

## INTRODUCTION

### Humic substances:

Their definition. Humic substances have attracted the attention of soil chemists for over two centuries; in spite of intensive investigations, knowledge of the nature of these materials is still limited, however.

Part of the difficulties experienced are caused by inaccuracy and inconsistency of definitions used. Over the years the terms humus, soil organic matter and humic substances have been redefined continuously. The large number of definitions has lead to many confusions and is partly caused by attempts of many investigators to relate definitions of these materials with their structural features. Because these materials have a very complex, mainly macromolecular nature it is understandable that analytical data of all kinds related to partial structures present in these materials are interpreted in many different ways.

For a detailed historic review of these and other terms the reader is referred to Waksman (1936), Kononova (1966), Stevenson (1982), Aiken et al. (1985), Frimmel and Christman (1988). The most recent definitions after Stevenson (1982) and Aiken et al. (1985) are given in Tables 1 and 2.

Based on their solubility in alkali and acid, humic substances are usually divided into three main fractions: humic acids, which are soluble in dilute alkali but precipitate on acidification of the alkaline extract; fulvic acids which comprise that humic fraction which remains in solution when the alkaline extract is acidified; humins, which comprises humic fractions that cannot be extracted directly from the soil by dilute alkali or acid but by alkali only after HF/HCl treatment. A certain number of subfractions can also be obtained by solvent extraction or addition of electrolytes (Figure 1). Although the definitions of these humic fractions have undergone changes over the years as well they cause much less confusion because they are operationally defined without reference to their structural

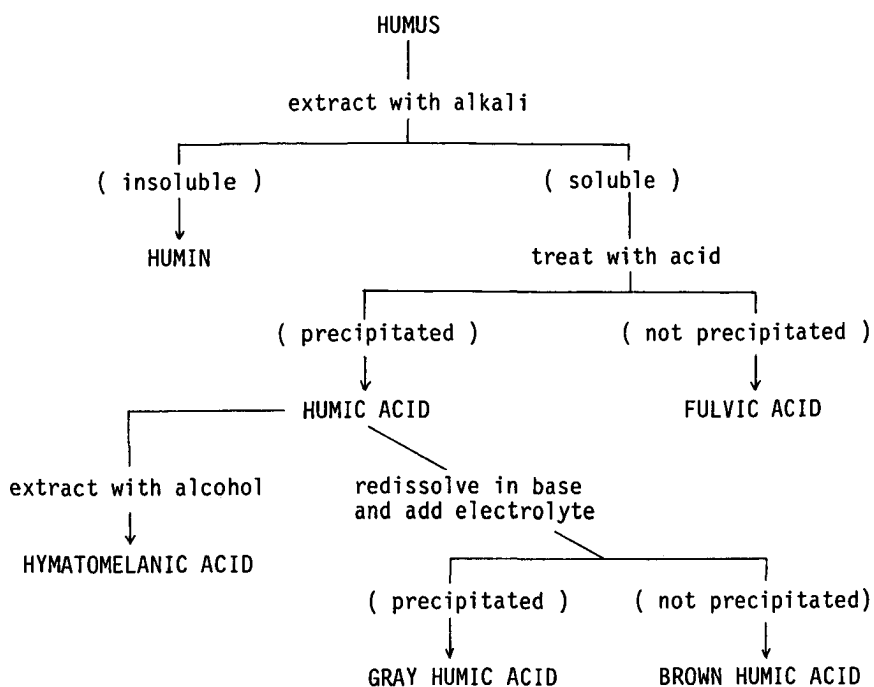
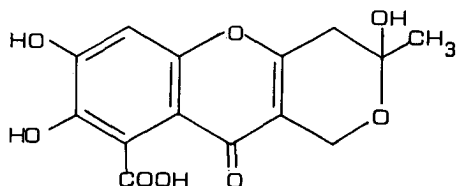


Figure 1. Scheme for the fractionation of humic substances, after Stevenson (1982)

properties. Figure 2 shows a guide to nomenclature of humic substances recently published by Thurman et al. (1988).

The classification schemes for defining components of humic substances have been questioned from time to time. As early as 1936, Waksman stated that it is not justified to draw conclusions on the chemical structures of various types of humic fractions obtained by a separation based upon solubility. In other words, humic acids, fulvic acids and humins are operationally defined terms as already indicated above.

Humic and fulvic acids obtained by different procedures or from different sources may differ significantly in their chemical composition (Saiz-Jimenez et al., 1979; 1986). Therefore, these fractions do not correspond to a unique chemical entity and they cannot be described in terms of precise chemical structure. These definitions have survived because of their practical utility, in spite of striking facts, as for instance, the existence of a well defined compound synthesized by Penicillium griseofulvum, P. flexuosum and P. brefeldianum with the structural formula



called fulvic acid (Turner, 1971).

Because of this vagueness, many controversies about humic fractions have originated over the years. However, whereas the fractionation scheme is arbitrary to some extent it has nonetheless been widely accepted because the fractions are in general more suitable for further analysis than unfractionated humic substances.

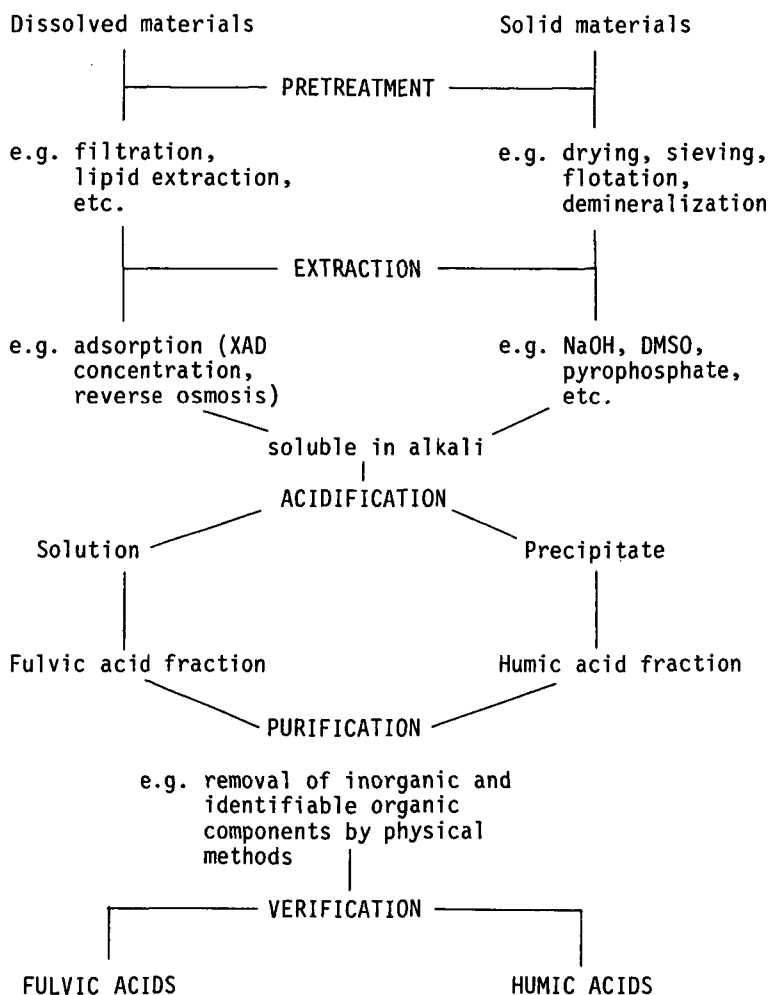


Figure 2. A guide to nomenclature of humic substances,  
after Thurman et al. (1988)

### Humic substances:

Their origin. The term humus dates back to the time of the Romans, when it was used to designate the soil as a whole.

Wallerius first defined humus, in 1761, in terms of decomposed organic matter. The first historical reference to the isolation of a compound similar to that which became later known as humic acid dates to Achard, who, in 1786, extracted a brown substance from soil and peat, by using alkaline solutions. Upon adding sulphuric acid to the alkaline extract, he obtained a dark brown to almost black precipitate.

In 1804, de Saussure introduced the term humus, the latin equivalent of soil, to designate the dark-coloured organic matter in soil.

The first comprehensive study on the origin and chemical nature of humic substances was carried out by Sprengel (1837). Many of the procedures he developed for the preparation of humic acids became generally adopted, such as pretreatment of the soil with dilute mineral acids prior to the extraction with alkali.

The modern foundations of humus chemistry are attributed to Sven Oden (1919). Oden considered humic compounds as the light-brown to dark-brown substances of unknown constitution which are formed in nature by decomposition of organic matter through the actions of atmospheric agencies or in the laboratory by chemical reagents, and humic acids as those humic substances which show acid properties and thus form salts with strong bases.

Years before, Maillard (1912) suggested that humus is the product of a condensation reaction between carbohydrates and amino acids, in which microorganisms are not involved.

Eller (1921) stated that the oxidation of phenol, quinone and hydroquinone in an alkaline solution yields compounds similar to humic acids.

Beckley (1921) pointed out that the action of mineral acids on carbohydrates results in the formation of hydroxymethyl furfural, which on condensation gives rise to humus.

The most generally accepted theory is that humic substances are derived from lignin. According to this theory, lignin is

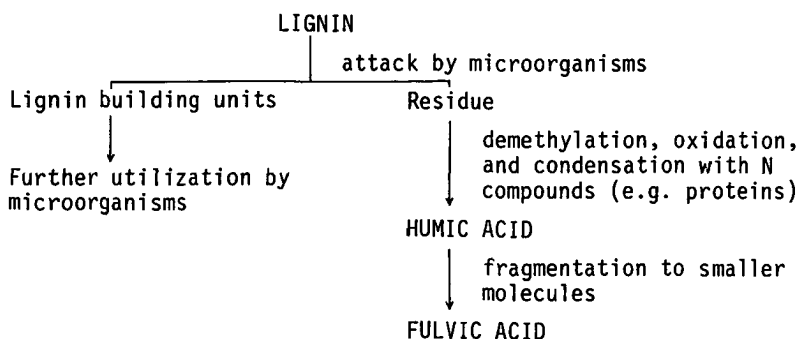


Figure 3. Schematic representation of the lignin theory of humus formation, after Stevenson (1982)

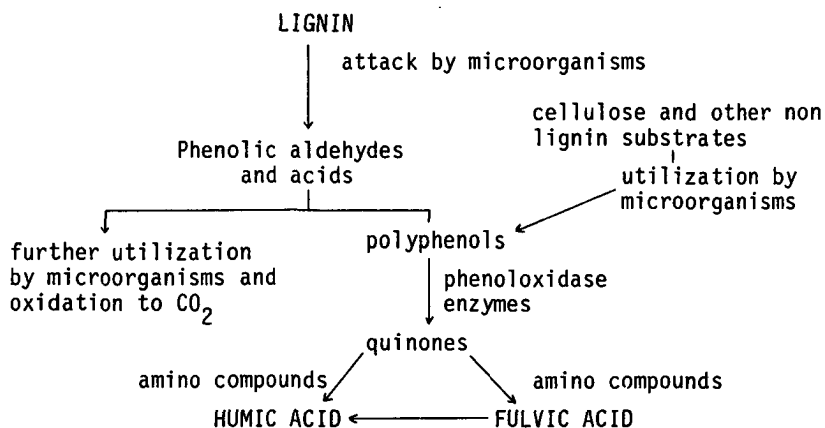


Figure 4. Schematic representation of the polyphenol theory of humus formation, after Stevenson (1982)

incompletely degraded by microorganisms and the residual moiety becomes part of the soil humus (Figure 3).

Wehmer reported in 1915 that, during fungal degradation of wood the lignocellulose is attacked, the cellulose is metabolized and the lignin is converted into humic substances.

Waksman (1936) stated that the lignin theory is substantiated by a number of facts, summarized as follows:

1. Decomposing wood and peat increase in lignin content with time.
2. Lignin, as opposed to cellulose, gives rise to aromatic transformation products. Humic acids are found to contain aromatic moieties as well.
3. Both lignin and humic acids contain methoxyl groups; the methoxyl content decreases with proceeding decomposition.
4. Both lignin and humic acids are acidic in nature; both can react with bases and both are characterized by their capacity of cation exchange, all be it to different degrees.
5. Both lignin and humic acids are insoluble in cold concentrated acids, the degree of insolubility increasing with proceeding decomposition.
6. When lignins are heated in aqueous alkaline solutions, they are transformed into methoxyl-containing humic acids, which does not hold for cellulose.
7. On oxidation under pressure, lignin gives rise to humic acids and finally to aromatic carboxylic acids, but cellulose is changed to other products.
8. Oxidation of brown coal under pressure gives benzenecarboxylic acids and no furancarboxylic acids.
9. Both lignin and humic acids are oxidized by oxidizing agents, such as permanganate and hydrogen peroxide.
10. Both lignin and humic acids are soluble in alkalis and precipitated by acids.
11. Both lignin and humic acids are partly soluble in alcohol and pyridine, depending also on the method of preparation; some lignin and some humus preparations are completely soluble in alcohol.

12. Both lignin and humic acids are decomposed with great difficulty or not at all by the great majority of fungi and bacteria.

Further, Waksman presumed that the nitrogen contained in humic acids resulted from a condensation of modified lignin with microbially derived proteins and other nitrogen-containing compounds.

These days, the so called polyphenol theory as put forward by Stevenson (1982) had become the more accepted one (Figure 4). It states that quinones of lignin origin, together with those synthesized by microorganisms polymerize either autooxidatively or by phenol oxidases in the presence of amino compounds to form humic macromolecules. Stevenson mentioned that because lignins are major plant constituents and relatively resistant to microbial decomposition they are sometimes considered to be the major, if not the primary, source of phenolic units in humic substances.

#### Humic substances:

Their chemical structure. The study of the chemical nature of humic substances has been hampered not only by their insolubility and macromolecular structure but also by the fact that they are a complex conglomerate of a variety of subunits and do not constitute a uniform molecule.

Much work has been devoted to the elucidation of the chemical structure of soil humic substances over a long period of time. Because of the complexity mentioned, degradative methods have been used with the aim of producing compounds that could be identified and whose structures could be related to those of the starting materials.

The degradative methods that have been used are mainly oxidations, reductions and hydrolyses. The oxidative reagents are often strong and could lead to significant alterations of the original building blocks and to the formation of artifacts. These artifacts are defined according to Norwood (1988) as identified



degradation product whose formation pathway is incorrectly interpreted leading the investigator to false structural inferences. This phenomenon has been demonstrated in the case of permanganate oxidation (Hayes and Swift, 1978). Recently, Martin et al. (1981) described persulfate as a mild oxidant which degrades about 50% of soil humic acids, leaving a residue that can easily be recovered and subjected to further analysis.

In general, much of the work on oxidative degradation is of limited value, because the structures of the reaction products only shed some light on those of the building blocks and not so much on their interconnections. The interpretation can even be incorrect because, in many instances, the naturally occurring units could be altered before or after their release from the macromolecular structure.

Reductive degradations e.g. by sodium amalgam have been successfully applied by Martin et al. (1974) to a diversity of soil humic substances and phenolic polymers. The method seems to work with samples with a certain number of aromatic ether linkages or with biphenyl structures where activating (hydroxy or methyl) substituents are ortho and/or para to the connecting bond (Hayes and Swift, 1978). Most of the compounds identified in humic substances are similar to those obtained by degradation of lignins or microbial phenolic polymers suggesting that these types of materials are also present in soil humic substances.

Hydrolytic procedures are effective in removing protein and polysaccharide constituents associated with humic substances. However, the rest of the molecule is not affected (Riffaldi and Schnitzer, 1973).

As a result of the identification of building blocks obtained by various degradative and non-degradative methods some model structures have been proposed for humic substances. Thus, Haworth (1971) concluded that the e.s.r. signal of humic acids is due to a complex aromatic core to which polysaccharides, proteins, simple phenols and metals are chemically and/or physically bound (Figure 5).

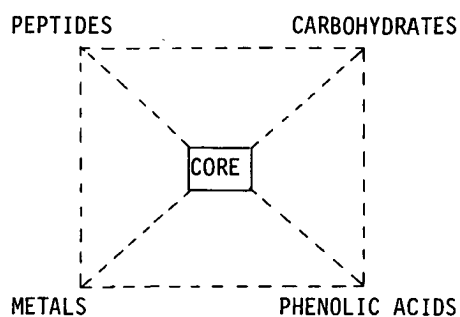


Figure 5. Diagrammatic representation of a humic acid, after Haworth (1971)

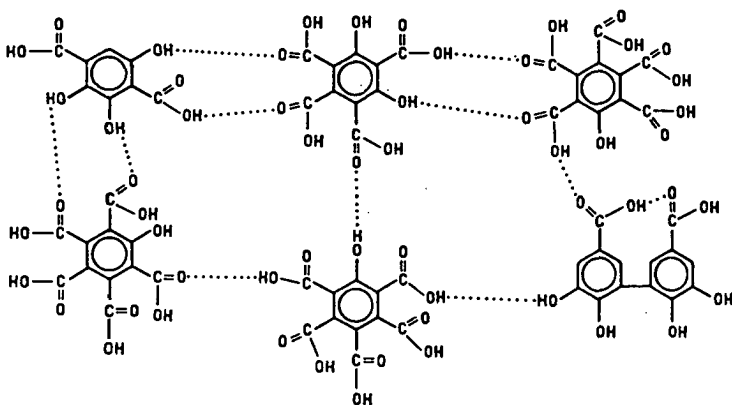


Figure 6. Structure of a fulvic acid, after Schnitzer (1978)

Based on degradative and non degradative methods and assuming that the major products thus obtained are building blocks, Schnitzer (1978) proposed a model structure (Figure 6) in which the phenolic and benzenecarboxylic acids could be held together by hydrogen bonds, Van der Waal's forces, and  $\pi$ -bonding.

According to Stevenson (1982) humic substances consist of a heterogeneous mixture of compounds for which no single structural formula will suffice. Each fraction must be regarded as consisting of a series of molecules of different sizes, few having precisely the same structural configuration or array of reactive groups. As far as the structure of humic acids is concerned, contemporary investigators favour a "model" consisting of micelles of a polymeric nature, the basic structure of which is an aromatic ring of the di- or trihydroxy-phenol type bound by -O-, -CH<sub>2</sub>-NH-, -N=, -S- bridges and other groups. These structures may contain attached proteinaceous and carbohydrate residues.

#### Analytical pyrolysis.

Curie point pyrolysis in combination with either low voltage electron impact mass spectrometry or gas chromatography-mass spectrometry has been used for differentiating between microorganisms and to study the chemical nature of synthetic polymers, bio- and geopolymers (Meuzelaar et al., 1982).

Nagar et al. (1975) indicated that pyrolysis-mass spectrometry was promising for soil humus research. A more extensive study was made by Meuzelaar et al. (1977) by comparing humic acids from soils, peats and composted straw, with fungal melanins and lignins. Humic acids from different soils and peats and most of the fungal melanins gave similar pyrolysis mass spectra with typical ion series, related to proteins, polysaccharides and aromatic compounds. Furthermore, pyrolysis mass spectra of humic acids showed ion series typical of lignin (Haider et al., 1977).

Saiz-Jimenez et al. (1979) studied the different organic matter fractions present in a soil. The pyrolysis mass spectra of humic acid fraction showed prominent mass peaks related to polysaccharides, proteins and lignins. The spectrum of the humin fraction resembled those of the humic acid ones although peaks thought to originate from complex polysaccharides were more evident. The pyrolysis-mass spectrum of the polysaccharide fraction showed the characteristic pattern of the pyrolysate of a complex polysaccharide together with fragments from polymers of amino acids or amino sugars. The pyrolysis mass spectrum obtained from the fulvic acid fraction showed clear dissimilarities to those of the humic acid fraction; signals from proteins as well as those related to phenols were low. Depending upon the isolation methods, pyrolysis mass spectra of fulvic acid preparations showed different suites of peaks related to polysaccharide and phenolic materials. Based on pyrolysis mass spectrometry humatomelanic acid fraction gave the impression that it consisted of material rich in polysaccharides and lignins removable from humic acids by extraction with ethanol. This assumption was based on mass spectra with a limited mass range ( $m/z$  below 180). However, upon pyrolysis-gas chromatography-mass spectrometry analysis the humatomelanic acid fraction was shown to consist mainly of lipid materials (see chapter 3).

Bracewell (1973) and Robertson (1977) have shown that pyrolysis-mass spectrometry enables the recognition of variations in humus type and differences of the genetic horizons in a soil profile. Pyrolysis-mass spectrometry has shown great promise in studies on the structures of soil organic matter and on the humification processes involved. This analytical approach can be used as a fingerprinting technique to clearly show similarities and dissimilarities between different fractions and samples. Preliminary tentative structural information is obtained as well.

To obtain more detailed insight into the structural composition of soil humic substances firmer identifications of pyrolysis products which reflect structural units present within the macromolecular matrix, are required. This can be achieved by

application of pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS).

Previous Py-GC-MS studies (Martin et al., 1977, 1979) have shown that common pyrolysis products of soil humic acids are aliphatic hydrocarbons and compounds related to polysaccharides, proteins, lignins. Further, Faix et al (1987) stated that pyrolysis of lignins gives similar results to those obtained by destructive (nitrobenzene oxidation) and non destructive (FTIR) methods.

#### **Framework of the thesis.**

In this thesis several soil organic matter fractions and composted humic acids are investigated in order to elucidate their composition and origin. Moreover, different types of lignins and woods and some of their degraded counterparts have been studied in an attempt to understand the complex structural changes occurring to woods and lignins by biotic and abiotic diagenesis.

In Chapter 2 the results of investigations of soil fulvic acids, a soil polysaccharide and polymaleic acid are presented. The soil fractions and the synthetic polymer were analysed by pyrolysis-gas chromatography-mass spectrometry in order to determine whether or not fulvic acids are mainly composed of polysaccharides and to what extent polymaleic acid-like structures occur in this humic fraction.

A detailed analysis of the soil organic matter fractions obtained from a representative soil, following the classical method of fractionation of humic substances, is summarized in Chapter 3. The data allow for an easy discrimination of these soil fractions based on the major characteristic series of pyrolysis products of each fraction.

Because lignin appears to be an important contributor to some of the soil humic fractions, a description of the pyrolysis products obtained from a spruce milled wood lignin, before and after fungal degradation, is presented in Chapter 4. The data

were also compared to those of a synthetic lignin and an industrial lignin.

Chapter 5 stresses the significance of lignin pyrolysis products as biomarkers. Pyrolysis permits a differentiation of the three types of lignins based on the presence or absence of characteristic phenols.

In Chapter 6 wet chemical and spectroscopic data of buried and present-day woods are compared with analytical pyrolysis data. The results obtained indicate that the pyrolysis data are in good agreement and are also complementary with the other data.

Because soil humic acids are complex mixtures of a broad variety of materials, acid hydrolysis was applied to remove the polysaccharide and proteinaceous moieties followed by solvent extraction of lipids. The residual humic acid fractions were pyrolysed and the significance of the greater part of the pyrolysis mixture, consisting of homologous series of straight chain alkanes, alk-1-enes and  $\alpha$ ,  $\omega$ -alkadienes is discussed in Chapter 7. A more detailed study of the most resistant part of soil humic acids is described in Chapter 8. The residues obtained after acid hydrolysis and persulfate oxidation gave similar chromatograms of pyrolysis products, dominated by homologous series of aliphatic hydrocarbons, which might be related to the highly aliphatic biopolymers encountered in plant cuticles and suberins (Nip et al. 1986).

The chemical characterization and the pyrolysis data of the humic acid fraction of a sludge obtained from waste water of olive mills after disposal in lagoons is presented in Chapter 9. This and other wastes or composted materials are being used to fertilize agricultural soils. Chapter 10 describes some of the chemical and pyrolysis data of humic acids extracted from vermicomposts (manure composted by earthworms). The results show that lignin constitutes an important part of the organic matter present in the composted manures and can be isolated in the humic acid fraction.

TABLE 1

Glossary of terms after Stevenson (1982)

Terms	
Humus	Total of the organic compounds in soil exclusive of undecayed plant and animal tissues, their partial decomposition products, and the soil biomass
Soil organic matter	Same as humus
Humic substances	A series of relatively high-molecular-weight, brown to black coloured substances formed by secondary synthesis reactions. This term is used as a generic name to describe the coloured material or its fractions obtained on the basis of solubility characteristics. These materials are distinctive to the soil (or sediment) environment in that they are dissimilar to the biopolymers of microorganisms and higher plant (including lignin)
Nonhumic substances	Compounds belonging to known classes of biochemistry, such as amino acids, carbohydrates, fats, waxes, resins, organic acids, etc. Humus probably contains most, if not all, of the biochemical compounds synthesized by living organisms
Humic acid	The dark-coloured organic material which can be extracted from soil by various reagents and which is insoluble in dilute acid

Fulvic acid	The coloured material which remains in solution after removal of humic acid by acidification
Humin	The alkali insoluble fraction of soil organic matter or humus
Hymatomelanic acid	Alcohol soluble portion of humic acid.



TABLE 2

Glossary of terms after Aiken et al. (1985)

Terms	Definition
Humus	The organic portion of soil, brown or black in colour, consisting of partially or wholly decayed plant and animal matter, that provides nutrients to plants and increases the ability of soil to retain water. This term is not entirely synonymous with humic substances, although it is often used as a synonym.
Humic substances	A general category of naturally occurring, biogenic heterogeneous organic substances that can generally be characterized as being yellow to black in colour of high molecular weight, and refractory.
Humification	The process of fermentation of humic substances; generally the decomposition of organic material.
Humic acid	That fraction of humic substances that is not soluble in water under acid conditions (below pH 2), but becomes soluble at greater pH.
Fulvic acid	That fraction of humic substances that is soluble under all pH conditions.
Humin	That fraction of humic substances that is not soluble in water at any pH value.

## REFERENCES

- Achard, F.K. (1786) Chemische Untersuchung des Torf. Crell's Chem. Ann. 2, 391-403.
- Aiken, G.R., McKnight, D.M., Wershaw, R.L. and MacCarthy, P. (1985) Humic Substances in Soil, Sediment and Water. Geochemistry, Isolation and Characterization. Wiley, New York.
- Beckley, V.A. (1921) The preparation and fractionation of humic acid. J. Agric. Sci. 11, 66-68.
- Bracewell, J.M. and Robertson, G.W. (1973) Humus type discrimination using pattern recognition of the mass spectra of volatile pyrolysis products. J. Soil Sci. 24, 421-428.
- Bracewell, J.M. and Robertson, G.W. (1977) Pyrolysis studies on humus in freely drained Scottish soils. In Analytical Pyrolysis, C.R.C. Jones and C.A. Cramers (eds.), pp. 167-178, Elsevier, Amsterdam.
- Eller, W. (1921) Künstliche und natürliche Huminsäuren. Brennstoff. Chem. 2, 129-133.
- Faix, O., Meier, D. and Grobe, I. (1987). Studies on isolated lignins and lignins in woody materials by pyrolysis-gas chromatography-mass spectrometry and off-line pyrolysis-gas chromatography with flame ionization detection. J. Anal. Appl. Pyrol. 11, 403-416.
- Frimmel, F.H. and Christman, R.F. eds. (1988) Humic Substances and their Role in the Environment. Wiley, Chichester.
- Haider, K., Nagar, B.R., Saiz, C., Meuzelaar, H.L.C. and Martin, J.P. (1977) Studies on soil humic compounds, fungal melanins and model polymers by pyrolysis mass spectrometry. In Soil Organic Matter Studies, vol. 2, pp. 213-220, I.A.E.A., Vienna.
- Haworth, R.D. (1971) The chemical nature of humic acid. Soil Sci. 111, 71-79.

- Hayes, M.H.B. and Swift, R.S. (1978) The chemistry of soil organic colloids. In *The Chemistry of Soil Components*, D.J. Greenland and M.H.B. Hayes (eds.), pp. 179-230, Wiley, Chichester.
- Kononova, M.M. (1966) *Soil Organic Matter*, Pergamon Press, Oxford.
- Maillard, L.C. (1912) Formation d'humus et de combustibles minéraux sans intervention de l'oxygène atmosphérique, des microorganismes, de hautes températures, ou de forte pressions. *C.R. Acad. Sci.* 155, 1554-1556.
- Martin, F., Saiz-Jimenez, C. and Cert, A. (1977) Pyrolysis-gas chromatography-mass spectrometry of soil humic acids. I. The low boiling point compounds. *Soil Sci. Soc. Amer. J.* 41, 1114-1118.
- Martin, F., Saiz-Jimenez, C. and Cert, A. (1979) Pyrolysis gas-chromatography-mass spectrometry of soil humic acids. II. The high boiling point compounds. *Soil Sci. Soc. Amer. J.* 43, 309-312.
- Martin, F., Saiz-Jimenez, C. and Gonzalez-Vila, F.J. (1981) The persulfate oxidation of a soil humic acid. *Soil Sci.* 132, 200-203.
- Martin, J.P., Haider, K. and Saiz-Jimenez, C. (1974) Sodium amalgam reductive degradation of fungal and model phenolic polymers, soil humic acids and simple phenolic compounds. *Soil Sci. Soc. Amer. Proc.* 38, 760-765.
- Meuzelaar, H.L.C., Haverkamp, J. and Hileman, F.D. (1982) *Pyrolysis Mass Spectrometry of Recent and Fossil Biomaterials. Compendium and Atlas*. Elsevier, Amsterdam.
- Nagar, B.R., Waight, E.S., Meuzelaar, H.L.C. and Kistemaker, P.G. (1975) Studies of the structure and origin of soil humic acid by Curie-point pyrolysis in direct combination with low voltage ionization mass spectrometry. *Plant Soil* 43, 681-685.
- Nip, M., Tegelaar, E.W., de Leeuw, J.W., Schenck, P.A. and Holloway P.J. (1986) A new non-saponifiable highly aliphatic and resistant biopolymer in plant cuticles. *Naturwissenschaften* 73, 579-585.

- Norwood, D.L. (1988) Critical comparison of structural implications from degradative and nondegradative approaches. In *Humic Substances and their Role in the Environment*, F.H. Frimmel and R.F. Christman (eds) pp. 133-148, Wiley, Chichester.
- Oden, S. (1919) *Die Huminsäuren*. Kolloidchem. Beihefte. 11, 75-260.
- Riffaldi, R. and Schnitzer, M. (1973) Effects of 6 N HCl hydrolysis on the analytical characteristics and chemical structure of humic acids. *Soil Sci.* 115, 349-356.
- Saiz-Jimenez, C., Haider, K. and Meuzelaar, H.L.C. (1979) Comparisons of soil organic matter and its fractions by pyrolysis-mass spectrometry. *Geoderma* 22, 25-37.
- Saiz-Jimenez, C., Hawkins, B.L. and Maciel, G.E. (1986) Cross polarization, magic angle spinning  $^{13}\text{C}$  nuclear magnetic resonance spectroscopy of soil humic fractions. *Org. Geochem.* 9, 277-286.
- Saussure, Th. de (1804) *Recherches chimiques sur la vegetation*. Paris. As quoted by Waksman (1936).
- Schnitzer, M. (1978) Humic substances: chemistry and reactions. In *Soil Organic Matter*, M. Schnitzer and S.U. Khan (eds.), pp. 1-64, Elsevier, Amsterdam.
- Sprengel, C. (1837) *Die Bodenkunde oder die Lehre vom Boden*. Müller, Leipzig.
- Stevenson, F.J. (1982) *Humus Chemistry*, Wiley, New York.
- Thurman, E.M., Aiken, G.R., Ewald, M., Fischer, W.R., Förstner, U., Hack, A.H., Mantoura, R.F.C., Parsons, J.W., Pocklington, R., Stevenson, F.J., Swift, R.S. and Szpakowska, B. (1988). Isolation of soil and aquatic humic substances. Group Report. In *Humic Substances and their Role in the Environment*, F.H. Frimmel and R.F. Christman (eds) pp. 31-43, Wiley, Chichester.
- Turner, W.B. (1971) *Fungal Metabolites*, Academic Press, London.
- Waksman, S.A. (1936) *Humus*, Williams & Wilkins, Baltimore.
- Wallerius, J.G. (1761) *Agriculturae fundamenta chemica spez. De Humo*. Diss. Upsala. As quoted by Waksman (1936).

Wehmer, C. (1915) Zum Abbau der Holzsubstanz durch Pilze. Ber.  
Deut. Chem. Gesell. 48, 130-134.