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EVOLUTION OF CONCEPTS OF ENVIRONMENTAL NATURAL NONLIVING ORGANIC MATTER

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1.1. INTRODUCTION

The importance of soil organic matter (SOM) has been recognized since the dawn of agriculture. There were extensive writings about agriculture in Roman times, and the literature that had accumulated was assembled in one volume by Petrus Crescentius (ca. 1240), a senator in Bologna (Russell, 1973). Applications to soil of by-products of vegetable, animal, and human origins have always been made. Even the process of maturing, prior to soil applications, of what we would now regard as composts has been known for over 2000 years. Columella (Lucius Iunius Moderatus Columella, Cadice, Spain, 1st century A.D.) described how organic wastes had to be processed before their use as soil amenders.

Russell (1973) referred to a communication by Palissy around 1563 that states: "You admit that when you bring dung into a field it is to return to the soil something that has been taken away." In reference to straw he writes "being burnt on the ground it serves as a manure because it returns to the soil those substances that had been taken away." It was known then that the application of char, or the residue from the pyrolysis or burning of organic materials and wastes under restricted air conditions, enhanced soil fertility greatly. For several millenia in pre-Columbian times the Indians of the Amazon region had burned their organic refuse slowly in restricted air supplies and returned the char to the soil. That char gave rise to an amazing fertility that is still very evident in comparison to the infertile unamended neighboring oxisol soils (Woods and Glaser, 2004). These chars in the Amazonian Dark Earth (ADE), or the Terra Preta de Indio soils, will inevitably have some mineral elements, and the slow oxidation of the chars has given rise to humic-type materials. However, the amazing fertility of these soils cannot be attributed to the elementary compositions of the chars. The char, as well as trapping and holding nutrients and growth promoting substances that could be released in the pyrolysis processes, is considered to provide refuge for such soil microorganisms as arbuscular mycorrhizal fungi (Rillig et al., 2001). These form symbiotic relationships with plant roots. The fungal hyphae effectively extend the root system and transport nutrients from the soil to the plant roots. The hyphae are considered to liberate and to make available for the plant phosphate complexed in the iron oxides associated with oxisols, and such fungi are known to secrete mucopolysaccharides that strongly adsorb to mineral surfaces and in this way help to promote and to stabilize soil aggregates. In return the fungi receive their organic sustenance from the plants of the Amazon region. The modern interest in the pyrolysis and the gasification of organic substrates and the residuals from second-generation biorefining processes (which give bio-oil and biochar products) has awakened interest in the benefits of biochar as soil amenders (Marris, 2006; Hayes, 2006a).

The early statement that "corruption is the mother of vegetation" doubtless arose from the observation that manures, composts, dead animal bodies, and parts thereof such as blood, hair, hoofs, and so on, increased plant growth. John Woodward (cited by Russell, 1973), in a paper published in the *Philosophical Transactions of the Royal Society* (Vol. 21, p. 382), observed that the falloff in yields of crops grown in successive years on unmanured land could be rectified when "supplied with a new fund of matter, of like sort with that it first contained; which supply is made in several ways, either by the ground's being fallow some time, until the rain has poured down a fresh stock upon it; or by tiller's care in manuring it." He considered that the best manures were parts either of vegetables or of animals, which ultimately are "derived from vegetables."

Applications of the sciences to the study of soils began slowly around the middle of the 18th century. Because there was not the instrumentation needed for studies of complex mixtures, such as SOM, progress was slow until the second half of the 20th century, and the most striking advances have been made in the present generation.

1.2. ORGANIC MATTER AT THE "DAWN" OF SOIL RESEARCH

In Chapter 1 of her book *Soil Organic Matter*, Kononova (1966) has provided a good treatise on the evolution of the chemical and biological approaches to the study of soil organic matter. The mixture of organic materials in soils presents problems in separation and in compositional and structural studies. Thus significant verifiable advances in aspects of the compositions and structures of the organic components in soils had to await the development of separation and analytical procedures and instrumentation. In the same way, awareness of the processes of organic matter transformations in soil was retarded because the development of the science of soil microbiology lagged behind that of the chemistry that had allowed some significant advances to be made. By the middle of the 18th century, there was appropriate awareness that humus has an important bearing on soil fertility. Lomonosov (1763) recognized that soil humus had its origins in the rotting with time of plants and animal bodies, and Komov (1789) associated the desirable agronomic properties and fertility to the presence of humus. He stressed the importance of applying farmyard manure and recommended the sowing of perennial grasses.

The book by Wallerius (1761) is regarded as the first scientific guide to agricultural chemistry. In it he recognized the formation of humus during the decomposition of plants; and he was aware of some of its important properties, such as its capacity to absorb water and retain nutrients. However, at that time humus was considered to be a plant food. deSaussure (1804) was first to show that humus had more C, H, and O than the original plant residues. However, he took the view that plants take their carbon chiefly from carbonic acid in the air. He considered that it was taken directly into plants as a food. The experimental data of von Thaer (1809) led him to consider that soil humus was a direct source of plant food. The book by Sir Humphrey Davy (Davy, 1813), who studied the hypotheses of other writers, set out in the new chemical language the accepted knowledge of the time. He did not totally accept de Saussure's concept of carbonic acid from the air, though he did concede that some plants could derive their C from that source. In general he considered that the carbon was taken in through the roots. German (1836, 1837) was among the last to accept the concept of humus as a source of plant nutrients. However, Liebig (1846) cast scorn in the theory of humus as a plant nutrient. Russell (1973) describes how Liebig, "with polished invective," scorned the plant physiologists of his day for their widely held view that plants derive their carbon from the soil. Russell quotes Liebig as follows: "All explanations of chemists must remain without fruit, and useless, because, even to the great leaders in physiology carbonic acid, ammonia, acids, and bases are sounds without meaning, words without sense, in terms of an unknown language, which awake no thoughts and no associations."

The experiments quoted by the physiologists were considered by Liebig "to be valueless for the decision of any question. These experiments are considered by them as convincing proofs, whilst they are fitted only to awake pity." Liebig's ridicule killed the theory of humus as a nutrient.

1.3. SCIENTIFIC APPROACHES TO STUDIES OF SOIL HUMIC SUBSTANCES

1.3.1. The Emergence of Procedures for the Isolation of Soil Humic Components

Chemical knowledge advanced significantly during the 18th and 19th centuries, and these advances had a significant bearing on studies of the chemistry of SOM. It was recognized that the isolation and fractionation of components of SOM was a prerequisite for studies of their compositions.

Achard (1786) is regarded as the first to isolate and to make a fractionation of humic substances (HS). He treated peat with potassium hydroxide, added acid to the dark solution, and obtained an amorphous dark precipitate. More precipitate was obtained from the darker (more humified) layers than from the "less rotted" residues in the upper layers. Later, Vauquelin (1797, 1798) isolated in alkali solution humic-type substances from elm wood infected by fungi, and Thomson (1807) proposed the term *ulmin* for the isolates.

Toward the middle of the 20th century, it was considered generally that HS were polymeric or at least macromolecular, as well as being polyelectrolytes. It was realized that at the pH of most fertile agricultural soils the polyelectrolytes were rendered insoluble through ion-exchange reactions with divalent and polyvalent metal cations. Mineral acids exchanged the metal cations for H^+ to give rise to H^+-exchanged HS, allowing the molecules to remain associated through hydrogen bonding and van der Waals forces mechanisms. In this state the humic materials would have the properties of H^+-exchanged polyelectrolytes. Raising the pH would give rise to dissociation of the acidic functionalities, allowing the conjugate bases to solvate in aqueous media.

Bremner (1950) showed that significant oxidation of organic (humic) matter took place in basic media. Earlier, Bremner and Lees (1949) had shown that a sodium pyrophosphate solution (0.1 M), neutralized to pH 7, was an effective solvent for humified material. The pyrophosphate forms a complex with the charge-neutralizing divalent and polyvalent cations and the freed conjugate bases dissolve in the aqueous medium. This system is successful in dissolving the more highly oxidized humic components (with greater carboxyl functionalities), but it will not dissolve the less humified materials in which phenolic groups (which dissociate at higher pH values) contribute significantly to the charge density.

Hayes et al. (2008) described the uses of 0.1 M sodium pyrophosphate (Pyro) solutions for exhaustive extractions of soil organic matter at pH 7, pH 10.6, and at pH 12.6 (Pyro + 0.1 M NaOH). They showed that the fractions were compositionally different, with the most transformed (oxidized) fractions isolated at the lower pH value. However, only about 26% of the organic matter was isolated in the sequential process.

When the International Humic Substances Society (IHSS) was founded at a meting in the US Geological Survey in September 1981, it was decided to hold symposia at which designated persons would present state-of-the-art information about various aspects of the humic sciences. This new society aimed to unite the soil and water humic scientists. Intensive studies on aquatic HS had arisen from the observation by Rook (1977) that coloured waters when chlorinated gave rise to mutagenic chlorinated hydrocarbons. This caused the US Geological Survey, led by Drs. Bob Avert and Ron Malcolm, to initiate intensive studies into all aspects of aquatic HS. At the International Soil Science Society meetings in Edmonton in 1978, Dr. Malcolm and Dr. Patrick MacCarthy (Colorado School of Mines) introduced soil humic scientists to the initiatives being taken by the USGS, and that led to the eventual formation of the IHSS in 1981. It was determined at the founding meeting (which the writer attended) that funding from the USGS would be used to provide a set of IHSS Soil and Water Standards, and procedures were agreed for the preparation of these standards. The writer, then at the University of Birmingham, England, was given the task of isolating humic and fulvic acids from a Florida Sapric Histosol; and Professor R. S. Swift, then at Lincoln College of the University of Canterbury, NZ, agreed to isolate these fractions from an Elliott Mollisol from Illinois. Dilute NaOH was the solvent of choice for the extraction of the IHSS Soil Standards, and Swift (1996) has outlined the procedures used. Invited contributions at the first meeting of the IHSS at Estes Park in 1983 dealt with the geochemistry, isolation and fractionation, and characterization of HS (Aiken et al., 1985a). Hayes (1985) presented the information that was available then about the isolation of HS from soil, and Aiken (1985) did likewise for their isolation from waters.

Hayes (1985) drew up a set of criteria that he considered to be important for good organic solvents for HS. Earlier, Whitehead and Tinsley (1964) had outlined criteria that they considered to be important for effective solvents for HS. These were:

1. A high polarity and a high dielectric constant to assist the dispersion of charged humic molecules
2. A small molecular size to penetrate through the humus matrix
3. The ability to disrupt existing hydrogen bonds, and to provide alternative groups to form humic–hydrogen bonds
4. The ability to immobilize metallic cations.

Hayes (1985, 2006b) has listed the properties of organic solvents that might be considered for the isolation of soil humic components. He checked the extents to which selected organic solvents dissolved H^+-exchanged humic acids (HAs), and he concluded that good organic solvents have electrostatic factor (the product of relative permittivity and dipole moment) values greater than 140 and have pK_{HB} (the measure of the strength of a solvent as an acceptor in hydrogen bonding) values greater than 2. Dimethylformamide (DMF) and dimethylsulfoxide (DMSO) meet these requirements; both of these were shown to be good solvents for the HAs, with DMSO being the better of the two. Hayes also discussed applications of solubility parameter data. The best of the organic solvents tested had δ_p (dispersion force), δ_h (hydrogen bonding), and δ_b (proton acceptor) parameters greater than 6, 5, and 5, respectively. Solvation is greatest when the product of δ_a (solvent) × δ_b (solute), or vice versa, is maximum (Hayes, 1985).

The major solvent systems still involve base, and 0.1 M NaOH is the solvent of choice of the IHSS (Swift, 1996). Song et al. (2008) used a modification of the IHSS procedure and extracted a Mollisol soil exhaustively in 0.1 M NaOH adjusted to pH 7, then at pH 10.6, and then with the unadjusted solution (pH 12.6). Subsequently the residual soil material was exhaustively extracted with 0.1 M NaOH + 6 M urea (see also Hayes, 2006b). The NMR data show significant differences between the humic components isolated at the different pH values, but the extract in the NaOH/urea solvent (which would be humin in the classical definitions) was similar to that isolated at pH 12.6. This would-be humin (Section 1.3.3) material in the classical definitions was in fact composed of HAs and fulvic acids (FAs) trapped within the humin matrix (Song et al., 2008). Subsequently the residual organic matter associated with the fine clay (humin material) was exhaustively extracted with DMSO + 6% concentrated H_2SO_4, and 93% of the humin residue was solvated and recovered. The remaining clay–humin associations can be released by dissolving the silicates in HCl/HF (Preston and Newman, 1992).

1.3.2. The Emergence of Procedures for the Fractionation of Soil Humic Components

Berzelius (1806) was the first to consider the humic fractions that are still extensively worked with. His *humic acids* were soluble in aqueous base and precipitated upon acidification of the media. He regarded as humin the inert material that was not dissolved in base. Light yellow materials were left in solution following the precipitation of the humic acids, and Berzelius called these *crenic* and *apocrenic acids* (he considered the latter to be an oxidation product of crenic acid), components that effectively complexed ammonia and various metals to give these elements greater mobility (compared with the salts of humic acids). Crenic and apocrenic acids would be covered by the term *fulvic acids* introduced subsequently. The mobility in the soil profile of salts of crenic and apocrenic acids was later used by Sibirtsev (1900, 1901) to explain aspects of podzolization. Berzelius ascribed to the thesis that soil fertility and plant nutrition were associated with the presence of humus and that, because crops deplete soil humus, it is necessary to apply organic manures. The various views held at the time are incorporated in his textbook of chemistry (Berzelius, 1839).

Fractionation on the basis of solubilities at different pH values has always been a major procedure for the fractionation of HS, and thus there arose the primary fractionations into HAs and FAs. Further fractionations on the basis of different solubilities in alcohol gave rise to additional components, as outlined in Section 1.3.3.

The development of electrophoretic techniques afforded possibilities for fractionations based on charge density differences. Duxbury (1989) has reviewed applications of different electrophoretic separation methods, including zone electrophoresis, moving boundary electrophoresis, isotachophoresis, and isoelectric focusing (IEF). Preparative column electrophoresis (Clapp, 1957) and continuous flow paper electrophoresis (Hayes, 1960; summarized by Hayes et al., 1985) methods have been used to separate components isolated from sapric histosol soils. These techniques allowed separation of polysaccharides from the colored components; the electrophoretograms of the colored components were diffuse, showing a continuum of components of different charge densities.

The availability of gel filtration techniques during the 1960s allowed fractionation to be achieved on the basis of size differences. The most noteworthy work using these techniques is attributed to Cameron et al. (1972) (see Swift, 1985). They, using gel filtration and discrete pore size membranes, fractionated a HA extract into 11 different size fractions and determined the molecular weight of the fractions using ultracentrifugation techniques (see Section 1.4.7).

Leenheer (1985) has reviewed procedures used by water scientists for the fractionation of aquatic HS. Water scientists introduced the Rohm and Haas resins XAD-8 [(poly)methylmethacrylate] and XAD-4 (styrenedivinly benzene) for the separation and isolation of HAs, FAs, and XAD-4 acids. The less polar HA and FA components sorb on XAD-8, and the polar components elute through the resin but are held by XAD-4. The HAs and FAs are recovered during back elution in dilute base, and the HAs are then precipitated at pH 2. The XAD-4 acids are also back-eluted in base, H^+-exchanged using IR-120 H^+-exchanged resin, and freeze-dried. The resin techniques are applicable to soil extracts, and they have been used successfully by Hayes et al. (2008) for the fractionation of extracts from soils and their drainage waters.

Techniques for the isolation and fractionation of carbohydrate and peptide components of SOM are discussed in Sections 1.5.2 and 1.6.

1.3.3. Definitions of Soil Humic Components

Mulder (1861–1862), who had been a student of Berzelius, classified the HS he isolated as:

Ulmin and *Humin*, the components insoluble in alkali
Ulmic acid (brown) and *Humic acid* (black), the components soluble in alkali
Crenic acid and *Apocrenic acid*, the components soluble in water

These definitions were essentially the same as those put forward by Berzelius. Mulder considered, however, that, besides humus substances, products from the decomposition of organic residues, such as leucine, butyric acid, valeric acid, and formic and ethanoic acids, could exist in soil. These observations are of interest because of the information that has emerged in the past half-century about growth inhibitors and stimulators from low-molecular-weight extracts from SOM and composts.

Mulder considered that the different isolates were chemically individual compounds, and on the basis of elemental analyses data he assigned to the different fractions the empirical folmulae:

Humin, $C_{10}H_{30}O_{15}$	Ulmin, $C_{10}H_{32}O_{11}$
Humic acid, $C_{10}H_{24}O_{12}$ (or $C_{10}H_{30}O_{15}$)	Ulmic acid, $C_{10}H_{30}O_{15}$
Crenic acid, $C_{10}H_{21}O_{16}$	Apocrenic acid, $C_{21}H_{12}O_{12}$

The numbers of humic fractions continued to increase, and terms such as *"mucic acid," lignoic acid*, and *hymatomelanic* acid were introduced; all of these were considered to represent chemically individual compounds, which of course they were not. Sprengel (1826) promoted fractionation on the basis of solubility characteristics in aqueous media, and then the terms *humic acids*, *fulvic acids*, and *humin* became generally recognized.

Definitions (based on the solubility criteria) of soil humic components have not changed much in the last 200 years. In the definitions of the International Humic Substances Society, as stated by Aiken et al. (1985b), *humic substances* are "a general category of naturally occurring, biogenic, heterogeneous organic substances that can generally be characterized as being yellow to black in color, of high molecular weight, and refractory." They classified formally the three major fractions as *humin*, "that fraction of humic substances that is not soluble in water at any pH value"; *humic acid*, that fraction of humic substances that is not soluble under acid conditions (below pH 2), but becomes soluble at greater pH"; and *fulvic acid*, "that fraction of HS that is soluble under all pH conditions." In the classification of Kononova (1966, 1975) adapted by Hayes and Swift (1978), SOM is grouped into:

1. Unaltered materials, which include fresh debris and nontransformed components of older debris;
2. Transformed products, or humus, bearing no morphological resemblances to the structures from which they were derived.

The transformed, or humified, components consist of humic and nonhumic substances. The humic substances are defined by Aiken et al. (1985b), as described above. The nonhumic substances belong to recognizable classes, such as polysaccharides, polypeptides, and so on. These can be synthesized by microorganisms or can arise from modifications of similar compounds in the original debris. It is questionable, on the basis of emerging information, that HS can be considered to be of high molecular weight (Piccolo, 2001; Simpson, 2002). Inevitably, these substances will have high molecular weight components, but there is support for the concept of molecular associations that give rise to pseudo-macromolecular properties.

The major solvent systems still involves base, and 0.1 M NaOH is the solvent of choice of the IHSS (Swift, 1996). Hayes (1985, 2006b) reviewed the principles and the procedures for the isolation of HS, and the more recent publication refers to solvent systems that isolate additional HA and FA materials using exhaustive extractions at increasing pH values, followed by exhaustive extractions with 0.1 M NaOH + 6 M urea (see Section 1.3.1). The components in intimate associations with the clays, isolated in DMSO/H_2SO_4 in the solvent sequence, were largely biological molecules (see Sections 1.4.7 and 1.7) and would not, in the classical definitions, be HS.

1.3.4. The Need to Redefine the Fractions of Soil Humic Substances

It would be pointless to draw up a classification system that takes account of several fractions based on charge density differences, or even differences in solubilities in organic solvent systems. Consideration might be given to the hymatomelanic acid, or the alcohol-soluble component described by Hoppe-Seyler (1889). It would be important to distinguish between the FA fraction (or the material that is soluble in acidic and basic media) and the FAs as defined by the IHSS (or the fractions recovered when the FA fraction in solution at pH 2 is passed on to XAD-8 resin). The XAD-4 acids are not true humic components. These are rich in carbohydrate and peptide biological molecules, which are, of course, components of SOM but should be considered to be outside of the definitions of HS as referred to above. Similarly, nonhumic components associated with the HAs can be recovered by dissolving the HAs in dilute base, diluting the solution to <20 ppm and passing on to XAD-8 resin. Again the polar components wash from the resin (Hayes, 2006b). Thus it would be appropriate to consider as the HA fraction the materials precipitated at pH 1 or 2 and to be aware that polar nonhumic substances will be associated in the precipitates. Consideration should be given to the humin fraction. On the basis of the recent compositional studies referred to above, humin materials will, for the most part, be biological molecules in association with other organic components and with the mineral colloids.

The terms HAs, FAs, and humin are general terms that refer to broad differences between soil organic components. In order to provide more discrete definitions, careful considerations need to be given to additional fractionation procedures that can give more discrete and distinct fractions.

1.4. EMERGENCE OF CONCEPTS OF COMPOSITIONS AND STRUCTURES OF SOIL HUMIC COMPONENTS

1.4.1. Pre- and Some Early 20th-Century Concepts of Soil Humic Components and Functions

As pointed out in Section 1.3.3, the organic fractions isolated in and fractionated from aqueous media were considered by the early workers to be individual compounds. The influences of microorganisms in the genesis were not recognized until the end of the 19th century, largely because the science of microbiology had not been developed. Thus the emphasis was on chemical synthesis. The prevailing concepts at the time considered that humus materials were formed from oxidation products of plant materials. Detmer (1871) considered that the oxidation of cellulose according to Eq. (1.1) gives rise to humic materials.

$$13C_6H_{10}O_5 + 36[O] \rightarrow C_{60}H_{54}O_{27} + 18CO_2 + 38H_2O \qquad (1.1)$$

van Bemmelen (1888) failed to isolate compounds that could be considered to be pure, and he concluded that crenic acids, apocrenic acids, ulmic acids, HAs, and humin were not homogeneous materials. Because of the developments in colloid chemistry toward the end of the 19th century, he was able to conclude that such materials were amorphous and colloidal and that the formulae suggested for these had no significance.

The emphasis on microbiology inspired by Pasteur focused interest on the roles that microorganisms have in the transformations of organic debris in the soil environment. Several studies—for example, those by vonPost (1862), Darwin (1881), Müller (1887), and Ramann (1888)—indicated that the genesis of humus is a biological, and not a chemical, process. That initiated the biological and chemical studies based on the release of building blocks from the degradations of plant components. Perhaps the definitive influence can be attributed to Dokuchaev (1883), who defined soil as a natural body formed through the combined action of natural factors and, in particular, the biological factors contributing to soil formation such as vegetation cover and the activities of living organisms. Humus was considered to have an important role in soil formation and in soil fertility. These concepts introduced a new era of soil humus studies.

At the end of the century there was general acceptance that HS are complex compounds of a synthetic nature formed as the result of decomposition involving two or more plant-derived materials. For example, Dehérain (1902) considered that HS synthesis involves interactions between proteins and "encrusting substances," mainly lignin. This concept was later developed as the "ligno-protein complex" of Waksman and Iyer (1932, 1933) (see Section 1.4.5) and as described by Waksman (1936).

Schreiner and Shorey (1909, 1910) of the US Department of Agriculture regarded soil humus as a complex mixture of organic substances arising from the decomposition of materials of plant and of animal origins. Because they considered HS to be artificial products formed in the processes of extractions with alkali, they focused on the isolation of organic substances using the techniques of organic chemistry. They used the acid filtrates after the precipitation of HAs, along with the ethanol extracts of the precipitated materials. In this way they isolated and identified more than 40 compounds that included hydrocarbons, sterols, fats, organic acids, carbaldehydes, organophosphorus, and N-containing compounds.

In a review of the work of Schreiner and Shorey, Shmuk (1924) considered that their approach tended to divide the humus concept into small groups of peripheral units and overlooked the major reserve of organic substances in the soil.

Trusov (1914) carried out a systematic study of humus formation. Initially he subjected plant components—proteins, cellulose, plant oils, and tannins—to treatment with strong acids. Later, recognizing the importance of biological processes, he (Trusov, 1915) studied the humification of plant components under normal biological conditions and then (Trusov, 1916) studied the transformations of plant residues, leaves, grass, and woody species under similar conditions. He concluded that plant components most readily utilized by microorganisms are first converted to microbial plasma and this then participates to give rise to humus. The plant residues not utilized by microorganisms (such as lignin, tannins, etc.) were considered to be direct sources of HS. These concepts have relevance at the present time.

Trusov died prematurely, but his contemporary, Shmuk, advanced the approaches initiated by him. He (Shmuk, 1924) was first to establish that soil HAs contained benzenoid structures, although Hoppe-Seyler (1889) had shown that peats and coals had aromatic units in their compositons. By esterification procedures, Shmuk showed that humic substances had hydroxyl (of phenolic origins) as well as carboxylic functional groups. He proposed that two components were contained in the HA molecule; one of these was an organic N-containing compound (Shmuk, 1914) of microbial origin, and the second one was the benzene ring. He regarded these components to be linked and not present as a mixture.

In a period of investigation and teaching, starting in 1902 and culminating in 1939, the studies of Williams (1939) led to conclusions for the era, as listed by Kononova (1966):

1. Humus substances exist in soil as a natural body.
2. Various plant materials that undergo complex biochemical transformations serve as sources of humus substances.
3. Plant materials decompose to more simple products from which the complex humic substances are synthesized.
4. Microbial enzymatic processes are involved in the decomposition and in the synthesis processes.

Thus it was accepted at the beginning of the 20th century that microorganisms had an important role in the synthesis of humus substances. Some considered the synthesis to be wholly biological, but there was a growing concept suggesting that the compounds released in the microbial breakdown of organic substrates could condense to give products from chemical synthesis processes.

Concepts of the compositions of coals influenced many in considerations of the structures of soil HS. For example, the proposal of Fuchs (1931) for structures of coal HAs (Figure 1.1) influenced soil humic scientists. The proposed structure is composed of heterocyclic aliphatic functionalities, some phenol-derived units, and considerable amounts of carboxylic and hydroxyl acidic functionalities. It may be possible that such structures could arise under conditions of elevated temperature and pressure, with oxidation taking place subsequently. Whereas such conditions might prevail during the synthesis of coals, they would be most unlikely to take place during the transformations of organic materials in the soil environment.

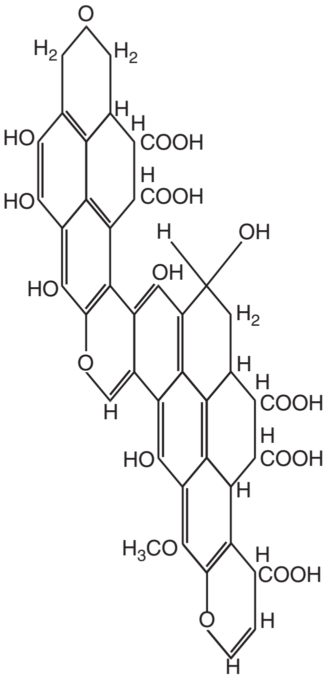

Figure 1.1. Structure of humic acid as proposed by Fuchs (1931).

1.4.2. The Maillard (Melanoidin) Concept of Humic Substances

The Maillard reaction is discussed in considerable detail Section 2.5.2 of Chapter 2 of this book. This section deals only with relevant work involving this reaction that was carried out prior to 1960.

Ellis (1959) has defined the Maillard reaction as the "reaction of the amino group of amino acids, peptides, or proteins with the glycosidic hydroxyl group of sugars," and he and Hodge (1953) presented excellent reviews of the chemistry of the Maillard–Browning reaction. The initial reaction of glucose with glycine is followed by other more complex changes that result eventually in the formation of brown pigments and polymers (see Section 2.5.2, Chapter 2). The formation of the brown pigmented products is generally regarded as the Browning reaction. It was argued that browning is brought about by the effects of pH on sugars, and that this can take place over a wide range of pH values, whereas the Maillard reaction requires alkaline media (Maillard, 1912, 1916). He (Maillard, 1912) deduced that the CO_2 evolved was from the glycine; and Wolfrom et al. (1953), using ^{14}C-labeled glucose, deduced that 90–100% of the CO_2 was from the glycine. Maillard (1917) showed that products of the reaction of glucose with glycine gave materials that resembled in many respects those of natural humic materials from soil. That stimulated interest in the abiotic synthesis of HS from sugars and amino acids liberated from the hydrolysis of polysaccharide and peptide materials.

Burdon (2001) has raised questions that would suggest that the Maillard reaction is not a significant contributor to the synthesis of soil HS. He has pointed out that there are not sufficient concentrations of reducing sugars or of amino acids in the soil solution to allow the reaction to take place to any great extent; and since the Maillard reaction proceeds best under alkaline conditions (Ellis, 1959), there should be more humic substances in alkaline soils, which there are not. Burdon referred to the spectroscopic data which show that lignin is a major contributor to the aromaticity of humic materials, whereas the aromatic components in Maillard reaction products are largely composed of heterocyclic N-containing substances.

Hayes (1960) observed that products from heating glucose with glycine in aqueous media under reflux conditions had some compositional properties and reactivities similar to those of the HAs isolated in aqueous base from a sapric histosol. The techniques used for the comparisons included differential thermal analysis (DTA) (see Figure 1.2). However, that work was done before the introduction of NMR to such studies.

1.4.3. The Enders Concept of Humic Compositions

Enders and Fries (1936) observed what they considered to be a relationship between melanoidins (or browning products) and humic acids. Later, he and Marquardt (Enders and Marquardt, 1941) showed that methylglyoxal [$CH_3C(O)C(O)H$] could give rise to melanoidins and caramel products. Then, based on his experiments to establish mechanisms of formation of methylglyoxal from hexose sugars, Enders (1942, 1943a) showed that glucose in aqueous solution was in equilibrium with the triose, and the triose could give rise to methylglyoxal. Enders and Sigurdsson (1944) found this equilibrium to be pH- and temperature-dependent under test tube conditions.

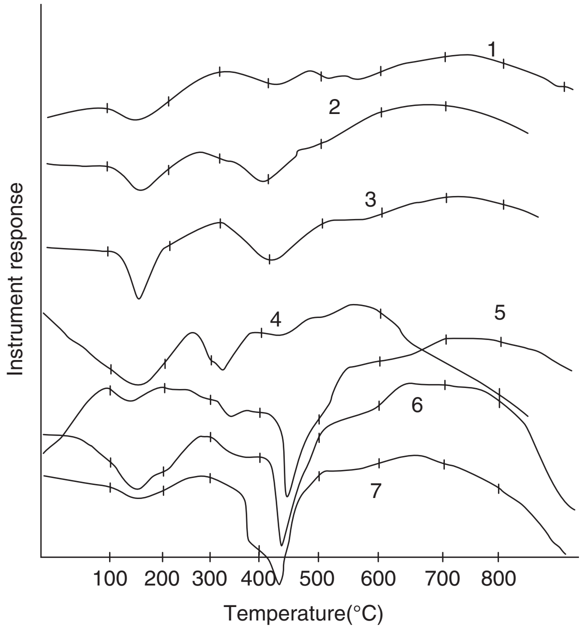

Figure 1.2. Differential thermal analysis for the humic acid fraction isolated in NaOH from a sapric histosol (1), from the acid precipitate isolated from products of the reaction of methylglyoxal with glycine (2), and from the acid precipitate formed from the reaction of glucose with glycine (3), alkali lignin (4), casein (5), lignin–casein 3:1 complex (6), and lignin–casein 6:1 complex (7).

Enders and Sigurdsson (1943) postulated a pathway from methylglyoxal to acetaldehyde (via pyruvic acid) and acetaldehyde could polymerize via the aldol condensation mechanism. In order to show how HAs could be formed under physiological conditions, Enders (1943b) postulated that methylglyoxal could be released by soil microorganisms under conditions unfavorable 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 HS.

Schuffelen and Bolt (1950) found that the C/N ratio in the product from the reaction of methylglyoxal and glycine varied with the concentrations of the reactants used. The product with a C/N ratio of 9.2 had titration curves and exchange capacity values similar to those for a Dalgrund peat. Hayes (1960) reacted equimolar (1.25 molar) concentrations of methylglyoxal and glycine at 97 °C in an atmosphere of O_2 and under N_2. In the course of synthesis of the browning product from the methylglyoxal/glycine system, 72% decarboxylation had taken place in the aerobic system in 40 h, and the HA-type product formed had 60% C, 3.3% N, and a C/N ratio of 18.2. The comparable data for the HA isolated from a sapric histosol were 59%, 3.0%, and 19.7%, respectively. The brown components in both products had the same electrophoretic mobility and relatively comparable differential thermal analysis (DTA) thermograms (Figure 1.2). The products formed from similar reactions of glucose and glycine, sodium glucuronate and glycine, and xylose and glycine had lesser similarities to the histosol HA.

Based on the reasoning of Burdon (2001), the extents to which methylglyoxal polymerization products and the methylglyoxal/glycine reaction products are synthesized will await NMR evidence for the nature of the aromatic components in the synthetic structures.

1.4.4. Phenols and the Synthesis of Humic Substances

The polyphenol pathway for the synthesis of HS is discussed in Section 2.5.1 of Chapter 2.

Trusov (1915, 1916), as indicated in Section 1.4.1, introduced the concept that polyphenols and quinones contributed to the synthesis of HS. Subsequently, as the result of enzymatic oxidation (with oxidase enzymes from microorganisms), the phenols oxidize to quinones; and these, through further condensation, are converted into dark-colored humic-type substances.

The writer, when an undergraduate in University College, Dublin, happened, purely by chance, to enter the Physics lecture theatre when Wolfgang Flaig (1960) was addressing attendees at the International Peat Conference in 1952. There the writer was introduced to the concept of synthesis from polyphenol and quinone structures. He was not ready at that time to appreciate the mechanistic inferences but was in a better position to do so six years later when Professor Flaig visited The Ohio State University. The concepts involved in the synthesis processes are outlined in the chapter by Flaig et al. (1975).

Martin et al. (1967) cultured the fungus *Epicoccum nigrum* on a glucose/asparagine medium containing yeast extract and inorganic salts. After five weeks of incubation at pH 2, they isolated mycelium-free “HA-type” substances. Some of these were similar to leonardite HAs in terms of elementary composition, total acidity, cation-exchange capacity, and carboxyl, phenolic, and hydroxyl contents, as well as in terms of molecular weight distributions. Haider and Martin (1967) illustrated a plausible scheme for the genesis from nonaromatic precursors of phenols identified from the *E. nigrum* culture. There is strong evidence from that era to indicate that fungi contribute significantly to the genesis of soil humus. Intracellular enzymatic synthesis would seem to be likely, but extracellular enzymatic catalytic synthesis cannot be ruled out (Hayes and Swift, 1978). Burdon (2001) has posed a very relevant question when he asked “Why should a micro-organism expend energy and resources making a material that it has no use for? Any organism that did this would become extinct because of competition by organisms that did not waste energy and resources in this way.” His succeeding arguments leave room to consider that the “humic-type” materials are formed by the enzymatic catalysis that render waste products innocuous to the organism and are stored inside the fungal mycelium.

The structural proposal for humic acids by Stevenson (1982) had significant logic, based on the state of information at that time. The structure (Figure 1.3) has compositional aspects of phenols derived from lignin and from tannins, and paper chemistry exercises would allow reactions and interactions needed to give structures of the type predicted. The structure shown is highly aromatic, and the proposed aliphatic moieties are saccharide- and peptide-derived. Modern analytical procedures invariably show peptide and saccharide components to be associated with

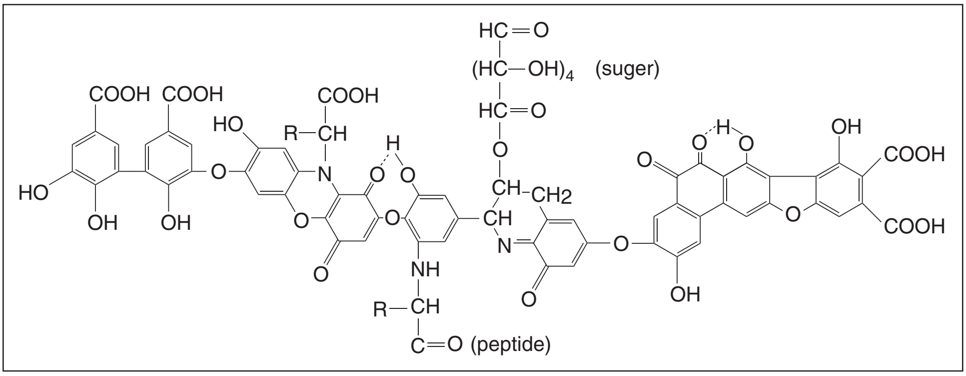

Figure 1.3. Humic acid-type structure, as postulated by Stevenson (1982).

HAs, but there has not been convincing evidence so far for covalent linkages between these and the lignin-derived components that are accepted as major contributors to soil HA structures.

1.4.5. The "Ligno-Protein" Concept of Humic Acids

As seen in Section 1.4.1, various authors had considered the involvement of lignin and of proteins in the genesis of HS. Specifically, Dehérain (1902) saw HS as products of interactions between proteins and "encrusting substances," mainly lignin. The ligno-protein theory that evolved is generally attributed to Waksman and his colleagues (Waksman and Iyer, 1932, 1933; Waksman, 1936). Their work indicated that oxidized lignin, when reacted with protein (casein), gave a product similar to HAs; and based on their work, the concept of HAs as complexes of oxidized lignin with protein predominated for a generation.

In the latter part of the 1950s, this author (Hayes, 1960) attempted to repeat the experimentation used by Waksman and Iyer (1932, 1933). He exhaustively washed powdered wheat straw with boiling water, then with hot dilute hydrochloric acid, and extracted twice for 5 h in an autoclave at 120 °C, each time with a 4% sodium hydroxide solution. The combined filtrates were acidified to pH 4 with hydrochloric acid and the precipitate formed was washed free of chloride and freeze-dried. A "ligno-casein complex" was formed by reacting three parts of the lignin extract and one part casein in a 0.1 M solution of sodium hydroxide and collecting the precipitate formed when the pH was adjusted to 4. This "complex" was washed free of chloride and freeze-dried. A 6:1 lignin–protein complex was formed in the same way.

The DTA thermograms shown in Figure 1.2 are for the HAs isolated in base from a sapric histosol, from the product from the reaction of methylglyoxal with glycine, for that for glucose with glycine, for alkali lignin, for casein, and for "lignin–casein 3:1 and 6:1 complexes." The thermograms show a degree of similarity between the sapric histosol HA and the methylglycol/glycine and glucose/glycine products, but definite differences are evident between these and the thermograms for the alkali lignin and casein. The thermograms for the lignin–casein complexes are composites of those for lignin and for casein and are very different from the HAs. That ended any adherence to the “ligno-protein complex” theory on the part of this author. However, on the basis of the information that was available at the time, the ligno-protein complex theory was logical and was based on the evidence we have today that lignin derivatives and peptides are important components of SOM, and they may well be present in associations, though not as materials linked covalently to significant extents (see Sections 1.4.7 and 1.7).

1.4.6. The Haworth Concept of Humic Structures

On the basis of the fused aromatic structures identified in the digests of the zinc dust distillation and fusion reductions of “acid boiled” HA, the Haworth Group (Haworth, 1971; Cheshire et al., 1967) proposed that humic molecules have a polycyclic aromatic core to which polysaccharides, simple phenols, proteins or peptides, and metals are attached by chemical or physical bonding processes, as summarized in Figure 1.4. The zinc dust distillation and fusion procedures are very harsh, and the yields are always low. It has become accepted that the procedures can lead to excessive bond breaking, and the recombination of fragments can give fused aromatic structures (see Clapp et al., 2005; Hayes and Swift, 1978). The polycyclic aromatic core thesis was based on the premise that yields of these aromatic materials were greater from the HAs than from nonhumic precursors. However, the formation of artifacts under these conditions is inevitable (Burdon, 2001). Polyhydroxyaromatic compounds, quinones, and furfurals, for example, would give rise to fused aromatic structures under the conditions applied. Cheshire et al. (1968) found that such compounds gave polycyclic aromatic structures from zinc-dust distillation at 500–550 °C, but at 400 °C only small yields of anthracene were obtained from hydroxybenzenes, and no aromatic structures were detected in the distillates from furfural and polymers of *ortho*- and *para*-benzoquinone. Because the same products were detected in the same proportions in the digests of the HAs at the higher and lower temperatures, it was concluded that the products released were largely from the humic materials. Long-chain hydrocarbons were identified in the digests, and these can be considered to have survived the distillation process.

The Haworth model (Figure 1.4) has been referenced widely, and in many instances it has been offered as an example of humic structures. Humic acid-type components containing fused aromatic structures can occur in soils. There is increasing awareness of char, black carbon, or charcoal products in soils that have been

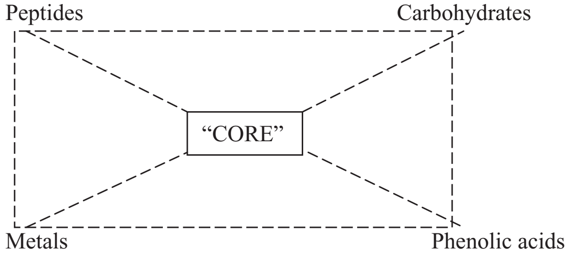

Figure 1.4. The Haworth concept of humic acid structure.

subjected to vegetation burning over time (Skjemstad et al., 2002). The char materials will, of course, be composed of fused aromatic structures, and there will be peripheral oxidation of the aromatic structures to give the acidic properties that could classify these under the operational definitions of HAs. However, in the cases of most soils, the char/oxidized char contents will be relatively small. Hence there is no convincing evidence to support the Haworth structural hypothesis.

1.4.7. Concepts of the Compositions of Humic Substances in the Modern Era

The availability of modern fractionation and spectroscopic instrumentation has allowed rapid advances to be made in our understanding of the compositions and aspects of the structures of HS. During the last half-century, Morris Schnitzer (Schnitzer, 2000) contributed greatly to our awareness of the compositions of HS. He commenced his degradation studies in the 1950s, "in the days of sail" insofar as modern instrumentation is concerned, with the oxidation of the HAs from the Ao and Bh horizons of a podzol from Prince Edward Island, Canada (Schnitzer and Wright, 1960). In terms of compositions, the greatest initial advance was made when GCMS instrumentation became available. Through extensive investigations, using a variety of chemical degradation procedures and GCMS instrumentation, Schnitzer and his colleagues provided indications of the types of molecules that could compose humic structures (reviewed by Schnitzer and Khan, 1972; Schnitzer, 1978, 2000). However, since many of the procedures used in degrading humic molecules are harsh, often involving vigorous oxidation and reduction procedures, the digest products in many instances may best be described as derivatives of the structures that compose the HS. Hayes and Swift (1978, 1990), repeated by Clapp et al. (2005), have reviewed the mechanisms involved in the different degradation reactions, and mechanistic considerations allow conclusions to be drawn about the types of structures that could give rise to the products identified in the degradation digests. Thus, through mechanistic awareness it has been possible to get indications of the types of structures that compose humic molecules.

The founding of the International Humic Substances Society (IHSS) in 1981 (as referred to in Section 1.3.1) has given a considerable boost to the humic sciences. Before that time there was little communication between soil and water scientists with interests in dissolved (DOM) or particulate (POM) organic matter in waters. The response of the US Geological Survey [Water Resources Division (USGS WRD, Denver, Colorado)] and the EPA (Cincinnati, Ohio) to the discovery by Rook (1977) of mutagenicity generated by the chlorination of FAs in natural waters brought about a major research effort in the WRD, led initially by Drs. Bob Avert and Ronald Malcolm. Following the first international meeting of the IHSS at Estes Park, Colorado (referred to in Section 1.3.1), a second meeting was convened in the University of Birmingham a year later. It was clear in Birmingham that soil and water humic scientists had learned from their associations of the previous year, and the book of invited contributions, *Humic Substances II: In Search of Structure* (Hayes et al., 1989a), covered in considerable detail the various degradative processes used in humic studies, listed the digest products identified, and referred to mechanistic interpretations that would suggest origins for the digest products. There were extended treatises on the different spectroscopic techniques used at that time for the characterization of humic molecules, and attention was also focused on the physico-chemical properties of HS, including studies of molecular sizes, shapes, and charges. In a chapter written after the meeting, consideration was given to the emergence of structural forms as seen on the basis of the data and concepts presented (Hayes et al., 1989b).

Two major IHSS publications (Aiken et al., 1985a; Hayes et al., 1989b) gave a comprehensive account of the states of the art in the humic sciences in the mid-1980s. However, very significant advances have been made in the past 20 years.

Awareness of sizes and of shapes is of major importance for considerations of the reactivities and interactions of humic molecules. Major advances were made when appropriate fractionation procedures became available. Advances in isolation, fractionation, and instrumental analytical methods have led to our current awareness of the compositions and aspects of the structures (including shapes and sizes) of humus materials. Thus, the development in the 1960s of gels of relatively discrete pore sizes allowed Cameron et al. (1972) to isolate 11 humic fractions from a sapric histosol. They considered these fractions to be reasonably homogeneous with respect to size. Molecular weight (MW) and frictional ratio values were determined from ultracentrifugation data. The plot of frictional ratio versus MW (Figure 1.5) gave a linear relationship for fractions with MW values up to ~400,000 Da, and the nonlinearity for the samples with the higher MW values was attributed to branching of the structures or to silicate contamination [see review by Swift (1989)]. The model based on the linearity of the MW versus frictional ratio plot suggested a random coil conformation for the HAs. That concept was remarkably convenient for explaining many of the interactions of HS. However, in the past 10 years there has been considerable emphasis on concepts of molecular associations that give rise to pseudo-macromolecular-type structures. The shift from concepts of macromolecular structures has been aided by the work of von Wandruszka (1998), which suggests the formation of intramolecular micelles, and by that of Wershaw (1999), which suggests molecular aggregation. Piccolo (2001) has provided evidence to

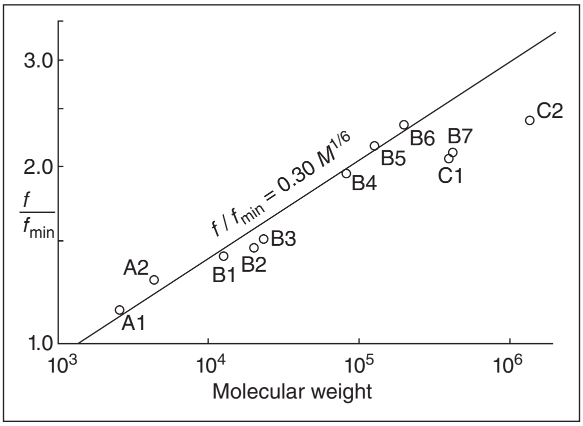

Figure 1.5. Relationship between the frictional ratio (f/f_{min}) and the molecular weight for different humic acid fractions isolated from a sapric histosol. The line is the theoretically derived relationship between frictional ratio and molecular weight for a randomly coiled polymer. After Cameron et al. (1972).

indicate how molecular associations can be disrupted to release from pseudo-macromolecular structures molecules of much lower sizes. Kenworthy and Hayes (1997) furthered that concept by showing that pyrene sorbed by what might be considered as HA aggregates or molecular associations was released by additions of very small amounts of ethanoic acid. Measurements of diffusion by Simpson (2002) and by Simpson et al. (2002), using diffusion-ordered spectroscopy (DOSY), have shown that additions of trace amounts of ethanoic acid (known to disaggregate proteins) gave different diffusivities for the lignin-derived, the carbohydrate, and the peptide components of an HA. These components gave a single diffusion coefficient in the absence of the ethanoic acid (see Figure 15.13, Chapter 15).

Senesi (1999) introduced and reviewed fractal geometry for the quantitative study of the morphology developed by HS under different experimental conditions, and he has discussed the underlying aggregation processes. The fractal approach may have limitations with regard to gaining direct evidence for the nature of the associations that are important for organic matter in the soil environment, but it can have applications for modeling the extents of the associations and the morphologies of the products.

Electron spin resonance (ESR) spectroscopy, as reviewed by Senesi and Steelink (1989), has given an awareness of the abundances of free radicals in humic fractions and of the possibilities for interactions that can arise from these.

Infrared spectroscopy was widely used in the second half of the 20th century, and this technique has allowed some advances to be made in awareness of functionalities in, and of complexes formed by, humic molecules. However, the greatest advances in determinations of functionalities, in aspects of compositions and structures, and now in aspects of humic interactions have been made since the introduction of solid-state ^{13}C NMR spectroscopy (Wilson, 1987; Malcolm, 1989). Chapter 15 in this book (by Simpson and Simpson) has reviewed in detail the applications of NMR in the solid and liquid states to studies of compositions and interactions of NOM. We now have a good indication of the types of functionalities that compose HS, and combinations of modern NMR technologies and principal component analysis (PCA) techniques allow us to deduce the origins of some of the functionalities (Novotny et al., 2007).

There is abundant evidence from NMR for the contributions of lignin to the compositions of the HA and FA fractions in the NOM of soils and waters. This is evident from the methoxyl resonance (56 ppm) and the O-aromatic resonance at 140–150 ppm. As humification proceeds, evidence for methoxyl diminishes, but the aromatic functionalities will still be derived mainly from altered lignin. The contributions of tannins to humic compositions have been recognised relatively recently, and tannins from decaying leaves and vegetation can be significant contributors to the HAs and FAs of soils and waters. Tannins come under the general category of phenolic extractives. These are a heterogeneous class of compounds consisting of: *hydrolyzable tannins*, which upon hydrolysis mainly yield glucose and gallic and ellagic acids; *flavonoids*, or polyphenols with a $C_6C_3C_6$ carbon skeleton, and their polymers are called *condensed tannins*; *lignans* formed from the oxidative coupling of two phenylpropane (C_6C_3) units; *stilbene* (1,2-diphenylethylene) derivatives which, because of their conjugated double bonds, are very reactive; and *tropolones*, characterized by an unsaturated seven-membered carbon ring (with a high resistance to biological degradation).

Tannins will be associated with lignin derivatives in the NMR spectra of HS. There can be significant resonance in the 100- to 110-ppm resonances, which hence can overlap with the anomeric carbon resonance (centered at 105 ppm) of saccharides. However, the uses of chemical shift anisotropy (CSA) and dipolar dephasing (DD) NMR techniques (Mao and Schmidt-Rohr, 2004) allow the distinction to be made between tannin and anomeric carbon resonances.

Humin has been regarded as the most intractable component of SOM. It must be considered to be a very important component, however, because typically it represents more than 50% of the organic carbon in a soil (Kononova, 1966; Stevenson, 1982, 1994) and more than 70% of the organic carbon in unlithified sediments (Durand and Nicaise, 1980; Rice, 2001). The definition of humin (Section 1.3.3) is similar to that of a protokerogen (Calvin and Philip, 1976; Rice, 2001), which is often used in petroleum geochemistry to describe insoluble organic matter in unlithified sediments.

Humin is considered by some to be at the stage in the generalised biogeochemical carbon cycle where organic carbon in the biosphere begins to become part of the geosphere (Rice, 2001). A part of this process would involve the removal of the more labile components of the organic carbon soil inputs. Consequently, humin would be regarded as the oldest of the three humic fractions (Rice, 2001). It was suggested that the mechanism by which nature slows the mineralisation of organic matter might involve the accumulation of an ill-defined, amorphous and heterogenous mixture of organic molecules (Swaby and Ladd, 1966; MacCarthy and Rice, 1991; Rice, 2001). This mixture would require either a very large assemblage of enzymes or an uncharacteristically versatile enzyme to effect its rapid mineralisation (Rice, 2001).

When considered from this perspective, humin has a significant role as a sink for carbon.

Solid-state NMR has done much to dispel the mysteries of humin compositions, and significant advances have recently been made using proton NMR in the liquid state (see Section 15.3.3 of Chapter 15). Based on solid-state ^{13}C NMR spectra, Hatcher et al. (1980) concluded that a repeating aliphatic structural unit, possibly attributable to branched and cross-linked algal or microbial lipids, is common to both soil and sediment humin samples. Hatcher et al. (1983) viewed the increase in humin relative to the other humic fractions as a “selective preservation” of the aliphatic compounds of the sediments and did not support condensation theories.

Derenne and Lageau (2001) have favored the concept of “selective preservation” of plant residues with a degree of resistance to microbial degradation. Studies of soils, sediments, and fossil fuels have shown that the organic components are rich in long-chain polymethylene functional groups (Deshmukh et al., 2005). These components are considered to be derived in terrestrial systems from plant cuticles containing the biopolymers cutin and cutan, as well as from suberized parts of plant organs containing suberin (Nierop, 1998). It has been shown that the aliphatic carbon content increases with increasing SOM decomposition (Baldock et al., 1997), which would indicate that these components are diagenetically resistant and survive unaltered over time (Deshmukh et al., 2005).

The two major polymeric lipid components found in plant cuticles are cutin and cutan. Whereas cutin is the polyester biopolymer that is solubilized upon saponification treatment, cutan is a nonsaponifiable and nonextractable polymeric substance found in certain cuticles. These molecules provide a protective barrier between the plant and its external environment and between different organs of the plant (Kolattukudy, 1980). Holloway (1984, 1994) has reviewed the structure of cutin.

Depolymerization of cutin using reactions such as alkaline hydrolysis, transesterification, and hydrogenolysis with $LiAlH_4$ in tetrahydrofuran (THF) gives monomers that are identified by gas chromatography–mass spectrometry (GC/MS) after derivatization (Kolattukudy, 1980). Such degradative studies suggest that cutin has a primary architecture comprised of C_{16} and C_{18} fatty acids, hydroxy fatty acids, and epoxy fatty acids (Kolattukudy, 1980). Deshmukh et al. (2005) have listed and given relevant ^1H (ppm) and ^{13}C (ppm) shifts for straight-chain and branched long-chain acids, alcohols, aliphatic hydrocarbons, epoxides, esters, and lesser amounts of aromatic esters in the cutan and cutin/cutan mixture in *Agave americana*. These components can be expected to persist in humin materials.

Suberins or polyestolides are related to cutins. These are complex polymers composed of ω-hydroxy monobasic acids linked by ester bonds. They also contain α,β-dibasic acids esterified with diols, as well as ferulic and sinapic acid moieties. Suberins are enriched with molecules having 16 and 18 carbon atoms. They also have ethylenic and hydroxyl functionalities, and ester and ether cross-linking can occur.

Although there is mostly indirect evidence, extensive ester cross-linking of these monomeric species gives cutins and suberins a three-dimensional architecture (Kolattukudy, 1984; Deshmukh et al., 2005).

Humin can no longer be considered to be the most intractable of the components of HS. As will be seen in Chapter 15, huge advances have been made, using proton NMR, in understanding the compositions of humin materials dissolved in DMSO/H_2SO_4. Evidence from solid-state (^{13}C) and liquid-state (proton) NMR has shown that humin material extracted from fine clays in DMSO + 6% H_2SO_4, after prior exhaustive exhaustive isolations at pH 7.0, 10.6, and 12.6 and in 0.1 M NaOH + 6 M urea, was largely composed of small amounts of oxidized lignin residues (trapped in the humin matrix), significant amounts of proteins/peptides and carbohydrate materials, peptidoglycan and lipoprotein structures, and large amounts of aliphatic waxes, cuticlar materials, and lipids (Simpson et al., 2007). The inference from that information is that the high affinities for the inorganic colloids of the biological molecules provides protection from microbial attack. Such biological molecules would mean that humin should not be classified as a humic substance, based on the classical definitions outlined in Section 1.3.3.

1.5. EVOLUTION OF STUDIES OF SOIL SACCHARIDES

The book by Cheshire (1979) has reviewed studies of soil polysaccharides, and he and his colleagues have contributed significantly to our awareness of the subject. Chapters by Hayes and Swift (1978), Cheshire and Hayes (1990), and Clapp et al. (2005) have reviewed relevant aspects of the chemistry and compositions of polysaccharides and have discussed the reactivities of saccharides in the soil environment.

Interest in soil saccharides, and especially in soil polysaccharides, is relatively recent, and their studies may be considered to be in the modern era of soil organic matter research. Martin (1945, 1946) established that the “slimy” bacterial products shown by Waksman and Martin (1939) to aggregate sand–clay mixtures were polysaccharides. Stacey and colleagues at the University of Birmingham, England (Haworth et al., 1946), and Geoghegan and Brian (1946, 1948) at the ICI Research Station, Jealotts Hill, Berkshire, England, were, at about that time, investigating the uses of bacterial dextran and levan polysaccharides for the improvement of soil structure and for the retention of water in soil. The Haworth Group, Geoghegan and Brian, and Martin, though working on a similar topic at about the same time, were unknown to each other.

The research that followed focused on statistical analyses (Rennie et al., 1954; Chesters et al., 1957) that correlated good soil structure with microbial gums and polysaccharide substances. Confidence in the roles of polysaccharides in soil stabilization was questioned when Mehta et al. (1960) showed that soil aggregate structures were preserved when a Swiss Braunerde was treated with periodate ($NaIO_4$). Periodate degrades polysaccharides. Hence it could be argued that soil crumbs stabilized by polysaccharides would disintegrate upon treatment with periodate. However, Greenland et al. (1961, 1962) showed that the periodate-treated soil crumbs were stabilized by fungal hyphae and myceliae (which resisted the periodate treatment). Also, crumbs in soils with free $CaCO_3$ were shown by Clapp and Emerson (1965) to resist degradation after treatment with periodate.

1.5.1. Origins of Soil Saccharides

Cheshire and his colleagues at the Macaulay Institute, Aberdeen, Scotland, carried out extensive studies on the origins of soil saccharides. When readily degradable ^{14}C-labeled carbohydrate substrates, such as glucose and starch, were incubated under field conditions, the labels were scrambled and the strongest labels in the newly synthesized sugars were in the hexoses glucose, galactose, and mannose and in the deoxyhexoses rhamnose and fucose, and there was much less labeling in the pentoses arabinose and xylose (Cheshire et al., 1969, 1971). That suggested that microorganisms were largely responsible for the hexoses and deoxyhexoses in soil. When the experiments were extended for up to two years, high levels of activity developed in the deoxyhexoses and especially in rhamnose. Because the compositions of the labeled sugars bore only a superficial resemblance to those in the whole soil, or in the soil extracts, the authors considered that sufficient time may not have elapsed to allow for differential degradation rates for the newly synthesized carbohydrates. The data would support the view that microbial synthesis is only a partial contributor to the origins of soil carbohydrates (Cheshire and Hayes, 1990).

In other soil incubation studies Cheshire et al. (1974, 1979) found that some sugars in straw in labeled plant material decomposed rapidly, but others decomposed relatively slowly, and about 15% of these remained after five years. There was little indication that xylose or cellulose was synthesized by soil microorganisms. However, studies by Cheshire and Anderson (1975) showed that plant residues are essential to maintain the soil carbohydrate levels. Total carbohydrate contents of soils fallowed for 10 years fell by as much as 50%. Because there was no significant change in the compositions of the soil carbohydrate as the result of the fallow, it was concluded that the sugars were equally susceptible to metabolism regardless of the management system.

Cheshire and Hayes (1990) have pointed out that although a particular sugar might be only a small proportion of the sugars in plants, this sugar could with time become predominant in a residue if it should have even a slightly greater resistance to degradation than the other sugars in the medium. This point was demonstrated earlier when Bacon and Cheshire (1971) showed that 2-*O*-methylfucose and 2-*O*-methylxylose are prominent among the methylated soil saccharides, despite the fact that these sugars are minor components of the hemicelluloses of some plants. The *O*-methyl functionality is considered to block enzymatic processes, leading to degradation of the sugars.

Sugar abundances ratios, such as (Mannose + Galactose)/(Xylose + Arabinose) and (Rhamnose + Fucose)/(Xylose + Arabinose), have been used to indicate the origins, plant or microbial, of sugars in soil saccharides (Oades, 1984; Murayama, 1984). The logic is based on the types of data, referred to above [and referenced in Clapp et al. (2005)], that indicate that xylose and arabinose are largely found in plants and that mannose, galactose, rhamnose, and fucose are more likely to be found in microbial cells. In the case of (Mannose + Galactose)/(Xylose + Arabinose), ratio values <0.5 would suggest origins in plants and values on the order of 2 would suggest microbial sources. It could also be meaningful to compare the ratios Mannose/Rhamnose and Galactose/Fucose for each sample. These and other ratio values have been used recently by Hayes et al. (2008) to show that the saccharides in fractions of HAs and FAs from the same soil have different origins (plant or microbial).

1.5.2. Isolation and Fractionation of Soil Saccharides

Procedures for the isolation of polysaccharides from soil and their fractionation have been reviewed by Mehta et al. (1961), Swincer et al. (1968), Greenland and Oades (1975), Hayes and Swift (1978), Cheshire (1979), Cheshire and Hayes (1990), Stevenson (1994), and Clapp et al. (2005). Sodium hydroxide is probably the best of the aqueous solvents used. Its efficiency might well be related to charges, arising predominantly from uronic acids. These are characteristic of many soil polysaccharides, though sulfonated polysaccharides (typically with SO_3H functionalities on C-6 of the hexose sugars) may also contribute to charge. Some of the dipolar aprotic solvents, especially DMSO, can be expected to be good solvents for soil saccharides. DMSO has the ability to break hydrogen bonds and can even dissolve cellulose. Häusler and Hayes (1996) have shown that considerable amounts of saccharides were removed from the HA fraction from a sapric histosol when that fraction was dissolved in DMSO/HCl and passed on to XAD-8 resin. The HA was sorbed by the resin, and saccharides were washed through. Thus the use of DMSO/HCl (or DMSO/H_2SO_4) and of XAD-8 and XAD-4 resins in tandem may well provide a useful method for the isolation of soil saccharides. Saccharides would be retained by the XAD-4 resin.

The bulk of the saccharides in soil aqueous extracts are contained in the FA fraction, or the organic fraction that remains in solution on acidification of soil extracts. The introduction of XAD resin technology (see Section 1.3.2) for the fractionation of soil organic extracts has allowed some separation of saccharides from what are considered to be the true FAs. Swincer et al. (1968) deserve to be credited with that concept. They used Polyclar-AT [a (poly)vinylpyrrolidone resin used for clarifying wine] to remove color (HS) from soil saccharide extracts, but XAD-8 has been found to be superior for that purpose. Barker et al. (1965, 1967) and Hayes et al. (1975) used gel chromatography and ion-exchange chromatography techniques to remove color successfully from their saccharide extracts.

Attempts to fractionate soil polysaccharides into components that would satisfy the criteria of purity have failed. That is not surprising because a vast, inhomogeneous mixture of polysaccharides are likely to exist because of the countless microbial species in soil. It has been possible to isolate components that were relatively homogeneous with regard to charge, and possibly size, but generally these had four or more component sugars and were likely to be mixtures. Clapp (1957), Finch et al. (1967), Barker et al. (1967), Clapp and Davis (1970), and Clapp et al. (1979) achieved a considerable degree of physicochemical homogeneity in their isolates, but all contained four or more sugars and must be regarded as mixtures (see Clapp et al., 2005). Hayes et al. (1975) isolated a polysaccharide material from a histosol that contained about 70% glucose, but it had six other sugars in concentrations ranging from 2% to 9% and hence must be considered to be impure.

1.5.3. Polysaccharides and Soil Aggregates

Pagliai et al. (1979) have shown that the stabilities of aggregates incorporating β-glycosidic-linked (poly)glucose (which gives a linear helical structure) are proportional to the molecular weights (MWs) of the polymers. Also, the stabilities could be related to the viscosities and the extents of adsorption. These authors have also shown that the relationship between MW and biodegradation is not straightforward. The first polymer segments to contact the clays would orientate flat on the sorbent. As the surface is covered, loops would extend away from the surface (Burchill et al., 1981), and these loops would not be protected from microbial attack if microorganisms or the enzymes they secrete gain access to the loops.

To stabilize preformed aggregates, the polysaccharide would need to diffuse to the adsorption site in the internal surfaces. Cellulose [β-(1→4) linked (poly)glucose] is not an effective binding agent when added to soil because the strong hydrogen bonds between the β-linked strands make the polymer insoluble in water. The polymer would dissolve, should these bonds be broken, and could then be expected to be a good aggregate former and stabilizer. Page (1980) demonstrated that cellulose xanthate (in which the –OH group on C-6 is replaced by $-OCS_2$), a water-soluble cellulosic polymer, is an excellent stabilizer of soil aggregates. Harrison (1982) showed that when the xanthate was introduced to clay media, CS_2 was given off and the cellulose remained sorbed to the clay.

Clapp and Emerson (1972) used a series of homo- and hetero-polysaccharides of varying intrinsic viscosities and charges (containing uronic acids) in studies of binding and the stabilization of aggregates of Ca^{2+}-montmorillonite preparations [see also Clapp et al. (1991)]. Sorption of the neutral polysaccharides increased with their intrinsic viscosity (η) values. When Na^+-exchange was used to disperse the aggregates, samples that adsorbed high levels of polysaccharide were dispersed only after prolonged treatments with 0.05 M periodate. Anionic polysaccharides adsorbed least, and it was clear that stabilization by the polyanions involved complexation with the polyvalent metals neutralizing the charges on the clay surfaces. Polysaccharide mixtures extracted from soils adsorbed to varying extents, yet failed to stabilize the aggregates. That was ascribed to their lower (than required) η and MW values and hence their inabilities to bridge between clay particles.

Finch et al. (1967) calculated that the plateau adsorption by Georgia Na^+-exchanged kaolinite of a soil polysaccharide corresponded to a surface coverage of $80\,m^2\,g^{-1}$. Surface area measurements by standard techniques gave a value of $96\,m^2\,g^{-1}$ for the clay, and this would suggest contamination by a clay of higher surface area (montmorillonite). Periodate oxidation of the adsorbed polysaccharide was greatly retarded, suggesting that the adsorbed material was held close to the surface. Less protection would be afforded to loops extending away from the surface. The adsorption data are in line with those of Moavad et al. (1974) for their Na^+- and K^+-kaolinites. Coordination provides one explanation for the vast increases in adsorption, especially by the Fe-exchanged clay, but the extents to which (hydr)oxides were involved in the adsorption mechanisms is not known.

Aggregate formation may be visualized as the interaction of mobile clay particles with mucigel from plant roots and extracellular polysaccharides from bacteria [see Oades (1990)]. Many plants during growth appear to give rise to a breakdown of microaggregates in the rhizosphere. This could result from a priming action of the indigenous organic matter.

It is inevitable that polysaccharides are important for the formation of microaggregates, but there is enough evidence to indicate that fungal hyphae and plant rootlets are important for the stabilization of macroaggregates. Interest is being focused on the symbiotic relationship which arbuscular mycorrhizal fungi have with plants and on the nature of the secretions by such fungi (Rillig et al., 2001). Peptidoglycan, mucopolysaccharide, and such secretions by these and other fungi will have close contact with the soil inorganic colloids and can be expected to aid in the formation and stabilization of soil aggregates.

Hayes and Swift (1978), Theng (1979), and Clapp et al. (1991, 2005) have dealt in depth with the adsorption of neutral and charged polysaccharides by clays.

1.5.4. General Conclusions from Studies of Soil Saccharides

The fact that polysaccharides are major aggregating agents in soils is well established, and there is a reasonable awareness of the compositions and aspects of the structures of the kinds of polysaccharides that can give rise to aggregate formation and stabilization. However, there is only a limited awareness of the compositions and the structures of the indigenous soil polysaccharides that promote the formation of soil aggregates. The study by Finch et al. (1967) has shown ways by which polysaccharides that interact with clays can be isolated. However, there must be the will to persist in extended studies, such as those that engaged Cheshire and his colleagues at the Macaulay Institute, if this important area of SOM studies is to be advanced. There was considerable emphasis on soil polysaccharides during the period between the late 1940s and the mid-1980s, but interest has not been maintained. These studies were carried out in an era when the instrumentation needed for rapid advances was limited.

Soil polysaccharides can amount to as much as 20% of the humic fractions isolated in aqueous media, and identification of the classes of components in humin materials in associations with the soil clays indicates that carbohydrates contribute significantly to those isolated in the DMSO/H_2SO_4 medium (see Sections 1.4.7 and

1.7). Qualitative and quantitative identification of the sugars in the hydrolysates of these humin isolates may give indications about their origins (plant or microbial), and identification of the configurations of the sugar linkages could lead to deductions about the sorption mechanisms. To achieve the latter objective will require isolation of the polysaccharide and/or mucopolysaccharide components. That will not be an easy task, but it is doable.

1.6. SOIL PEPTIDES

Amino acids, amino groups, amino sugars, and nucleic acid derivatives usually account for >95% of the organic N in soils (Anderson et al., 1989), and many other N-containing compounds have been reported in trace amounts (Stevenson, 1994). Anderson et al. (1989) have found traces of L-phosphatidic acid, choline, ethanolamine, and uric acid (the end product of N metabolism of many animals), which can be oxidized to allantoin, cyanuric acid, and urea.

It is inevitable that there will be considerable amounts of peptide and amino acids in soils at any time because of the inputs from plant roots or from plant materials that are directly added to the soil or that enter the soil as senescent matter from vegetative cover (introduced by organisms such as earthworms). Additions can also arise from the microfauna, composed of bacteria, fungi, and viruses, all of which can number in the region of 1×10^7 to $1 \times 10^{10}\,\mathrm{g}^{-1}$ of dry weight of soil (Burns, 1990), and estimates of living tissue microbial biomass range from 17 to $22\,\mathrm{g\,m}^{-2}$ of soil (Jenkinson and Ladd, 1981). Significant contributions also arise from protozoa, eelworms, earthworms, soil insects, burrowing animals, and so on. If not protected, protein and peptide materials in dead organisms are rapidly recycled by the soil biota. When conditions are right, some will interact with other organic species to give products such as melanoidins (referred to in Section 1.4.2). These can have a degree of resistance to microbial decomposition. Interactions with clays, as well as steric constraints provided by microaggregates, can also provide protection. McLaren (1954) and McLaren et al. (1958) were first to show how enzymes could interact with clays, and enter the interlamellar spaces. McLaren et al. (1975) also showed how the activity of enzymes is preserved in associations with humus.

Knicker (2000) has referred to her NMR evidence that shows that >80% of the organic N in soils is in peptide-like structures. Knicker et al. (2002) did detect a clear shoulder in the chemical shift region for pyrrole- or indole-heteroaromatic N (−145 to −220 ppm) in the ^{15}N-NMR spectrum of the deepest layer of a peat that was at least 10,000 years old. However, this peat could be considered to be at the beginning of the coalification stage, and there is abundant evidence for heterocyclic N in coal.

There are no accurate determinations of the overall contributions of peptide components to the compositions of HS and of SOM. The difficulties arise from the problems of separating these from other components of the SOM mixtures. Use of DMSO + 1% 12 M HCl and XAD-8 resin [(poly)methylmethacrylate] technology enabled Appelqvist et al. (1996) to decrease the amino acid content of a HA by 23%, and the decrease was uniform for the different amino acid groupings. This still left considerable amounts of peptide material in association with the HAs. Some may well have been in association with the humic matrix, and some may have been covalently linked to the humic core through, for example, the formation of Schiff base structures through interactions between free amino groups in argenine and lysine with carbaldehyde and keto groups in the humic core. Such amino functional groups can also form covalent linkages with carbon α to the keto group in the quinones in humic structures, as indicated by Hayes and Swift (1978).

The possibilities for the identification of forms of N, other than peptide N, in soil fractions have been greatly advanced by recent developments in solid-state NMR pulse sequences. Carbon directly bonded to N can now be observed through saturation-pulse induced dipolar exchange with recoupling (SPIDER), as described by Schmidt-Rohr and Mao (2002). That technique allows detection of N-substituents on aromatic nuclei. This has led to increasing evidence for organically immobilized N in SOM. For example, yields of lowland rice (*Oryza sativa* L.) have been shown to decrease by more than 35% during 20–30 years of double and triple cropping. The total soil N did not decrease. The SPIDER evidence suggested that the unavailable organic N was bonded to lignin residues that accumulate in the anaerobic conditions (Olk et al., 2000; Schmidt-Rohr and Mao, 2002).

The same amino acids are found in plants, microorganisms, and animals, and so it is difficult to assign origins to peptide materials on the basis of the amino acid compositions of hydrolysis digests. Beavis and Mott (1996, 1999) claimed that amino acid fingerprints could distinguish between different sources of amino acids. They studied the amino acid profiles of the Rothamsted Wilderness soils, of manured and unmanured soils from the Park Grass experiment, and from the manured and unmanured Broadbalk continuous wheat plots at the Rothamsted Experimental Station, Harpenden, England, and their data indicated that amino acid fingerprints could distinguish between grass and arable experiments, and the amino acid profile for the Wilderness wooded soil was distinguishable from those for the grassland and arable soils.

Hayes et al. (2008) identified the amino acids in a variety of fractions from grassland soils and their drainage waters and segregated these into acidic, basic, neutral hydrophilic, and neutral hydrophobic groups. They then calculated the ratios of the abundances of the different groups in the different fractions. By comparing the distributions of the ratios with those of the sugars (Section 1.5.1), it was possible to get some indications about the likely origins (plant or microbial) of the amino acids.

1.7. THE FUTURE FOR STUDIES OF ENVIRONMENTAL NATURAL ORGANIC MATTER

The advances that are being made this century in the studies of the compositions and of some aspects of the structures of components of the NOM of soils and of waters far outstrip those made in any previous relatively short period of time. This is largely attributable to the advances in instrumentation and in particular in NMR technology, as outlined in Chapter 15 of this book. NMR techniques such as dipolar dephasing (DD), chemical shift anisotropy (CSA), diffusion-ordered spectroscopy (DOSY), diffusion editing, and 2-D NMR techniques have given significant information about aspects of compositions, sizes, and associations of components of NOM materials. The uses of ultrahigh-resolution Fourier transform ion cyclotron mass spectrometry (FT–ICR MS), using electrospray ionization (ESI) is capable of resolving individual species in complex DOM mixtures with masses as high as

~1000 Da (Stenson et al., 2003). Because m/z values can be determined with very high resolution and precision, usually to the fifth decimal place, accurate m/z values can be calculated for each peak, allowing the determination of elemental formulae that can be assigned to within 1 ppm error (Sleighter and Hatcher, 2007). This technique will be especially applicable initially to DOM materials.

There is a good, but not detailed, awareness of aspects of the compositions of various components of NOM. Lignin would appear to be the source of the major components of transforming organic matter isolated from soils in aqueous basic media. There is clear evidence for lignin functionalities in the NMR spectra (O-aromatic and methoxyl substituents) of these. There is evidence also for significant amounts of carbohydrate and peptide materials, and in general there is less convincing evidence for contributions from aliphatic hydrocarbon moieties. The contributions of tannins to the humic components has not been widely appreciated, but information from dipolar dephasing (DD) and chemical shift anisotropy (CSA) NMR procedures has resolved the overlaps that occur in the 100- to 110-ppm region of the spectrum where tannins and anomeric carbon (from carbohydrates) resonances can overlap (see Section 1.4.7). As humification progresses, the definite evidence for lignin residues diminishes; and there are some indications of increasing inputs from microbial sources, especially to the carbohydrate and peptide components.

On the basis of data from DOSY and diffusion editing, we now know that the components soluble in aqueous base tend not to be macromolecular (Simpson et al., 2002), at least not to the extents considered previously. More detail needs to be known about the nature of the associations that give pseudo-macromolecular properties.

Humin (see Section 1.4.7) has been regarded as the most recalcitrant component of SOM. Until recently, it was considered to be an intractable mixture of materials that were outside the capabilities of methods used for the isolation and fractionation (without significant alteration) of components of SOM. The recent work by Simpson et al. (2007) and by Song et al. (2008) has shown that humin can indeed be isolated and, to some extent, fractionated. The exhaustive aqueous extraction processes used by these authors isolated classical HA and FA fractions, including those released by urea/NaOH systems, before humin components were isolated in DMSO + 6% concentrated H_2SO_4 [see also Hayes (2006b)]. Recent studies in the author's laboratory have shown that >90% of SOM can be solubilized using aqueous and nonaqueous media. Also, the residual materials in association with the soil inorganic colloids have been shown to be similar to the major component isolated in the DMSO/H_2SO_4 medium (Song et al., 2008).

Solid-state, liquid-state, and 2-D NMR have given good indications of the compositions of the humin materials, and these have been found to be rich in carbohydrate, protein/peptide, peptidoglycan, waxes, lipids, and aliphatic hydrocarbon components (see Chapter 15). The aliphatic hydrocarbon contributions from waxes, lipids, long-chain hydrocarbons, acids and esters, and cutins/cutans/suberins predominate. Cutins/cutans and suberins are relatively recalcitrant components of plants (Deshmukh et al., 2005) and are likely to be significant contributors to the aliphatic materials. The lignin-derived species in humin may be regarded as components trapped in the humin matrix. Humin materials are surprisingly low in aromatic components, and the major contributors to the aromaticity would seem to be from small amounts of lignin-derived residues, some tannin materials, and aromatic amino acids (Simpson et al., 2007; Song et al., 2008).

The humin components isolated in DMSO/H_2SO_4 media contain macromolecular components, such as peptides, peptidoglycans, and possibly mucopolysaccharides (Simpson et al., 2007), and these would appear to be strongly sorbed to the soil inorganic colloids. Such species can therefore be considered to have importance in soil particle interactions.

It can be said that we know in broad terms the aspects of the compositions and the origins of at least some of the components of SOM, but we lack detailed awareness of the ways in which the components are associated, how they interrelate and interact in providing degrees of resistance to decomposition, how they interact with the mineral colloids to provide the basis of soil structure, and how they interact with anthropogenic chemicals that enter the soil environment. There is much work to be done, and this work will still involve getting more complete information about compositions, aspects (though not necessarily details) of structure, and associations. That approach is more important than concerns about details of structures. It is likely that the compositions and awareness of structures of humin molecules will become known in the not-too-distant future because these are largely biological molecules. However, it will be highly challenging to resolve the structures of the biologically transformed molecules because of the difficulties faced in isolating materials of sufficient purity for structural studies, at least in the cases of soil HAs.

There is a need to resume studies of soil saccharides and peptides. These can compose as much as 30–40% (when account is taken of the compositions of humin materials). Much is known about how polysaccharides of known structures interact with soil colloids, but it has not been possible as yet to know in sufficient detail the structures of the polysaccharides that persist in the soil. Hence we do not know the mechanisms of their binding to soil mineral colloids. The same applies for the peptide materials, though it is clear that polysaccharides and peptides have important roles in soil structure formation and stabilization.

The need to place an urgent emphasis of NOM cannot be overemphasized. The study of NOM has never been given the priority emphasis that it deserves. Political and popular interest has been raised from time to time—as, for example, by (a) the dust bowls in the 1930s in the United States and (b) the realization in the 1970s that chlorinated aquatic humic substances can be carcinogenic. However, the level of interest that would lead to substantial support for NOM (and especially SOM) research has not been sustained. Essential lessons from the past are not heeded. It is known, for example, that the great civilisations in the plains of the Tigris and Euphrates did not perish or disband directly as the result of invasions by vandals. A thriving population relied on conservational agriculture in which the value of organic amendments was appreciated. When the irrigation systems were damaged and conservational emphasis was lost, the organic reserves became depleted and the population disbanded. Similar happenings are taking place throughout the world at this time.

There is now much emphasis on carbon sequestration, carbon sinks, and carbon "footprints," but there is only peripheral awareness of the vast and vital sink that the soil provides for carbon. Few seem to realize that, worldwide, there is more than three times the amount of carbon in SOM than there is in all living matter on the surface of the earth. Depletion of that reserve is more serious than is appreciated because vast amounts of carbon are released into the atmosphere as the result of soil mismanagement. In order to emphasize the vast reserves of carbon in Irish soils (where 90% of the arable soils are in grassland), this author has pointed out that the amount of organic matter in one hectare of local Limerick grassland soils is equivalent to the mass of 6000 Kerry Gaelic Football players (the current All-Ireland champions), each weighing ~85 kg. After 30 years of continuous nonconservational cultivation, the organic matter loss would be equivalent to the mass of ~2000 of these athletes. This loss must be regarded as fossil carbon, though it could be replaced over time should the land use be returned to grassland. The degradation of soil structure resulting from the biological oxidation of the organic matter is more serious than the release of the CO_2.

The current controversy surrounding the uses of food crops as source materials for fuel additives and substitutes, and the increasing demands for food for an increasing world population will inevitably focus interest on agriculture and eventually on soils. More pressure will be placed on soil resources, and it will be evident that soil degradation will have serious consequences for water quality. This should lead to an awareness of the essential role that NOM has on the quality of life and on the need to conserve SOM. It will also be realized that carbon lost from soils as the result of mismanagement is fossil carbon. Therefore, it is logical to expect that it will be seen that a comprehensive awareness is needed of the transformations that lead to the genesis and the losses of SOM, of the compositions and aspects of the structures of components of SOM, and of the mechanisms by which SOM influence vital reactions and interactions that take place in the soil.

Studies at the frontiers of NOM research in the present era require state-of-the-art instrumentation, and it will be very difficult for any one laboratory to have all the equipment needed to make comprehensive advances in certain aspects of NOM studies. Thus future frontiers research may involve a limited number of laboratories fully equipped with state-of-the-art equipment operated by experts. To obtain optimum advances and instrumentation, scientists might be encouraged to take their samples, prepared by state-of-the-art procedures, to Centres of Excellence equipped with the state-of-the-art equipment and operators.

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