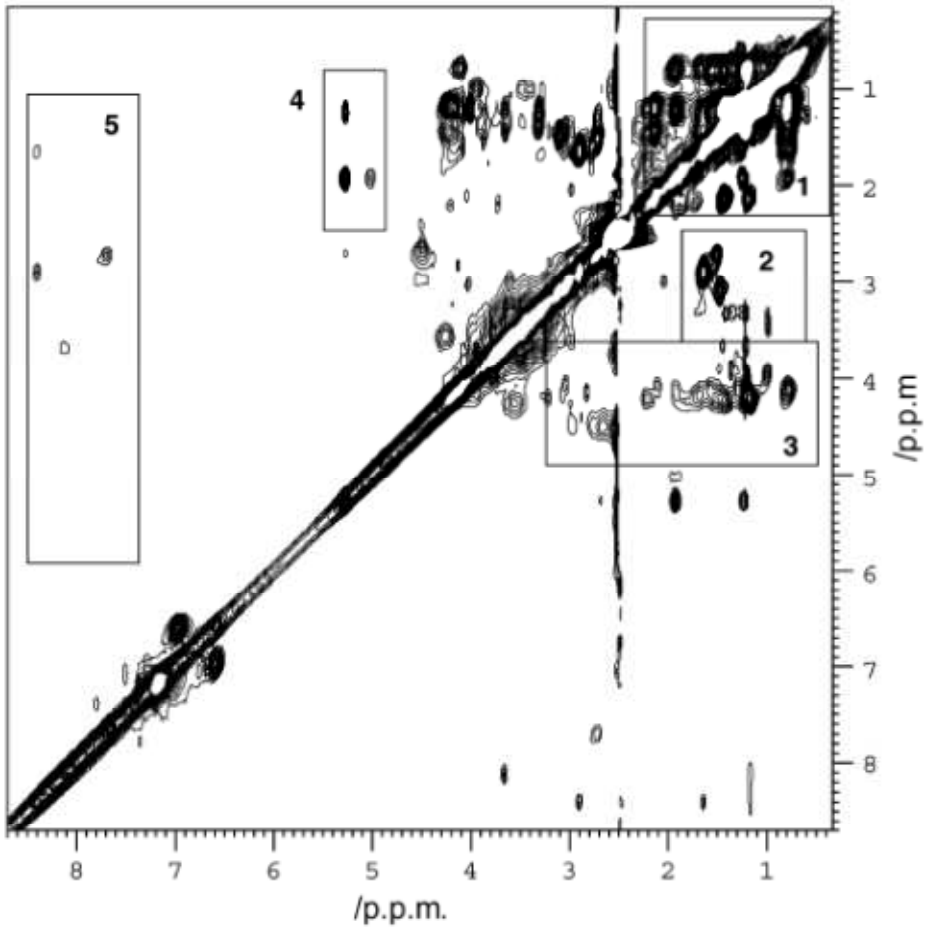
Heteronuclear Multible Bond Coherence (HMBC) Spectra

- These (^{1}H-^{13}C) experiments decrease a spectral overlap due to the additional dispersion provided by the 13C dimension, and that allows assignments to be made that are not possible in the 1D NMR.Heteronuclear spectra.
- Unlike those for 2D homonuclear experiments,these do not exhibit a spectrum diagonal.
- A ^{1}H–^{13}C bond will produce a resonance at the point of intersection of the ^{1}H and ^{13}C chemical shifts

Total Correlation Spectroscopy (TOCSY) of DMSO humin isolated from the Mollisol soil. General assignments: (1), aliphatic couplings; (2), couplings from aliphatic alcohols and ethers (some amino acid side chains overlap in this region); (3), couplings between α-protons and amino acid side chains in peptides/proteins (couplings from ester will also overlap in this region); (4), couplings from double bonds; and (5), couplings from amide in peptides. (these couplings are weak as most of the amides have been exchanged by the addition of D_2SO_4).



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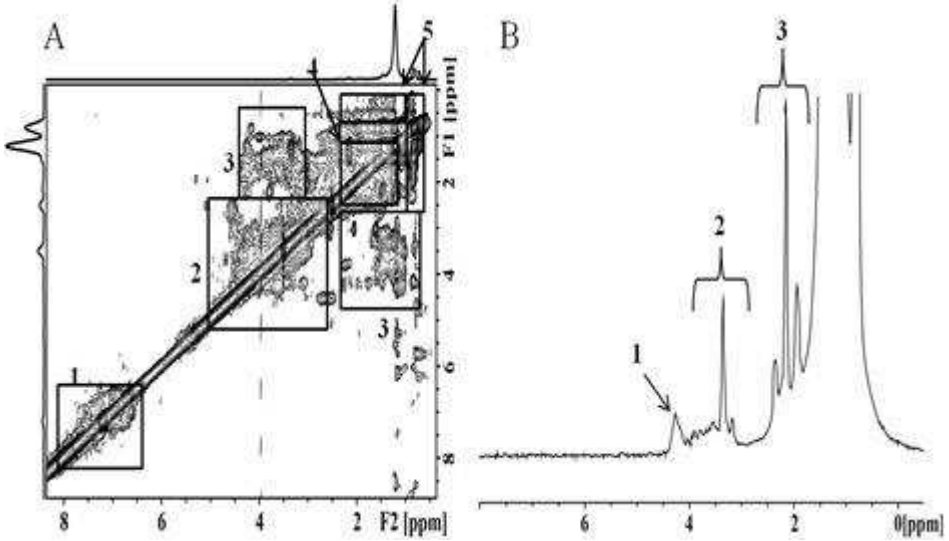
TOCSY (Homonuclear experiments)

- TOCSY provides information about protons that are in the same spin system
- Magnetization from the first proton is transferred to the next proton and so forth.
- The longer the mixing time, the greater is the likelihood that a cross-peak from a spin that is many bonds away will be observed).
- Taking a slice from the TOCSY experiment can show 1H 1D resonances in greater detail, thus aiding the assignment of peaks.

Total Correlation Spectroscopy (TOCSY) of DMSO/acid sol Humin (SHU) from the Top 0-1 m of Esturine Core

A. Assignments: (1), aromatics; (2), sugars, methine units bridging lignin aromatics, amino acids (α-β couplings); (3), methylene units adjacent to ethers, esters, and hydroxyls in aliphatic chains, amino acids (α-β-γ couplings); (4), methylene in aliphatic chains; and (5), CH_3 units in amino acids and aliphatic chains.

B: ^{1}H slice through the aliphatic region of the TOCSY spectrum. Assignments: (1), H attached to the O side of an ester; (2), methylene α to H in primary alcohols; and (3), methylene bonded to (a), free fatty acids, and (b), C=O side of an ester .



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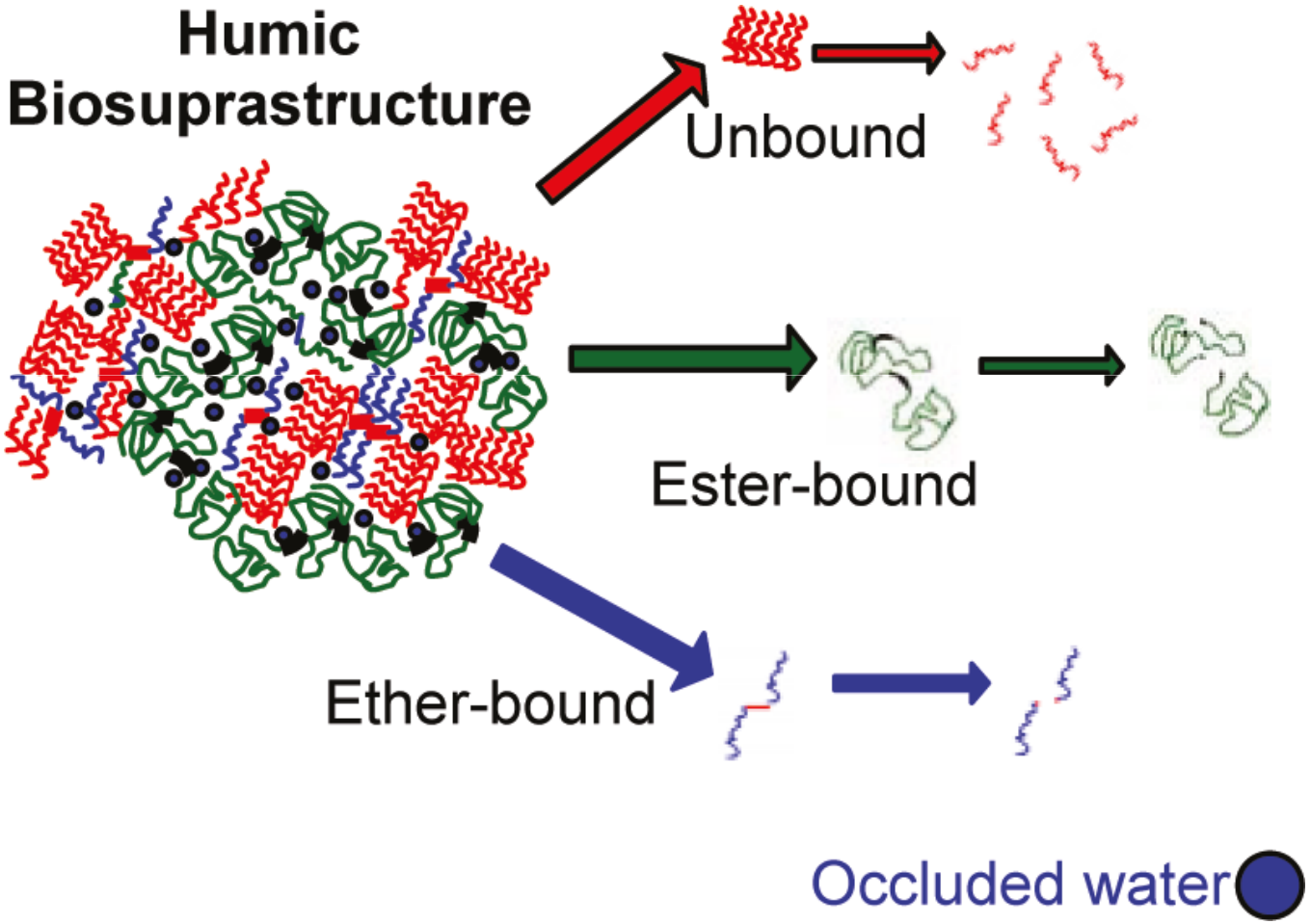
Summary of Humin Data

- A variety of solid and liquid state NMR procedures have given good indications of the types of molecules that compose humin structures. It is important to emphasise that the major components in the structures are aliphatic hydrocarbons, although there is abundant evidence for other types of structures present in small amounts.
- We have, therefore, a reasonable understanding of the types of components in humin structures, but we need to have a better awareness about the ways in which these are associated with each other and with the soil mineral components.
- Awareness of humic structures is less well defined. There is a need to achieve better fractionation based on size, shape, charge and polarity. The HUMEOMICS approach is, in principle, advanced, but it will need concerted research involving persons who are willing to cooperate and collaborate, and freely exchange views.

The Humeomics Approach

Seeks to separate the unbound from the covalently linked species in HSs, and to cleave and identify covalently linked species (See Nebbioso & Piccolo, *Bio*Macromolecules(2011) 12, 1187-1199))



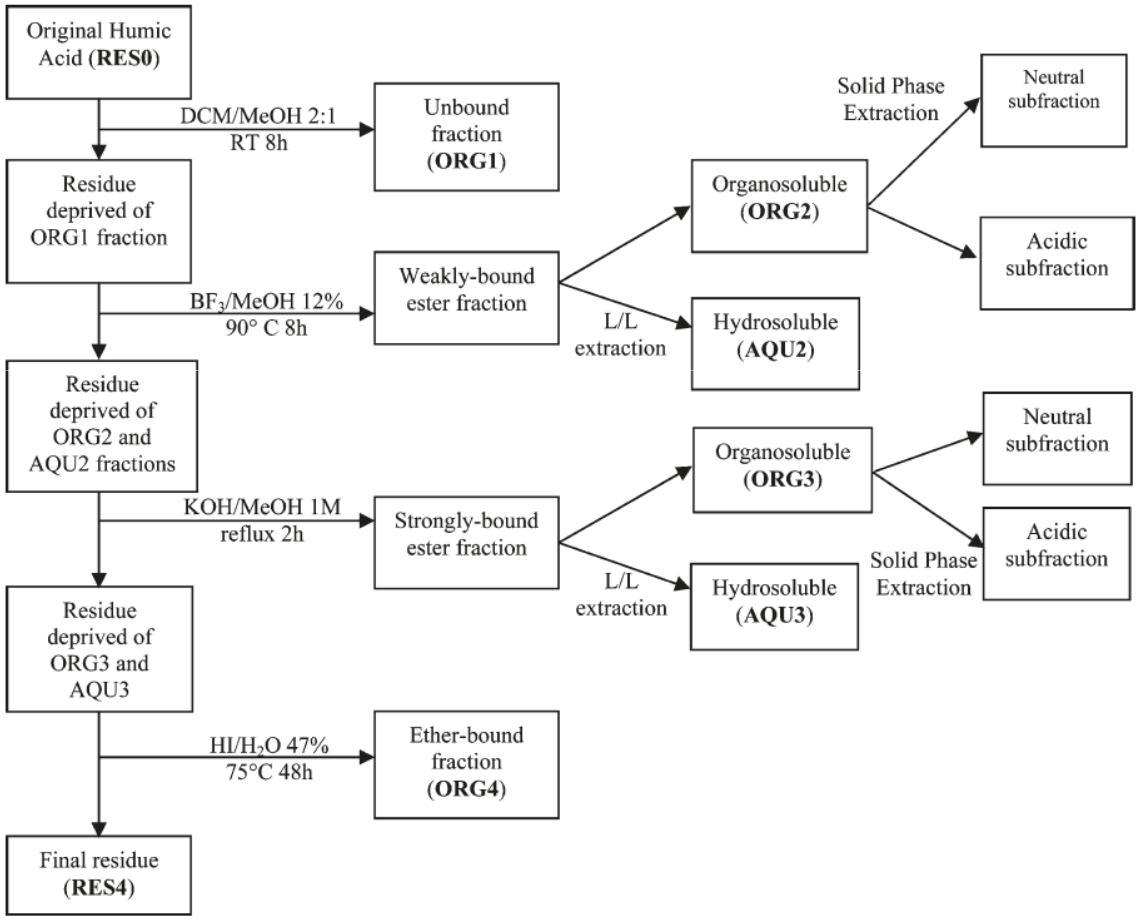
What is Humeomics?

- *(There will be significant coverage of this topic in the programme. Here I give a brief illustration and comment)*

- **Nebbioso and Piccolo (BioMacromolecu 12, 1187-1199, 2011) have described Humeomics as**
- **"A stepwise separation of molecules from humic suprastructures by progressively breaking intermolecular bonds and characterising their structure by advanced analytical instrumentation".**
- **It begins with the organic solvent extraction of unbound molecules associated with the structures.**
- **Then weakly bound esters, strongly bound esters and ether bound components are successively cleaved, recovered and analysed, as outlined in the next slide (74).**

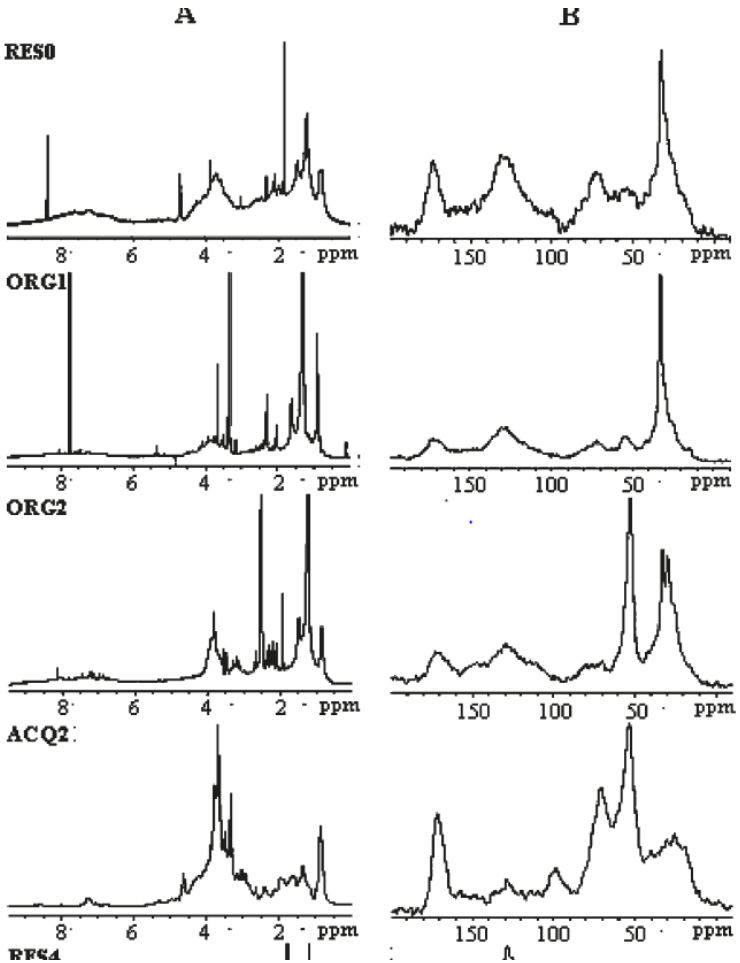
The Humeomics Approach (Continued)

(See Nebbioso and Piccolo, *Bio*Macromolecules (2011, **12**, 1187-1199)



NMR Spectra of HA Fractions Sequentially Fractionated from a Volcanic Soil (Nebbioso & Piccolo (2011)

A = Proton NMR: B = CPMAS 13C NMR



NMR (Continued)

(From Nebbioso and Piccolo, 2011)

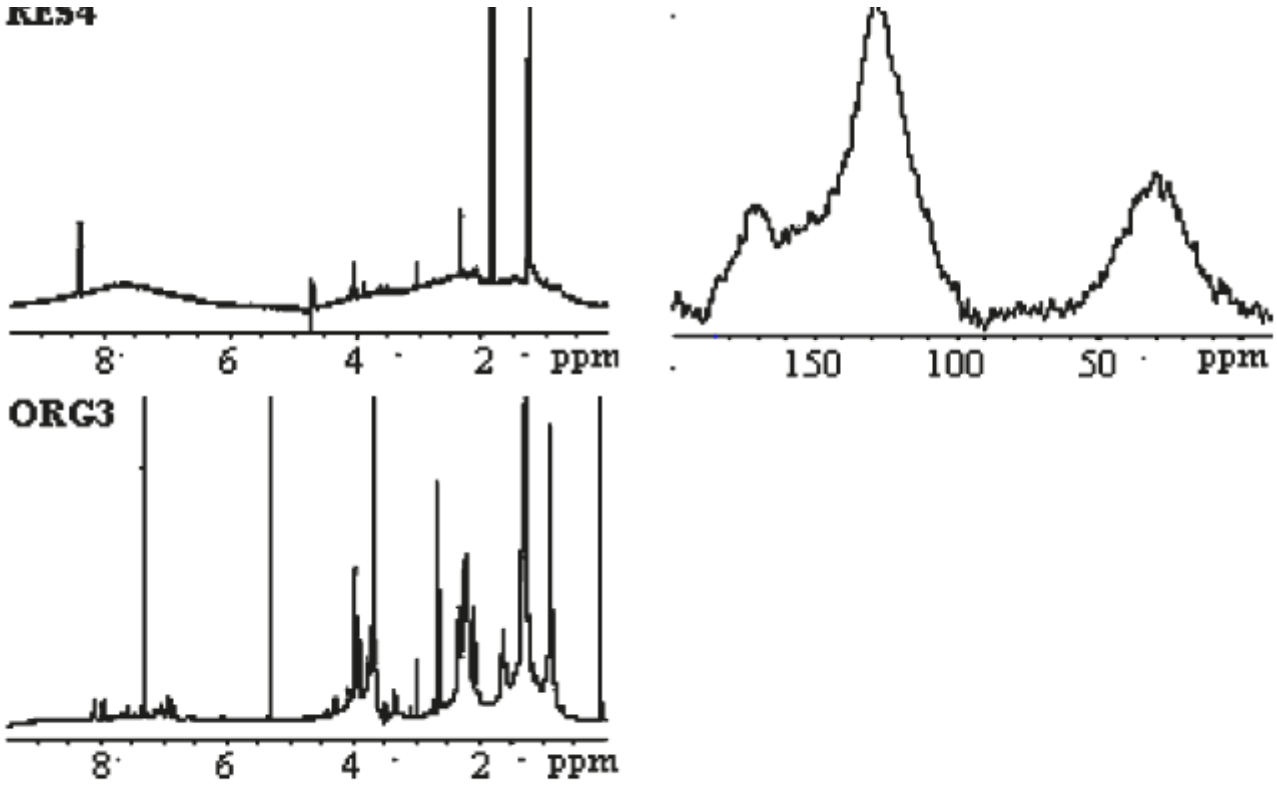


Figure 3. (A) ^{1}H NMR spectra and (B) ^{13}C−CPMAS−NMR of humic fractions separated by sequential fractionation.

Observations from Slides 74, 75,76

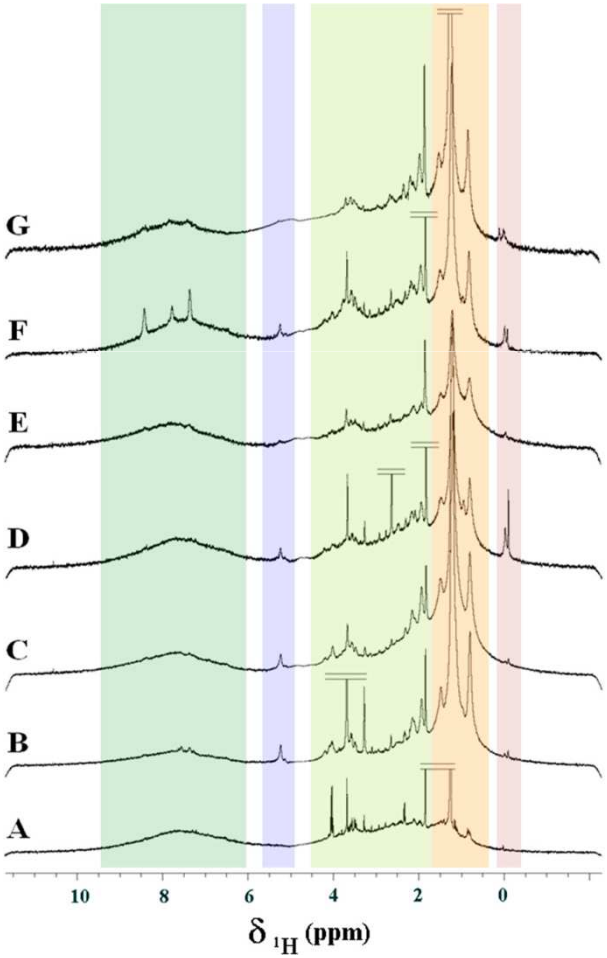
- Note clearly evident aliphatic hydrocarbon , possibly amino, carbohydrate aromatic and carboxyl components in RES0. The relative abundance of the hydrocarbon constituents is maintained in ORG1 and ORG2 , and the apparent resonances at ca 30 and 32 ppm in ORG2 would suggest amorphous and ordered methylene structures, respectively. Note in ORG3 the ^{1}H NMR evidence for aliphatic methylene and possibly carbohydrate, C/O C/N structures. The distinct resonances at ca 55 ppm in ORG2 and ACQ2 arise from methoxyl arising from methylation in the transesterification process.
- The ^{13}C NMR spectrum for RES4 emphasise the significant abundances of aromatic/olefinic resonance and the ill-defined but significant resonance in the aliphatic hydrocarbon (0-40 ppm) region.

Further Information About RES4

- **Nebbioso *et al* (2014, *Chemical and Biological Technologies in Agriculture (2014) 1:24 9 pages* *(Open Access, Springer http://wwwchembioagro.com/content/1/1/24))* fractionated, using HPSEC, RES4 (A in slide 79) into 6 fractions (B-G, slide 77), and the sets of protons from the different functional groups are highlighted. Note the abundant signals in the 3-5.2 pp range (hydroxy- or aminoalkyl. All fractions, B-G, have intense alkyl proton signals (0.7-1.4 ppm) suggestive of methylene chains or CH_2 in lipids. Note the three distinct resonance (7.4-8.4) in the aromatic range in Fraction 5 (F).**
- **Slide 80 shows 2D COSY and HSQC spectra for the bulk RES4.**
- **Slide 81 shows COSY and HSQC spectra for F1 (B in Slide 77).**
- **Slide 82 highlights the aromatic region of F5 9 (F in slide 77).**
- **The spectrum for aromatic region of F5 in slide 77 shows distinct resonances (7.3, 7.8, and 8.4 ppm in A), and these are bound to C signals at 124, , 136, and 149 ppm in B, respectively. There are indications of electron-withdrawing groups, but correlations at 7.2 and 7.7 would indicate electron-donating substituents,**

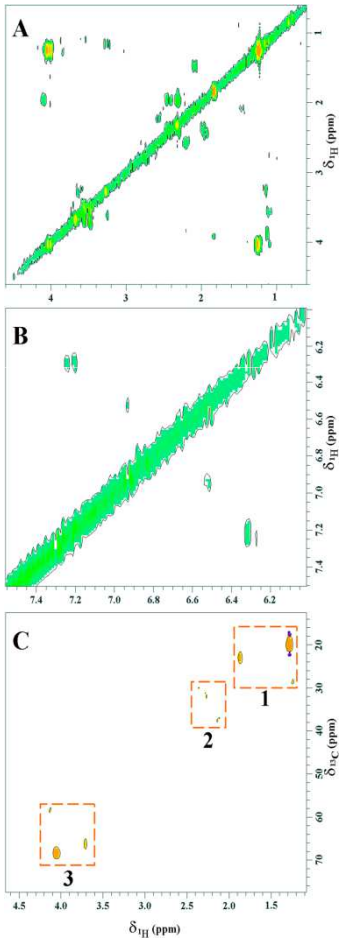
Proton NMR of 6 size fractions (HPSEC) (B-G) of RES4 (A)

(Aromatic , dark green: Olefinic, blue; CHnX (X=O/N) , light green; CHn orange))



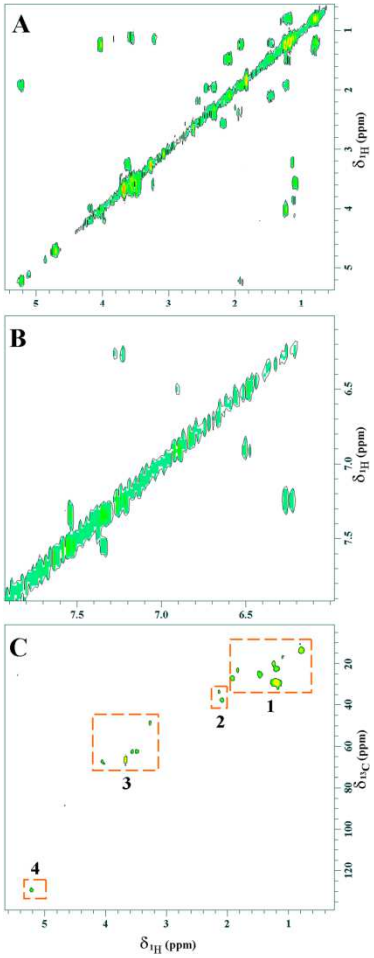
^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC NMR Spectra of RES4.

^{1}H-^{1}HCOSY (A), aliphatic and hydroxyalkyl region; (B), aromatic region; and ^{1}H-^{13}C HSQC (C) spectra of RES4. Rectangular regions in HSQC correspond to the following sets of proton signals: Alkyl CHn (1); CHC = O (2); CHn-X where X = electron-withdrawing group (3).



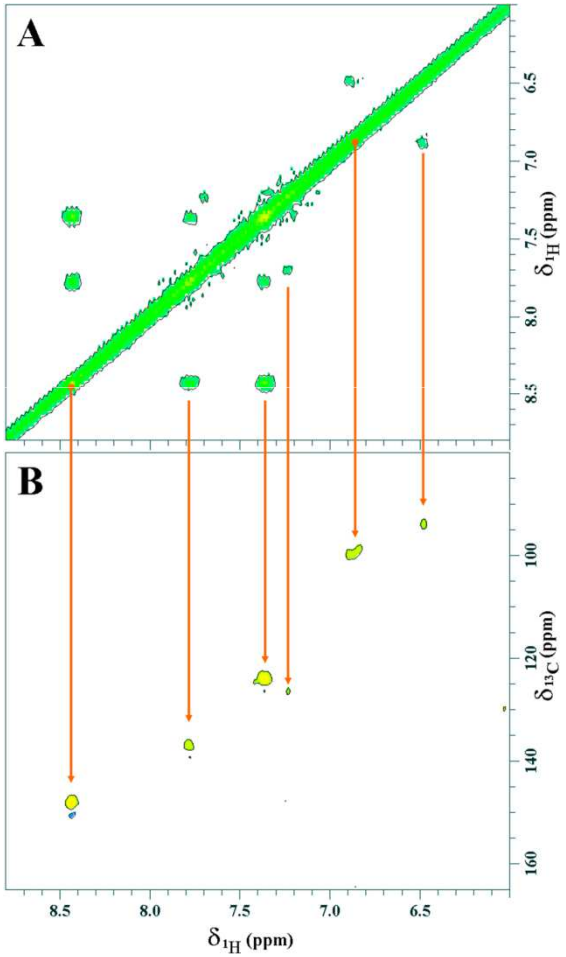
^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC Spectra of F1 (A, Slide 7)

^{1}H-^{1}H COSY (A), aliphatic and hydroxyalkyl region; (B), aromatic region), and (C), ^{1}H-^{13}C HSQC (Rectangular regions (1 to 4) in HSQC correspond tosets of proton signals: Alkyl CHn (1); CHC = O (2); CHn-X whereX = electron-withdrawing group (3); CHn-C = C (4).



2-D NMR Spectra of Aromatic Regions of F5 of RES4.

(A), ^{1}H-^{1}H TOCSY; and (B), ^{1}H-^{13}C HSQC

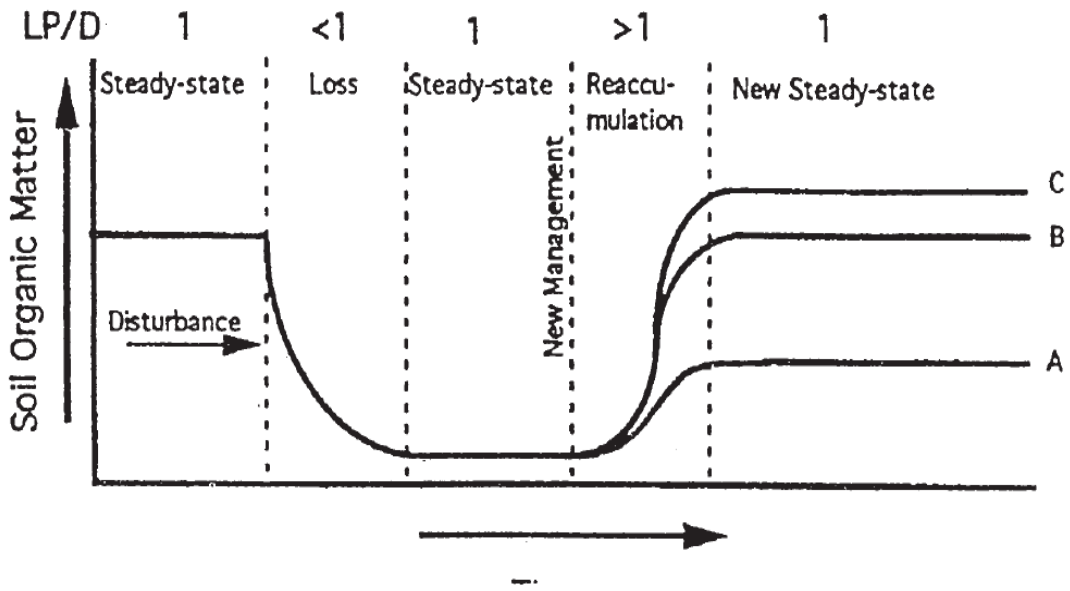


Inferences from Humeomics

- It has been evident to most of us that it would not be possible to get meaningful information about humic compositions and structures without appropriate isolation and fractionation procedures. The application of HPSEC shows promise of good fractionation in terms of molecular size.
- Could better fractionation be obtained with a longer column?
- The NMR data (slide 39) of resin-fractionated extracts isolated at different pH values show compositional differences.
- Would application of HPSEC fractionation of these fractions provide more homogeneous fractions for Humeomics studies?
- The Humeomics concept is excellent and a significant advance on the chemical degradation techniques referred to in slides 22-29.
- Sequential degradation is essential. The solvent extraction will not give rise to artefacts, and neither should ester cleavage with BF_3/methanol. It would be appropriate to carry out model studies of the more highly energetic degradation processes. HI will degrade carbohydrates as well as breaking ether linkages. Will the products released give rise to condensation reactions? It would be reassuring if RES4 is shown not to result from such.

Stabilising Soil Organic Matter

- **We all know how vital SOM is to soil structure, soil biota, and to soil fertility. Let us reflect again on the effects depicted by Johnson (1995). Clearly the objective should be to preserve the Steady-State that exists before llong-term cultivation is initiated. Various practises such as minimum till, no-till, etc, have been introduced, but results are not convincing. It would seem that we will need to solve the problem of how to preserve SOM under long-term cultivation conditions, perhaps well before mid-century.**



Bioimetic Catalysis and Stabilisation of Humic Suprastructures

- **This topic will be detailed in the programme, and is given only brief reference here. The Piccolo group in Universita di Napoli are the 'trail blazers' in this area. The fundamental study used soil HAs treated with water-soluble Fe(III) porphyrin formed from *meso*-tetra(2,6-dichloro-3-sulfonatophenyl)porphyrin and $FeSO_4$. The molecular size of the HAs was increased based, it was considered, on the formation of covalent intermolecular linkages via a free radical mechanism. The study has been extended to field soils. Note, in slide 86, the effects of oxidative biometic catalysis (Nuzzo et al., Biol. Technol. Agric. (2017, 4:12, 6pp)) (and the role of H_2O_2, though sunlight will have the same effect) on the degradation of SOM as indicated by the release of CO_2.**
- **Some will question costs, etc. Such is irrelevant. The work is at the beginning, and it shows that it will become possible in the future to stem SOM degradation.**
- **85**

Oxidative Bioimetic Catalysis of In Situ Polymerisation and Stabilisation of SOM

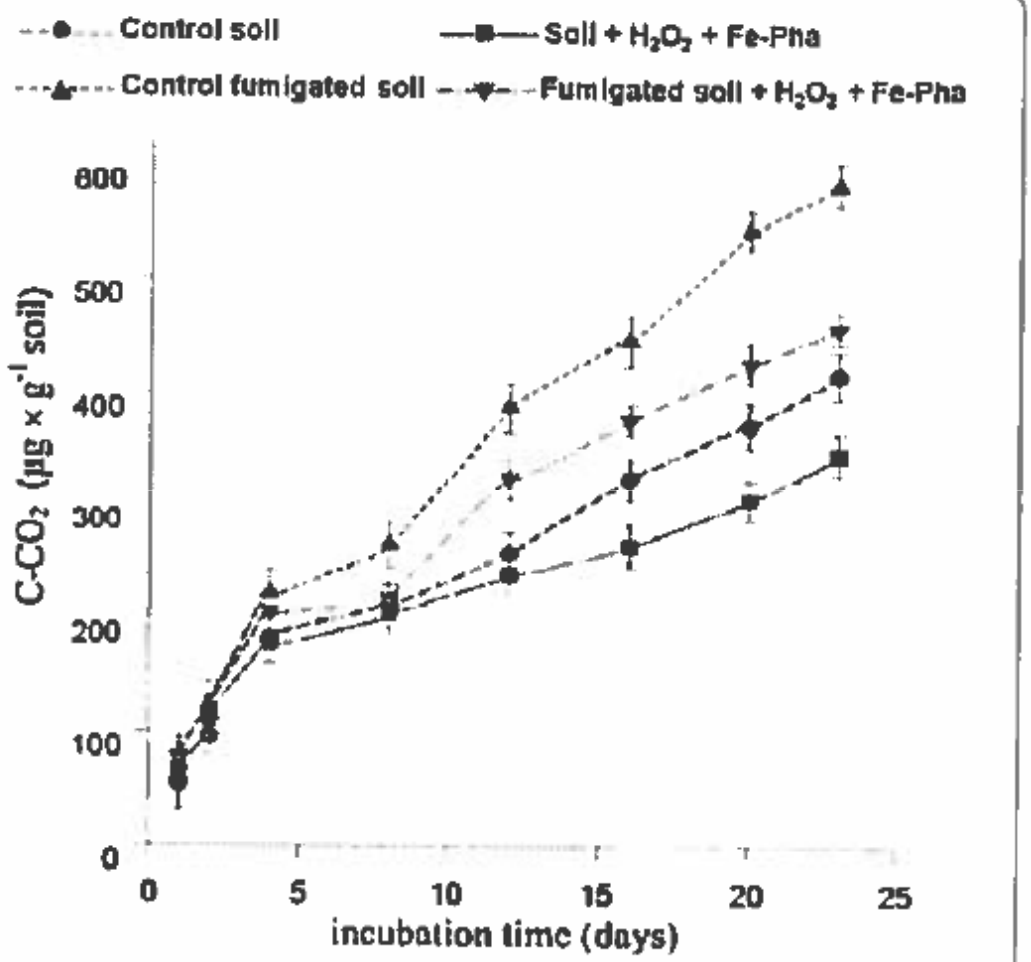


Fig. 4 Soil respiration (µg CO_2 g^{-1} of soil), during 23 days of incubation, before and after the catalyst-assisted oxidative polymerization catalyzed by Fe-Pha ($n = 3$), and before and after fumigation with chloroform: control soil, soil + H_2O_2 + Fe-Pha, control fumigated soil, fumigated soil + H_2O_2 + Fe-Pha. Standard deviation was less than 5%

Summary and Ways Forward

- We are at an exciting stage in studies of the most vital components of soil organic matter, and highly significant advances have been made in the present generation. There are some (and many who should know better) who will say that all we need to know about SOM is that it has polyelectrolyte functionalities that enable it to retain exchangeable cations, that it promotes the formation and stabilisation of soil aggregates, and that, on degradation, it supplies needed minor elements needed for plant growth. All that is true, but there must be widespread recognition of the fact that growing world population trends will continue to place more demands on soils and it is probable that one hundred annual crops from soils in long-term cultivation now will have lost their stabilising SOM and become degraded.
- There must be a concerted effort to stop that happening, and the remedy will follow when we understand fully the types of components we have in the soils in the different classification categories, how these react and interact with each other, and with the soil mineral colloids.
- We need to know more about the genesis of humic molecules. It would seem that the origins and the compositions of humins is reasonably straight forward because the evidence we have would indicate that these are composed of the recalcitrant components of plants (such as those in the cuticular material, and of long chain fatty acids/esters, and recalcitrant components of microbial cell walls, etc. All the humins we have observed contain also small

Summary (Continued)

amounts of carbohydrate and peptide components that we might expect not to be persistent. Thus we ask, why do they persist? Are they innately resistant, or are they preserved by their associations with the recalcitrant less polar hydrocarbon rich components and/or with the mineral colloids? And what is their role, if any?

It will be important to have updated definitions for humic substances and humin. Those outlined in Slide 3 will need adjustment in view of the fact that humin would not, based on the definitions given be a humic substance.

We have little reliable evidence about the compositions of the highly important humic and fulvic acids. It will be essential to have better fractionation procedures in order to separate from the humic core materials the peripherally associated carbohydrate, peptide, and other polar-type, non-covalently bound substances. The resin procedure outlined in slides 38, 39, 40 indicate that the polar, non-covalently linked compounds rich in carbohydrates and peptide can be separated, but (as will be seen from the references listed) carbohydrates and peptides still persist with the core and we do not know how.

The Humeomics approach is the best so far for studies of the compositions of what we will call the humic materials. The fact that significant amounts of the compounds identified have significant non-polar functionality is puzzling (but may be justified in terms that these give cohesion to the structures), and it is surprising that aromatic components were low. That stresses the need for studies on a broad spectrum of humic materials formed from different substrates in different soil and climatic conditions.

The need to improve the stabilities of SOM components is pressing. and it is hoped that the concepts implicit in Slide 85 are just at the beginning.

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