

DEFINITION OF SOIL ORGANIC MATTER

By Jerzy Weber

Definition of humic substances

Agriculturist since ancient times have recognized significant benefits of soil organic matter (SOM) to crop productivity. These benefits have been the subject of controversy for centuries and some are still debated today.

Many of the benefits of SOM have been well documented scientifically, but some effects are so intimately associated with other soil factors that it is difficult to ascribe them uniquely to the organic matter. In fact, soil is a complex, multicomponent system of interacting materials, and the properties of soil result from the net effect of all these interactions.

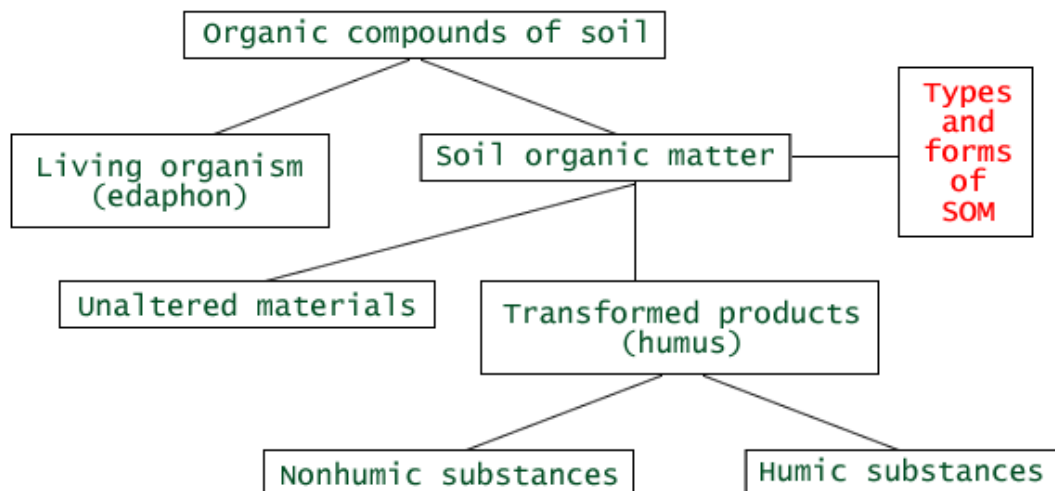
One of the major problems in communicating in the field of humic substances is the lack of precise definitions for unambiguously specifying the various fractions. Unfortunately, the terminology is not used in a consistent manner. Some soil scientists synonymously with soil organic matter that is to denote all organic material in the soil including humic substances use the term humus. Contemporary, the term **humus** is frequently used to represent only the humic substances.

The term **SOM** is generally used to represent the organic constituents in the soil, including undecayed plant and animal tissues, their partial decomposition products, and the soil biomass. Thus, this term includes:

1. Identifiable, high-molecular-weight organic materials such as polysaccharides and proteins,
2. Simpler substances such as sugars, amino acids, and other small molecules,
3. Humic substances.

It is likely that SOM contains most if not all of the organic compounds synthesized by living organisms.

SOM is frequently said to consist of **humic substances** and **nonhumic substances**. Nonhumic substances are all those materials that can be placed in one of the categories of discrete compounds such as sugars, amino acids, fats and so on. Humic substances are the other, unidentifiable components. Even this apparently simple distinction, however, is not as clear-cut as it might appear. Distribution SOM is shown on the picture:



Organic compounds of soil - live organisms and their undecomposed, partly decomposed and completely decomposed remains as well as products of their transformation.

Living organisms alive - edaphon.

Soil organic matter - non-living components which are a heterogeneous mixture composed largely of products resulting from microbial and chemical transformations of organic debris. Soil organic matter can exist in different morphological patterns, which are the bases of the classification of so called forms and types of humus.

Unaltered materials - fresh and non-transformed components of older debris.

Transformed products - (humus) - bearing no morphological resemblance to the structures from which they were derived. These transformed components are referred to as the humification process products.

Humic substances- a series of relatively high molecular weight, brown to black colored substances formed by secondary synthesis reactions. The term is used as a generic name to describe tocolored material or its fractions obtained on the basis of solubility characteristics:

- Humic acids (HA)
- Fulvic acids (FA)
- Humins

Nonhumic substances- compounds belonging to known classes of biochemistry, such as:

- Carbohydrates
- Lipids
- Amino acids

The chemical and colloidal properties of SOM can be studied only in the free state, that is, when freed of inorganic soil components. Thus the first task of the researcher is to separate organic matter from the inorganic matrix of sand, silt, and clay. Methods for the extraction of soil organic matter have evolved from the research and thinking of many scientists.

Function of organic matter in soil

Organic matter contributes to plant growth through its effect on the physical, chemical, and biological properties of the soil. It has a:

- Nutritional function in that it serves as a source of N, P for plant growth
- Biological function in that it profoundly affects the activities of micro flora and micro faunal organisms
- Physical and physico-chemical function in that it promotes good soil structure, thereby improving tilt, aeration and retention of moisture and increasing buffering and exchange capacity of soils.

Humus also plays an indirect role in soil through its effect on the uptake of micronutrients by plants and the performance of herbicides and other agricultural chemicals. It should be emphasized that

the importance of any given factor will vary from one soil to another and will depend upon such environmental conditions as climate and cropping history.

Availability of nutrients for plant growth.

Organic matter has both a direct and indirect effect on the availability of nutrients for plant growth. In addition to serving as a source of N, P, S through its mineralization by soil microorganisms, organic matter influences the supply of nutrients from other sources (for example, organic matter is required as an energy source for N-fixing bacteria). A factor that needs to be taken into consideration in evaluating humus as a source of nutrient is the cropping history. When soils are first placed under cultivation, the humus content generally declines over a period of 10 to 30 years until a new equilibrium level is attained. At equilibrium, any nutrients liberated by microbial activity must be compensated for by incorporation of equal amounts into newly formed humus.

Effect on soil physical condition, soil erosion and soil buffering and exchange capacity

Humus has a profound effect on the structure of many soils. The deterioration of structure that accompanies intensive tillage is usually less severe in soils adequately supplied with humus. When humus is lost, soils tend to become hard, compact and cloddy. Aeration, water-holding capacity and permeability are all favorably affected by humus. The frequent addition of easily decomposable organic residues leads to the synthesis of complex organic compounds that bind soil particles into structural units called aggregates. These aggregates help to maintain a loose, open, granular condition. Water is better able to infiltrate and percolate downward through the soil. The roots of plants need a continual supply of O₂ in order to respire and grow. Large pores permit better exchange of gases between soil and atmosphere.

Humus usually increases the ability of the soil to resist erosion. First, it enables the soil to hold more water. Even more important is its effect in promoting soil granulation and thus maintaining large pores through which water can enter and percolate downward. From 20 to 70% of the exchange capacity of many soils is caused by colloidal humic substances. Total acidities of isolated fractions of humus range from 300 to 1400 meq/100g. As far as buffer action is concerned; humus exhibits buffering over a wide pH range.

Effect on soil biological condition

Organic matter serves as a source of energy for both macro- and micro faunal organisms. Numbers of bacteria, actinomycetes and fungi in the soil are related in a general way to humus content. Earthworms and other faunal organisms are strongly affected by the quantity of plant residue material returned to the soil.

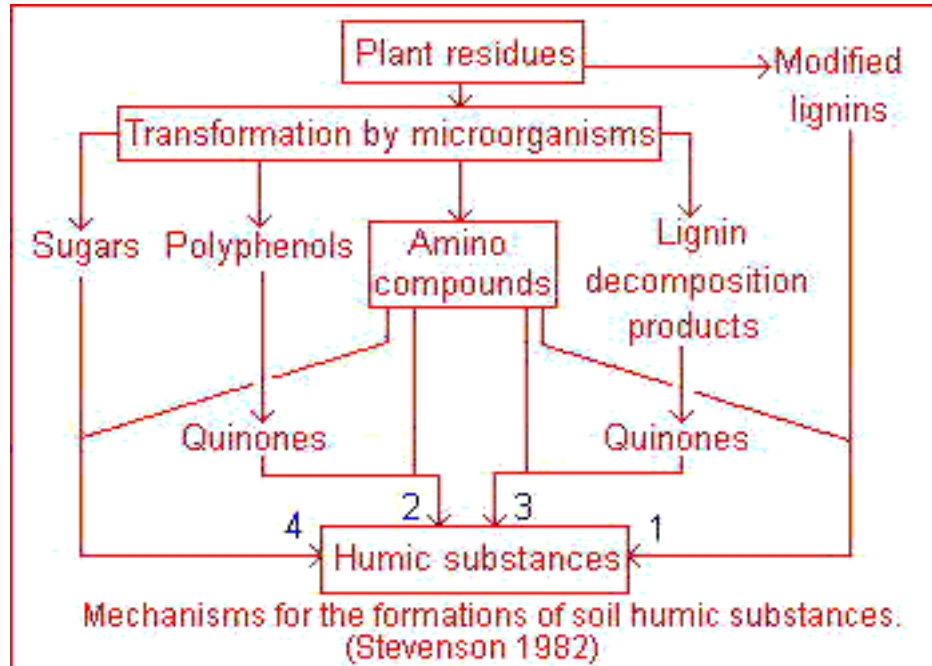
Organic substances in soil can have a direct physiological effect on plant growth. Some compounds, such as certain phenolic acids, have phytotoxic properties; others, such as the auxins, enhance plant growth.

It is widely known that many of the factors influencing the incidence of pathogenic organisms in soil are directly or indirectly influenced by organic matter. For example, a plentiful supply of organic matter may favor the growth of saprophytic organisms relative to parasitic ones and thereby reduce populations of the latter. Biologically active compounds in soil, such as antibiotics and certain phenolic acids, may enhance the ability of certain plants to resist attack by pathogens.

Formation of humic substances

The formation of humic substances is one of the least understood aspects of humus chemistry and one of the most intriguing. Studies on this subject are of long-standing and continued research can be justified on theoretical and practical grounds. Several pathways exist for the formation of humic substances during the decay of plant and animal remains in soil, the main ones being shown in the picture: The classical theory, popularized by Waksman, is that humic substances represent modified lignin's (pathway 1) but the majority of present-day investigators favor a mechanism involving quinones (pathway 2 and 3). In practice all four pathways must be considered as likely mechanisms for the synthesis of humic and fulvic acids in nature, including sugar-amine condensation (pathway 4). These four pathways may operate in all

soils, but not to the same extent or in the same order of importance. A lignin pathway may predominate in poorly drained soils and wet sediments (swamps, etc.) whereas synthesis from polyphenols may be of considerable importance in certain forest soils. The frequent and sharp fluctuations in temperature, moisture and irradiation in terrestrial surface soils under a harsh continental.



Pathway 1 - The lignin theory

For many years it was thought that humic substances were derived from lignin (pathway 1). According to this theory, lignin is incompletely utilized by microorganism and the residuum becomes part of the soil humus. Modification in lignin include loss of methoxyl (OCH₃) groups with the generation of o-hydroxyphenols and oxidation of aliphatic side chains to form COOH groups. The modified material is subject to further unknown changes to yield first humic acids and then fulvic acids. This pathway, illustrated on the picture, is exemplified by Waksman's lignin-protein theory.

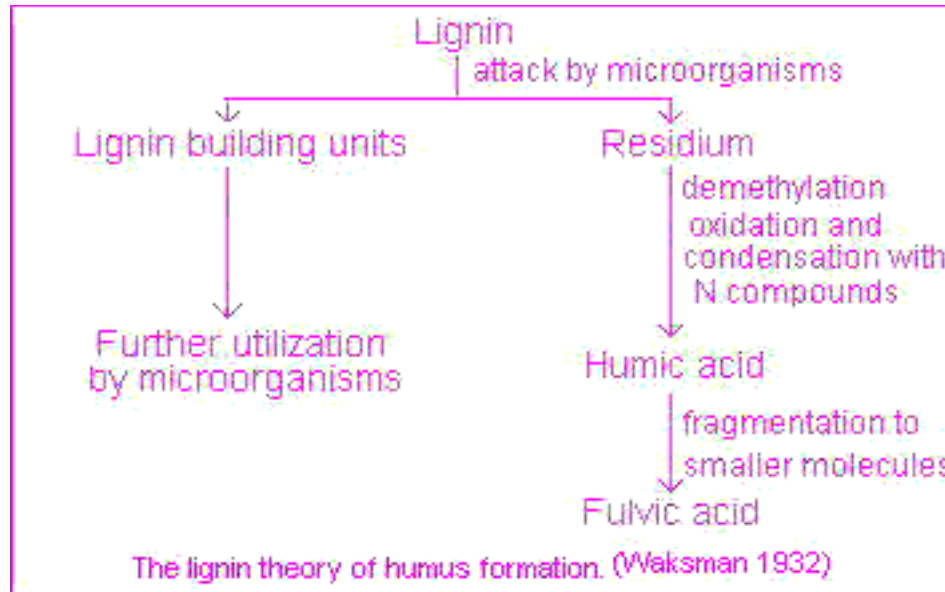
The following evidence was cited by Waksman in support of the lignin theory of humic acid formation:

- Both lignin and humic acid are decomposed with considerable difficulty by the great majority of fungi and bacteria.
- Both lignin and humic acid are partly soluble in alcohol and pyridine.
- Both lignin and humic acid are soluble in alkali and precipitated by acids.
- Both lignin and humic acid contain OCH₃ groups.
- Both lignin and humic acid are acidic in nature.
- When lignins are warmed with aqueous alkali, they are transformed into methoxyl-containing humic acids.
- Humic acids have properties similar to oxidized lignins.

Although lignin is less easily attacked by microorganisms than other plant components, mechanisms exist in nature for its complete aerobic decomposition. Otherwise undecomposed plant remains would accumulate on the soil surface and the organic matter content of the soil would gradually increase until CO₂ was depleted from the atmosphere. The ability of soil organisms to

degrade lignin has been underestimated in some quarters and its contribution to humus has been exaggerated.

In normally aerobic soils lignin may be broken down into low-molecular-weight products prior to humus synthesis. On the other hand, the fungi that degrade lignin are not normally found in excessively wet sediments. Accordingly, it seems logical to assume that modified lignins may make a major contribution to the humus of peat, lake sediments, and poorly drained soils.



Pathway 2 and 3 - The polyphenol theory

In pathway 3 lignin still plays an important role in humus synthesis, but in a different way. In this case phenolic aldehydes and acids released from lignin during microbiological attack undergo enzymatic conversion to quinones, which polymerize in the presence or absence of amino compounds to form humic like macromolecules.

Pathway 2 is somewhat similar to pathway 3 except that the polyphenols are synthesized by microorganisms from nonlignin C sources (e.g., cellulose). The polyphenols are then enzymatically oxidized to quinones and converted to humic substances. As noted earlier, the classical theory of Waksman is now considered obsolete by many investigators. According to current concepts quinones of lignin origin, together with those synthesized by microorganisms, are the major building blocks from which humic substances are formed.

The formation of brown-colored substances by reactions involving quinones is not rare event, but is a well-known phenomenon that takes place in melanine formation, such as in the flesh of ripe fruits and vegetables following mechanical injury and during seed coat formation.

Possible sources of phenols for humus synthesis include lignin, microorganisms, uncombined phenols in plants and tannins. Of these, only the first two have received serious attention.

Flaig's concept of humus formation is:

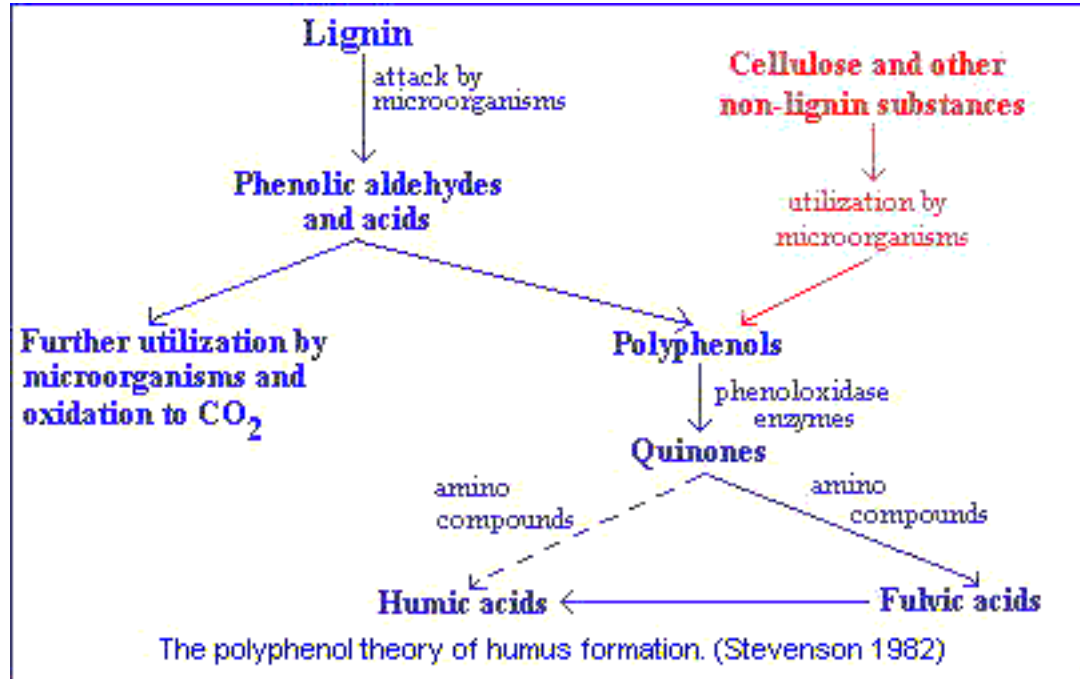
1. Lignin, freed of its linkage with cellulose during decomposition of plant residues, is subjected to oxidative splitting with the formation of primary structural units (derivatives of phenylpropane).

2. The side-chains of the lignin-building units are oxidized, demethylation occurs, and the resulting polyphenols are converted to quinones by polyphenoloxidase enzymes.
3. Quinones arising from the lignin (and from other sources) react with N-containing compounds to form dark-colored polymers.

The role of microorganisms as sources of polyphenols has been emphasized by Kononova. She concluded that humic substances were being formed by cellulose-decomposing myxobacteria prior to lignin decomposition.

The stages leading to the formation of humic substances were postulated to be:

1. Fungi attack simple carbohydrates and parts of the protein and cellulose in the medullary rays, cambium, and cortex of plants residues.
2. Cellulose of the xylem is decomposed by aerobic myxobacteria. Polyphenols synthesized by the myxobacteria are oxidized to quinones by polyphenoloxidase enzymes, and the quinones subsequently react with N compounds to form brown humic substances.
3. Lignin is decomposed. Phenols released during decay also serve as source materials for humus synthesis.



Pathway 4 - Sugar-amine condensation

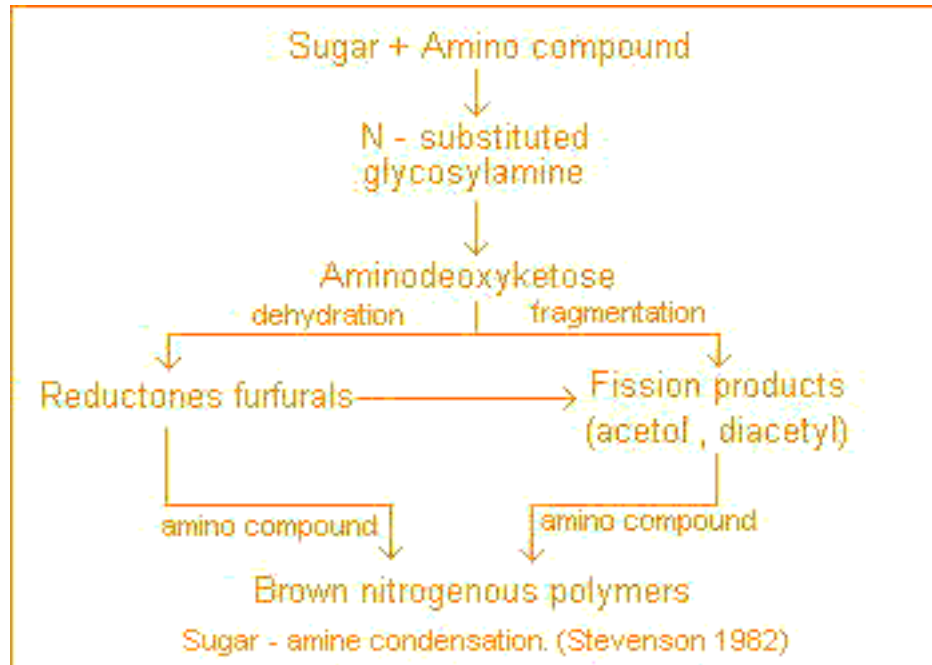
The notion that humus is formed from sugars (pathway 4) dates back to the early days of humus chemistry. According to this concept reducing sugars and amino acids, formed as by-products of microbial metabolism, undergo nonenzymatic polymerization to form brown nitrogenous polymers of the type produced during dehydration of certain food products at moderate temperatures.

A major objection to this theory is that the reaction proceeds rather slowly at the temperatures found under normal soil conditions. However, drastic and frequent changes in the soil environment (freezing and thawing, wetting and drying), together with the intermixing of reactants with mineral material having catalytic properties, may facilitate condensation. An attractive feature of the theory

is that the reactants (sugars, amino acids etc.) are produced in abundance through the activities of microorganisms.

The initial reaction in sugar-amine condensation involves addition of the amine to the aldehyde group of the sugar to form the n-substituted glycosylamine. The glycosylamine subsequently undergoes to form the N-substituted-1-amino-deoxy-2-ketose. This is subject to: fragmentation and formation of 3-carbon chain aldehydes and ketones, such as acetol, diacetyl etc.; dehydration and formation reductones and hydroxymethyl furfurals.

All of these compounds are highly reactive and readily polymerize in the presence of amino compounds to form brown-colored products.



Properties of humic substances

Humic acids - the fraction of humic substances that is not soluble in water under acidic conditions (pH < 2) but is soluble at higher pH values. They can be extracted from soil by various reagents and which is insoluble in dilute acid. Humic acids are the major extractable component of soil humic substances. They are dark brown to black in color.

Fulvic acids - the fraction of humic substances that is soluble in water under all pH conditions. They remain in solution after removal of humic acid by acidification. Fulvic acids are light yellow to yellow-brown in color.

Humins - the fraction of humic substances that is not soluble in water at any pH value and in alkali. Humins are black in color.

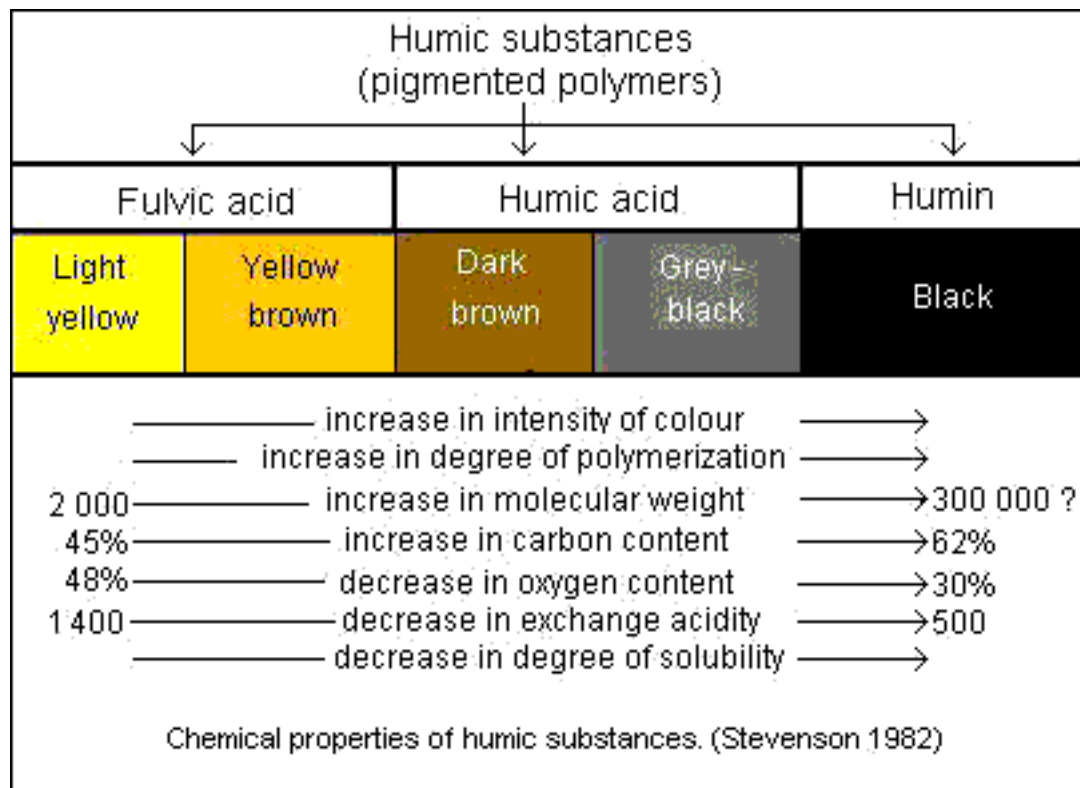
Many investigators now believe that all dark colored humic substances are part of a system of closely related, but not completely identical, high - molecular - weight polymers. According to this concept, differences between humic acids and fulvic acids, can be explained by variations in

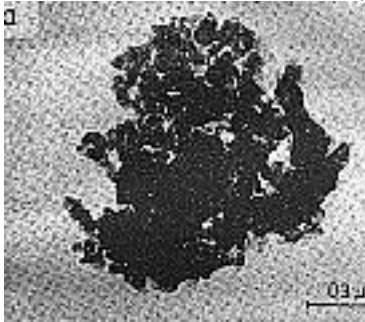
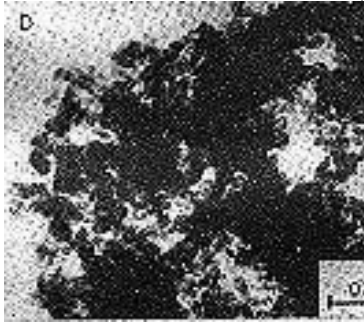
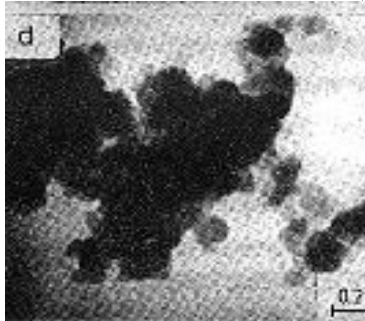
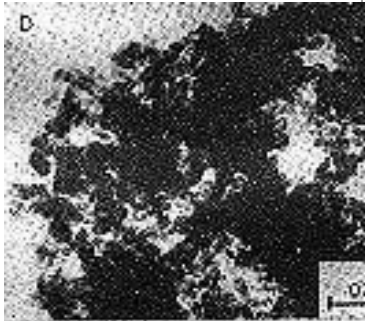
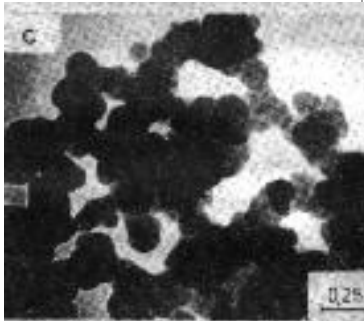
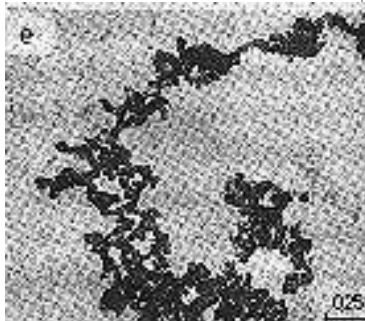
molecular weight, numbers of functional groups (carboxyl, phenolic OH) and extent of polymerization.

The postulated relationships are depicted in figure, in which it can be seen that carbon and oxygen contents, acidity and degree of polymerization all change systematically with increasing molecular weight. The low - molecular - weight fulvic acids have higher oxygen but lower carbon contents than the high - molecular - weight humic acids. Fulvic acids contain more functional groups of an acidic nature, particularly COOH. The total acidities of fulvic acids (900 - 1400 meq/100g) are considerably higher than for humic acids (400 - 870 meq/100g).

Another important difference is that while the oxygen in fulvic acids can be accounted for largely in known functional groups (COOH, OH, C=O), a high portion of the oxygen in humic acids seems to occur as a structural component of the nucleus.

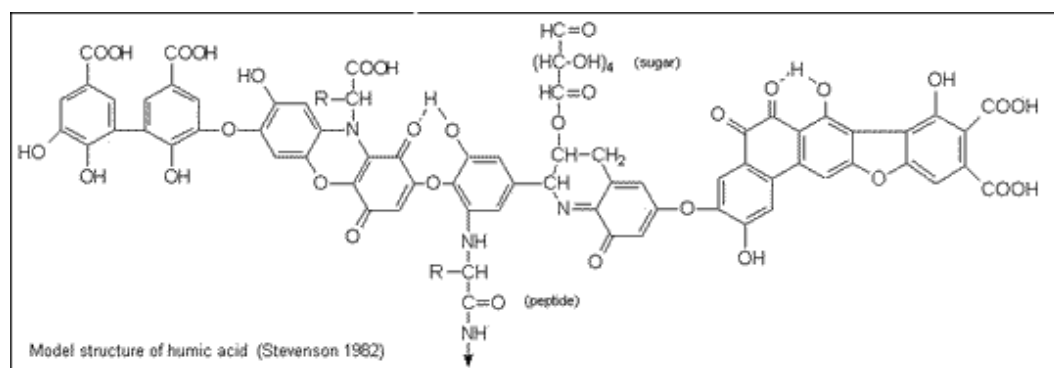
Electron microscope observations revealed the humic acids of different soils to have polymeric structure, appearing in form of rings, chains, and clusters. The sizes of their macromolecules can range from 60 - 500 A, what is mainly decided of by the occurring humification process, which also exerts an influence on their spatial structure. Compared to other taxonomic units, the polymers of podsol- earth soils showed to most loose structure.



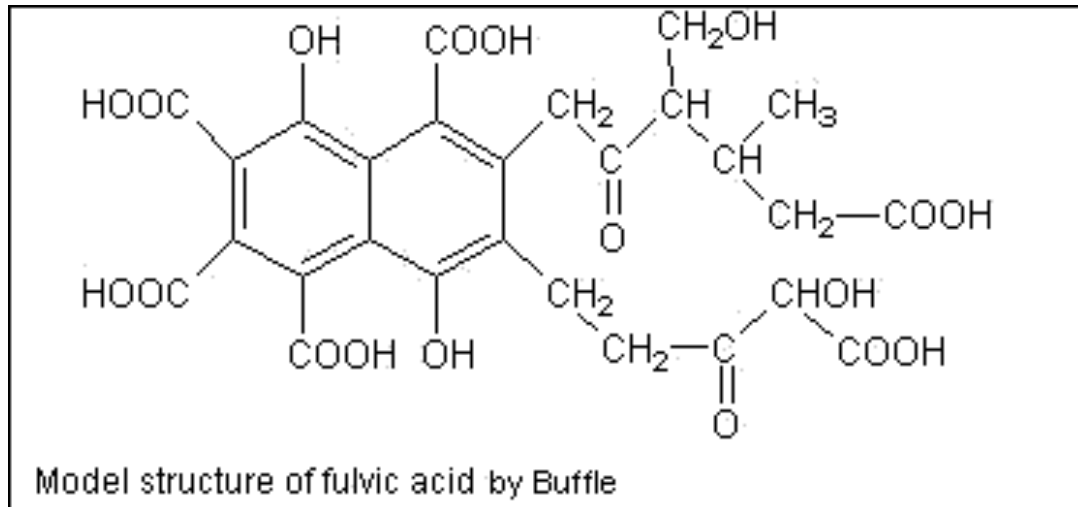
Electron microscope observations of humic acids (Drozd 1978)		
Humic acids of peaty sandy hydro earth soil	Humic acids of haplic phaeozems	Humic acids of podzolic soil
		
		

It is apparent that humic substances consist of a heterogeneous mixture of compounds for which no single structural formula will suffice.

Humic acids are thought to be complex aromatic macromolecules with amino acids, amino sugars, peptides, aliphatic compounds involved in linkages between the aromatic groups. The hypothetical structure for humic acid, shown in figure, contains free and bound phenolic OH groups, quinone structures, nitrogen and oxygen as bridge units and COOH groups variously placed on aromatic rings.



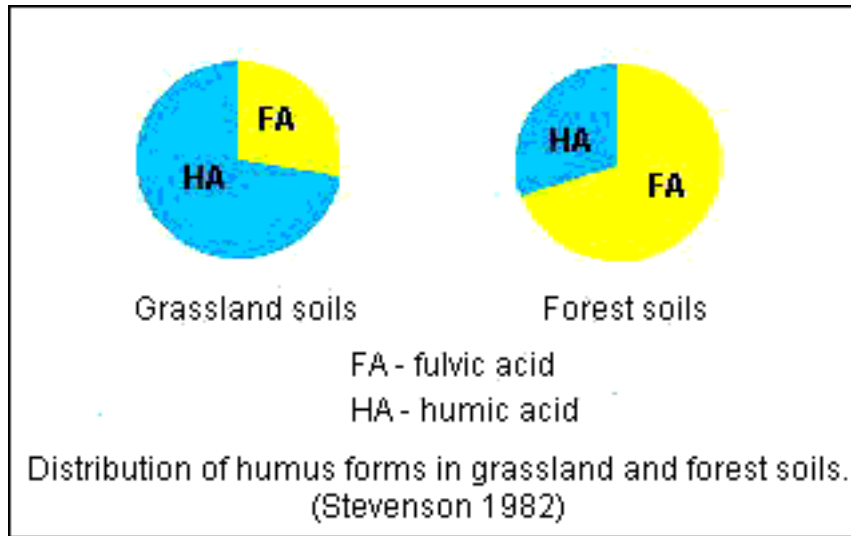
The hypothetical model structure of fulvic acid (Buffle's model) contains both aromatic and aliphatic structures, both extensively substituted with oxygen-containing functional groups.



Elemental composition of humic substances and several plant material (by Kononova)

Substances	% dry ash-free basis			
	C	H	O	N
Fulvic acids	44 - 49	3,5 - 5,0	44 - 49	2,0 - 4,0
Humic acids	52 - 62	3,0 - 5,5	30 - 33	3,5 - 5,0
Proteins	50 - 55	6,5 - 7,3	19 - 24	15,0 - 19,0
Lignin	62 - 69	5,0 - 6,5	26 - 33	-

The humic acid/fulvic acid ratio The percentage of the humus which occurs in the various humic fractions varies considerably from one soil type to another. The humus of forest soils is characterized by a high content of fulvic acids while the humus of peat and grassland soils is high in humic acids. (see figure) The humic acid/fulvic acid ratio usually, but not always, decreases with increasing depth.



Humic acid/fulvic acid ratios of some surface soils (by Kononova)			
Soil	Humic acid/ Fulvic acid ratio	Soil	Humic acid/ Fulvic acid ratio
Chernozem ordinary	2.0 - 2.5	Gray forest	1.0
Chernozem deep	1.7	Sod podzolic	0.8
Chestnut dark	1.5 - 1.7	Tundra	0.3

Properties of non-humic substances

Soil carbohydrates

Carbohydrates constitute 5 to 25% of the organic matter in most soils. Plant remains contribute carbohydrates in the form of simple sugars, hemicellulose, and cellulose, but these are more or less decomposed by bacteria, actinomycetes and fungi, which in turn synthesize polysaccharides and other carbohydrates of their own.

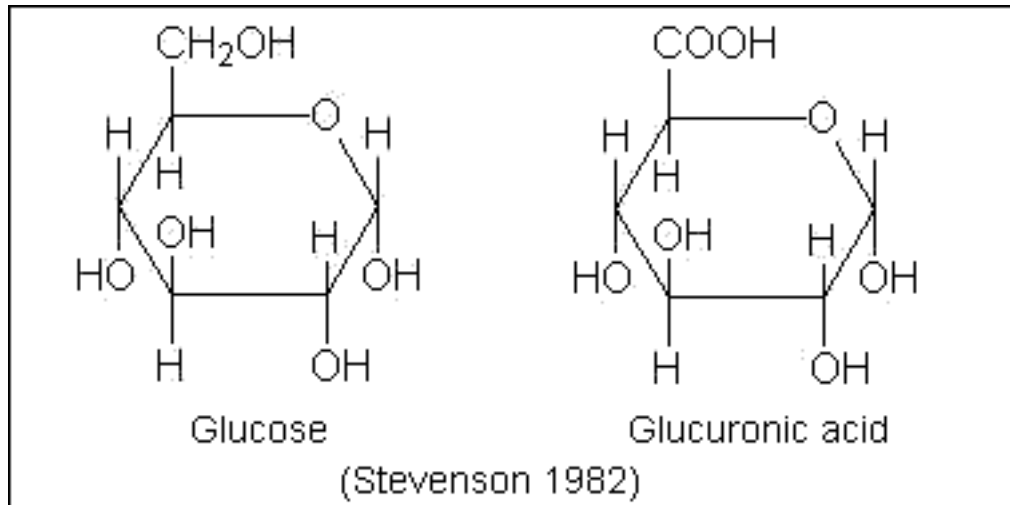
Significance of carbohydrates

The significance of carbohydrates in soil arises largely from the ability of complex polysaccharides to bind inorganic soil particles into stable aggregates. Carbohydrates also form complexes with metal ions, and they serve as building blocks for humus synthesis. Some sugars may stimulate seed germination and root elongation. Other soil properties affected by polysaccharides include cation exchange capacity (attributed to COOH groups of uronic acids), anion retention (occurrence of NH₂ groups), and biological activity (energy source for microorganisms).

The major groups of carbohydrates

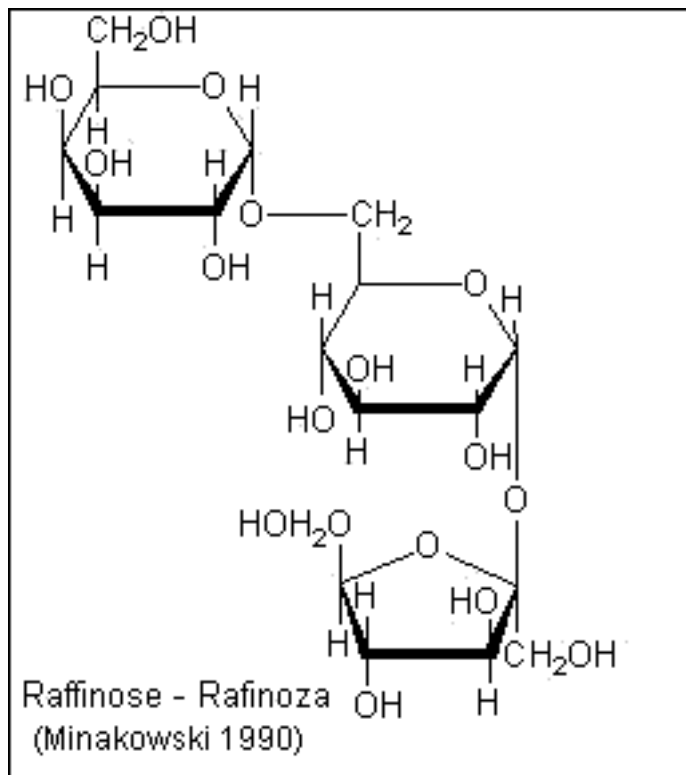
They can be divided into 3 subclasses:

1. **Monosaccharides**, which are aldehyde and ketone derivatives of the higher polyhydric alcohols.



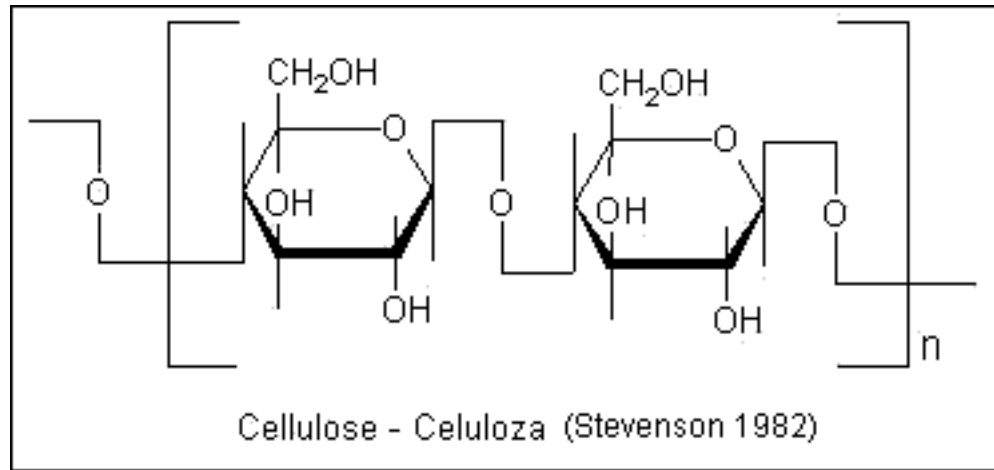
2.

3. **Oligosaccharides**, a large group of polymeric carbohydrates consisting of a relatively few monosaccharide units.



4.

5. **Polysaccharides**, contain many monomeric units (8 or more)



6.

The carbohydrates material in soil occurs as:

1. free sugars in the soil solution
2. complex polysaccharides
3. polymeric molecules of various sizes and shapes which are so strongly attached to clay and/or humic colloids.
- 4.

The contribution of individual sugar types to soil organic matter:	
Sugar	% of organic matter
Amino sugars	2-6
Uronic acids	1-5
Hexose sugars	4-12
Pentose sugars	<5
Cellulose	to 15
Others	trace

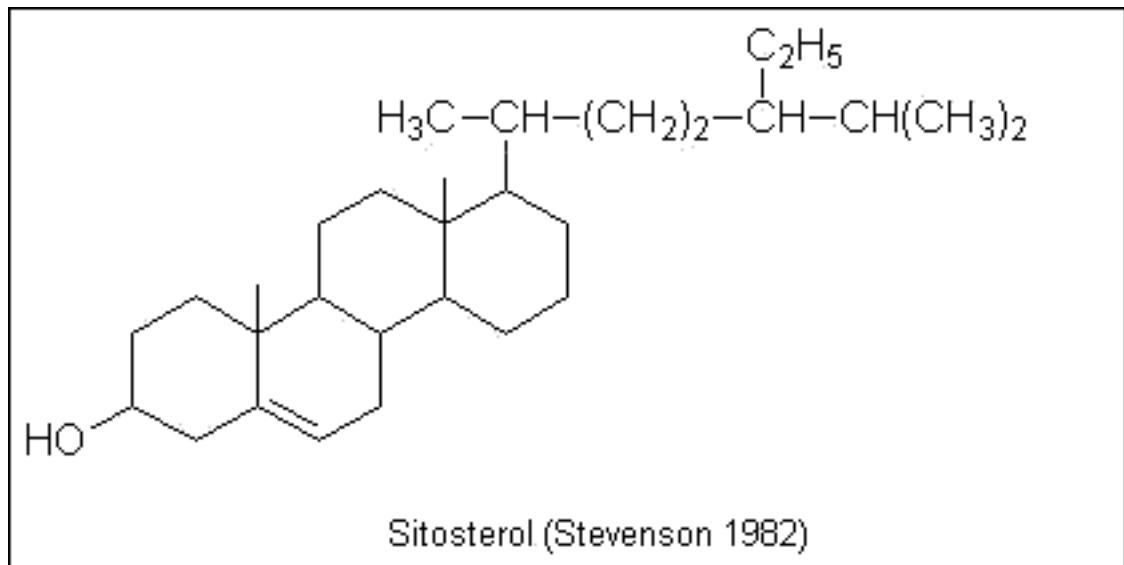
Soil lipids

The class of organic compounds designated as lipids represents a convenient analytical group rather than a specific type of compound.

They represent a diverse group of materials ranging from relatively simple compounds such as fatty acids to more complex substances such as the sterols, terpens, polynuclear hydrocarbons, chlorophyll, fats, waxes, and resins. The bulk of the soil lipids occurs as the so-called fats, waxes, resins.

In normal aerobic soil the lipids probably exist largely as remnants of plant and microbial tissues. From 2 to 6% of soil humus occurs as fats, waxes, resins.

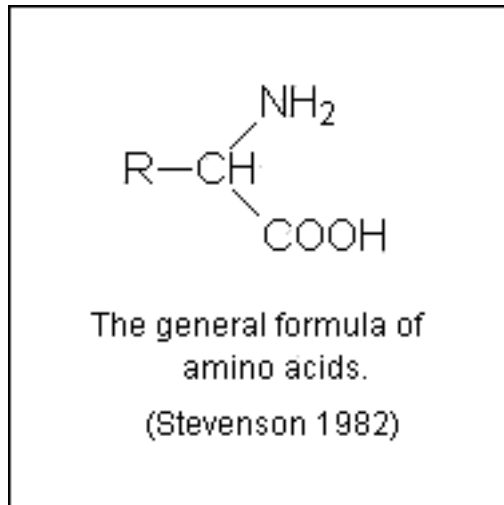
Lipids are physiologically active. Some compounds have a depressing effect on plant growth whereas others act as growth hormones. Waxes and similar materials may be responsible for the water repellent condition of certain sands.



Amino acids

Amino acids exist in soil in several different forms, like:

1. As free amino acids
 - in the soil solution
 - in soil micropores
2. As amino acids, peptides or proteins bound to clay minerals
 - on external surfaces
 - on internal surfaces
3. As amino acids, peptides or proteins bound to humic colloids
 - H-bonding and van der Waals' forces
 - in covalent linkage as quinoid-amino acid complexes
4. As mucoproteins
5. As a muramic acid



Amino acids, being readily decomposed by microorganisms, have only an ephemeral existence in soil. Thus the amounts present in the soil solution at any one time represent a balance between synthesis and destruction by microorganisms.

The free amino acids content of the soil is strongly influenced by weather conditions, moisture status of the soil, type of plant and stage of growth, additions of organic residues, and cultural conditions.

Humic substances complexes with mineral components

Connection of humic substances with mineral fraction

Extensive studies have shown that not much of the humic substances in soil is in free state but much is bound to colloidal clay.

The ways in which humic substances are combination with mineral portion of soil as follows:

1. As salts of low - molecular organic acids (acetate, oxalate, lactate and others).
2. As salts of humic substances with alkaline cations - humate, fulvate.
3. As chelate with metal ions.
4. As substances held on clay mineral surfaces.

Salts of low - molecular organic acids

Salts of low - molecular acids forms in result of action of acids (acetic acid, oxalic acid, fumaric acid, lactic acid) on minerals (magnesite, calcite, siderite and others) or salts of mineral acids with Ca, K and others cations.

Salts of humic substances with alkaline cations

Salts of humic substances with alkaline cations comprehensive of compounds:

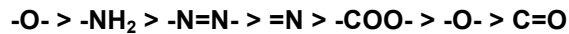
- humate (salts of humic acids)

- fulvate (salts of fulvic acids)

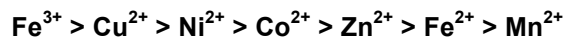
They are the most characteristic compounds of soil humic substances. The alkaline cations (Na^+ , K^+ , Ca^{2+} , Mg^{2+}) are held primarily by simple cation exchange with COOH groups (RCOONa , RCOOK etc.). The humate and fulvate occur in soil largely as mixture with hydroxide of Fe and Al.

Chelate with metal ions

A chelate complex is formed when two or more coordinate positions about the metal ion are occupied by donor groups of a single ligand to form an internal ring structure. In soil role of ligands fulfillment simple organic compounds and functional groups of humic substances. The order of decreasing affinity of organic groupings for metal ions is approximately as follows:



The order of decreasing ability of metal ions to chelating is as follows:



The complexing ability of humic and fulvic acids results largely from their content of oxygen-containing functional groups, such as COOH, phenolic OH and C=O group. Soil organic constituents form both soluble and insoluble complexes with metal ions and thereby play a dual role in soil. Low - molecular - weight compounds (biochemicals, fulvic acids) bring about the solubilization of metal ions and affect their transport to plant roots. In contrast, high - molecular - weight compounds (e.g. humic acids) function as a "sink" for polyvalent cations. Natural complexing agents are of considerable importance in weathering processes and in the movement of sesquioxides into the subsoil.

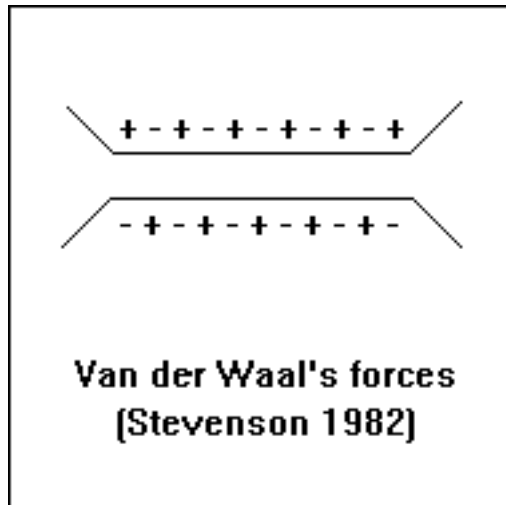
Clay - organic complexes

The interaction of organic substances with clay has a multitude of consequences that are reflected in the physical, chemical and biological properties of the soil matrix. Several mechanisms are involved in the adsorption of humic substances by clay minerals, the main ones being:

- van der Waals' forces
- bonding by cation bridging
- H - bonding
- adsorption by association with hydrous oxides
- adsorption on interlamellar spaces of clay minerals

Van der Waals' forces

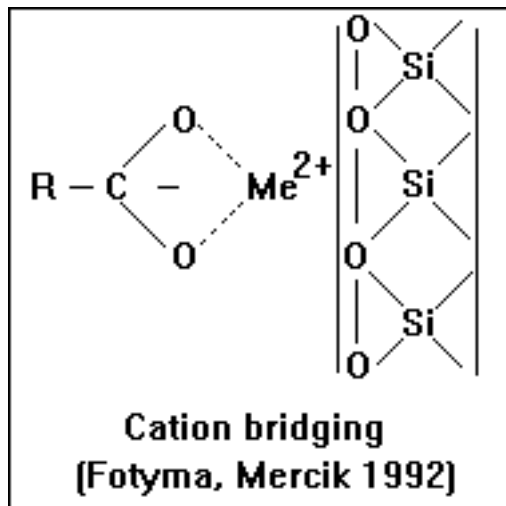
Van der Waals' forces operate between all molecules, but are rather weak. Essentially, these forces result from fluctuations in the electric charge density of individual atoms. An electrically positive fluctuation in one atom tends to produce an electrically negative fluctuation in a neighboring atom and a net attractive forces results. Attractive forces resulting from these fluctuations are every pair of atoms or molecules.



Adsorption caused by van der Waals' forces can be of considerable importance in the adsorption of neutral polar and nonpolar molecules, particularly those which are high in molecular weight.

Bonding by cation bridging

Since organic anions are normally repelled from negatively charged clay surfaces, adsorption of humic and fulvic acids by clay minerals such as montmorillonite occurs only when polyvalent cations are present on the exchange complex. Unlike Na^+ and K^+ , polyvalent cations are able to maintain neutrality at the surface by neutralizing both the charge on the clay and the acidic functional group of the organic matter (e.g. COO^-). The main polyvalent cations responsible for the binding of humic and fulvic acids to soil clays are Ca^{2+} , Fe^{3+} and Al^{3+} . The divalent Ca^{2+} ion doesn't form strong coordination complexes with organic molecules. In contrast Fe^{3+} and Al^{3+} form strong coordination complexes with organic compounds. The polyvalent cations act as a bridge between two charged sites.

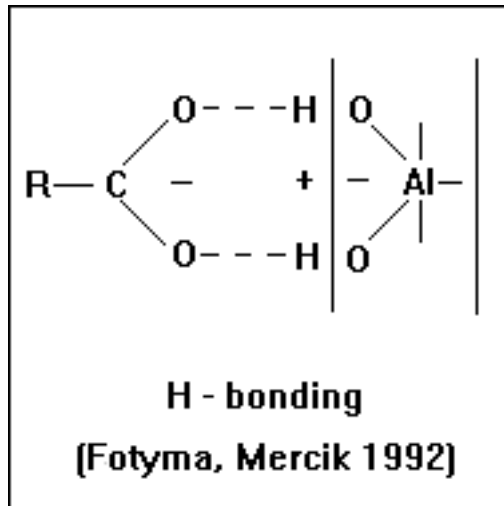


For a long chain organic molecule, several points of attachment to the clay particle are possible.

H – bonding

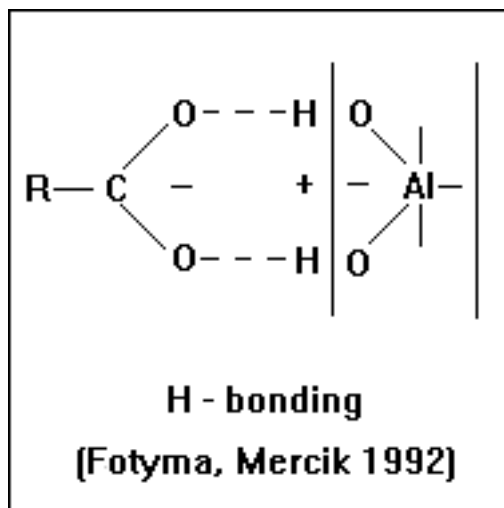
This is a linkage between polar groups of the organic molecule and adsorbed water molecules or oxygens of the silicate surface through bonding with a single H^+ ion. The strength of an individual

bond is small, but they are additive thus total adsorption energy can be appreciable. Rigorous drying, such as by desiccation at the soil surface or consumption of available moisture by plant roots, will tend to increase the bonding between humic material and clay by eliminating hydration water and bringing the humic matter in closer contact to the clay.



H – bonding

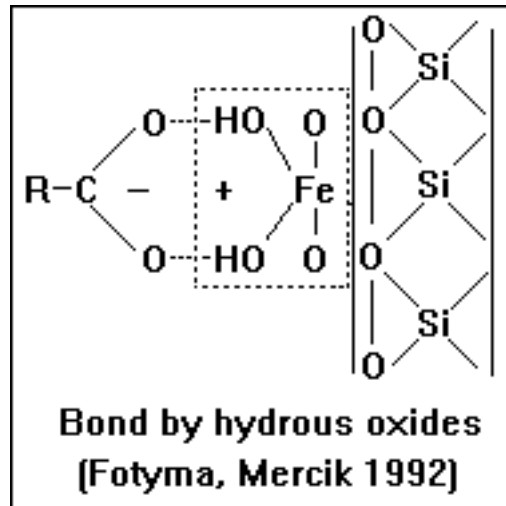
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Adsorption by association with hydrous oxides of Fe and Al.

For many soils, hydrous oxides are equal in importance to mica-type surfaces in sorbing humic substances. When clay minerals are coated with layers of hydrous oxides their surface reactions are dominated by these oxides rather than the clay. Organic anions can be associated with the

oxides by simple coulombic attraction. Anion associated with clay in this manner are readily removed by increasing the pH or by leaching with NaCl or NH₄Cl. The fact that very little humic material can be recovered from soils by these treatments suggest that most of the adsorbed organic matter is retained by supplementary mechanisms. Coordination or ligand exchange occurs when the anionic group penetrates the coordination shell of Al or Fe and becomes incorporated with the surface OH layer. The sorption of fulvic acid on oxide surfaces is accompanied by displacement of OH groups by COO⁻ ions. The organic anion is not easily displaced with simple salts, although adsorption is pH sensitive. As was the case with organic cations on clay mineral surfaces, a very strong bond will result if more than one group on the humic molecule participates.



Adsorption on interlamellar spaces of clay minerals

An important mechanisms for retention of proteins and charged organic cations by expandable-layer silicates is through adsorption on interlamellar spaces. Considerably controversy exists as to whether humic and fulvic acids are bound in this way in the natural soil. Evidence for interlamellar adsorption of fulvic acid by montmorillonite at pH < 5.5 has been given by Schnitzler and Kodama and Theng. The high - molecular - weight humic acids may be too large to penetrate interlamellar spaces.

Extraction of humic substances

Extraction of Soil Organic Matter The properties of soil organic matter, and especially humic substances, can be studied only in free state, that is, when freed of inorganic soil components. Therefore, the first task of the researcher is to separate organic matter from the inorganic matrix of sand, silt, and clay. A variety of techniques have been employed, depending upon the nature of the material to be examined. Thus nonpolar compounds like fats, waxes, resins, etc. can be extracted with such organic solvents as hexane, ether, alcohol-benzene mixtures, and others. Hydrolysis procedures have been used for isolating individual monomers, such as amino acids and sugars.

The ideal extraction method is one which meets the following objectives:

1. the method leads to the isolation of unaltered material
2. the extracted humic substances are free of inorganic contaminants, such as clay and polyvalent cations
3. extraction is complete, thereby insuring representation of fractions from the entire molecular-weight range

- the method is universally applicable to all soils

Reagents used for extraction of organic constituents from soil (Stevenson 1982)		
Type of material	Extractant	Organic matter extracted %
Humic substances	NaOH	to: 80%
	Mild extractants:	
	Na ₄ P ₂ O ₇ and other	to: 30%
	Organic chelates: acetylacetone, cupferron, hydroxyquinoline	to: 30%
	Formic acid (HCOOH)	to: 55%

Alkali extraction

NaOH solution of 0.1 to 0.5N concentration in water and a soil to extractant ratio of from 1:2 to 1:5 (g/ml) have been widely used for recovering organic matter. Repeated extraction is required to obtain maximum recovery. Leaching the soil with dilute HCl, which removes Ca and other polyvalent cations, increases the efficiency of extraction of organic matter with alkaline reagents. As a general rule, extraction of soil with 0.1 or 0.5N NaOH leads to the recovery of approximately two-thirds of the soil organic matter.

Undesirable features of alkali extraction are as follows:

- Alkali solutions dissolve silica from the mineral matter and this silica contaminates the organic fractions separated from the extract.
- Alkali solutions dissolve protoplasmic and structural components from fresh organic tissues and these become mixed with the humified organic matter.
- Under alkaline conditions, autoxidation of some organic constituents occurs in contact with air both during extraction and when the extracts are allowed to stand.
- Other chemical changes can occur in alkaline solution like condensation between amino acids and aldehydes or quinones.

The more alkaline the solution and the longer the extraction period the greater will be the chemical changes. The amount of organic matter extracted from soil with caustic alkali increases with time of extraction.

Mild extractants

Several milder and more selective extractants have been recommended in recent years as alternatives for the classical extraction with strong alkali. Included are salts of complexing agents (Na₄P₂O₇ and EDTA), organic complexing agents in aqueous media (acetylacetone), and organic solvents of various types. Whereas less alternation of organic matter may result, these extractants are much less effective than alkali hydroxides in removing organic matter. The main exception being the illuvial (B) horizon of the Spodosol. As was the case with alkali extraction, recovery of organic matter frequently can be increased by pretreating the soil with mineral acids to remove carbonates (HCl) or silicates (HCl-HF mixtures).

For certain investigations, a mild extractant is definitely preferred; for others, a more complete extraction is caustic alkali. At now, many investigators are using a sequence of extractants in which part of the organic matter is recovered by a mild reagent before alkali extraction.

Na₄P₂O₇ and other neutral salts

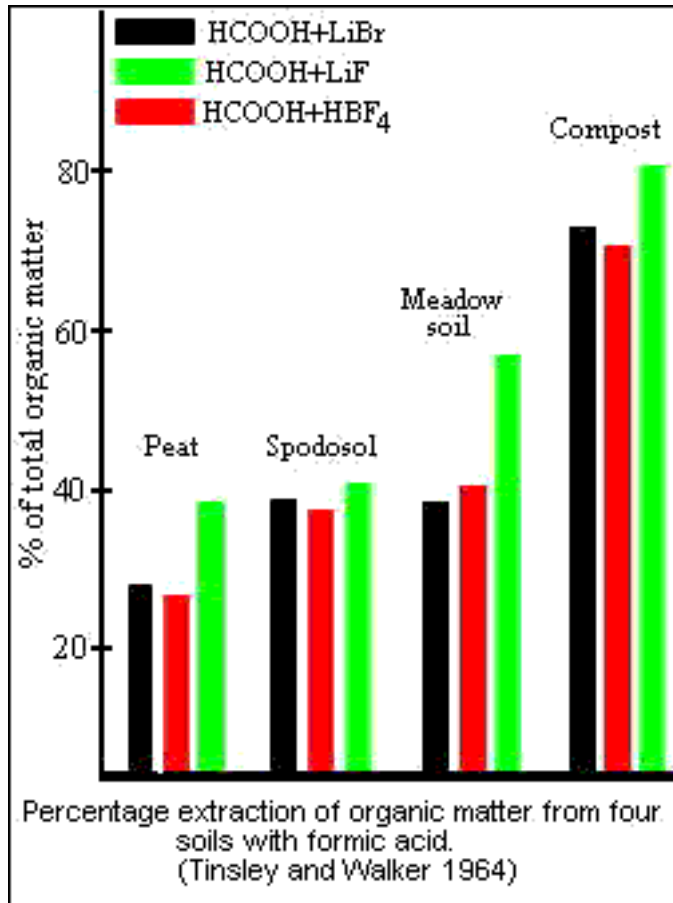
In many soils, Ca and other polyvalent cations (Fe, Al) are responsible for maintaining organic matter in a flocculated and insoluble condition. Accordingly, reagents that inactivate these cations by forming insoluble precipitates or soluble coordination complexes lead to solubilization of the organic matter. Such reagents as ammonium oxalate, sodium pyrophosphate and salts of weak organic acids have been used for this purpose. Of the various neutral reagents, Na₄P₂O₇ has been the most widely used. As note earlier, the amount of organic matter recovered (<30%) is considerably less than with caustic alkali, but less alteration occurs. To minimize chemical modification of the humic material, extraction should be carried out at pH 7.0.

Formic acid – HCOOH

Extensive research on the extraction of soil organic matter with formic acid shows that under certain circumstances up to 55% of the organic matter in mineral soils and as much as 80% of that in composts can be extracted with formic acid containing LiF, LiBr or HBF₄ (see picture).

Advantages of anhydrous formic acid for extraction of organic matter is that it is a polar compound that neither exhibits oxidizing nor hydrolytic properties. Furthermore, formic acid is a good solvent for a wide variety of compounds, including polysaccharides. Large quantities of Ca, Fe, Al and other inorganic components are dissolved from the soil along with the organic matter and thus far it has not been possible to remove the inorganic material completely.

Formic acid is most efficient with soils where much of the organic matter is only partially humified.



Organic chelating agents

Organic compounds such as acetylacetone, cupferron and hydroxyquinoline, which are capable of forming chelate complexes with polyvalent metal ions, have been used for extracting illuvial organic matter from Spodosols. The organic matter in the B-horizon of these soils occurs as complexes with Fe and Al and the complexing of these metals by chelating agents releases the organic matter to soluble forms. Organic chelating agents are rather ineffective for extracting organic matter from other soil types.

Outline of extraction procedures in IHSS method

Step 1. Equilibrate the sample to a pH value between 1-2 with 1 M HCl at room temperature. Adjust solution volume with 0.1 M HCl to provide a final concentration that has ratio of 10 mL liquid/1 g dry sample. Shake the suspension for 1 hour.

Step 2. Separate supernatant from the residue by decantation after allowing solution to settle (or by low speed centrifugation). Save supernatant for XAD-8 isolation.

Step 3. Neutralize the soil residue with 1 M NaOH to pH=7.0 then add 0.1 NaOH under an atmosphere of N₂ to give a final extractant to soil ratio of 10:1.

Step 4. Extract the suspension under N_2 with intermittent shaking for a minimum of 4 hours. Allow the alkaline suspension to settle overnight and collect the supernatant by means of decantation or centrifugation.

Step 5. Acidify the supernatant with 6 M HCl with constant stirring to pH=1.0 and then allow the suspension to stand for 12-16 hours.

Step 6. Centrifuge to separate the humic acid (precipitate) and fulvic acid (supernatant - FA Extract 2) fractions.

Step 7. Redissolve the humic acid fraction by adding a minimum volume of 0.1 M KOH under N_2 . Add solid KCl to attain 0.3 M (K^+) and then centrifuge at high speed to remove suspended solids.

Step 8. Reprecipitate the humic acid as in step 5. Centrifuge and discard supernatant.

Step 9. Suspend the humic acid precipitate in 0.1 M HCl/0.3 M HF solution in a plastic container. Shake overnight at room temperature.

Step 10. Centrifuge and repeat HCl/HF treatment(step 9), if necessary, until the ash content is below 1 percent.

Step 11. Transfer the precipitate to a Visking dialysis tube by slurring with water and dialyze against distilled water until the dialysis water gives a negative Cl^- test with the $AgNO_3$.

Step 12. Freeze-dry the humic acid.

Step 13. Pass the supernatant from step 2 through a column of XAD-8 (0.15 ml of resin per gram of initial sample dry weight at a flow rate of 15 bed volumes per hour). Discard the effluent, rinse the XAD-8 column containing sorbed fulvic acid with 0.65 column volumes of distilled water.

Step 14. Back elute the XAD-8 column with 1 column volume of 0.1 M NaOH, followed by 2-3 column volumes of distilled water.

Step 15. Immediately acidify with 6 M HCl to pH=1. Add concentrated HF to a final concentration of 0.3 M HF. Solution volume should be sufficient to maintain fulvic acid solubility.

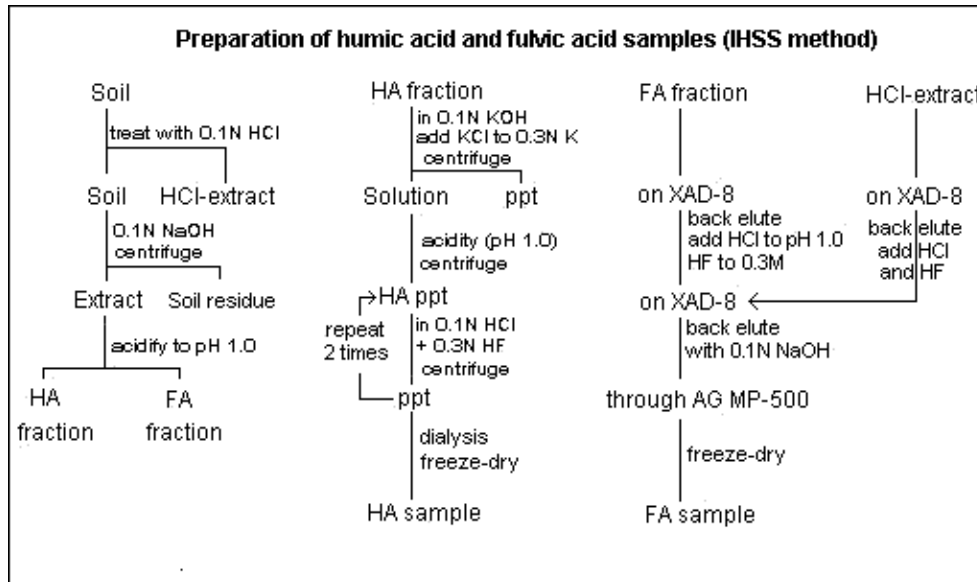
Step 16. Pass the supernatant from step 6 through a column of XAD-8 (1.0 mL of resin per gram of initial sample dry weight).

Step 17. Repeat steps 14 and 15

Step 18. Combine the final eluates from steps 15 and 17 and pass this solution through XAD-8 resin in glass column (column volume should be 1/5 of sample volume). Rinse with 0.65 column volumes of distilled water.

Step 19. Back elute with 1 column volume of 0.1 M NaOH followed by 2 column volumes of distilled water. Pass eluate through H^+ - saturated cation exchange resin (Bio-Rad AG-MP-5) using three times the mole of Na ions in solution).

Step 20. Freeze-dry the eluate to recover the H^+ - saturated fulvic acid.

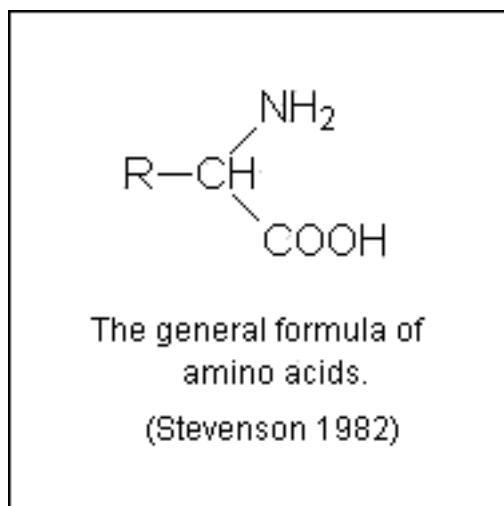


Organic forms of soil nitrogen

Organic form of soil nitrogen

Over 90% of the nitrogen N in the surface layer of most soils occurs in organic forms, with most of the remainder being present as NH_4^+ which is held within the lattice structures of clay minerals. The surface layer of most cultivated soils contains between 0.06 and 0.3% N. Peat soils have high N contents to 3.5%. Plant remains and other debris contribute nitrogen N in the form of:

Amino acids



Amino acids exist in soil in several different forms, like:

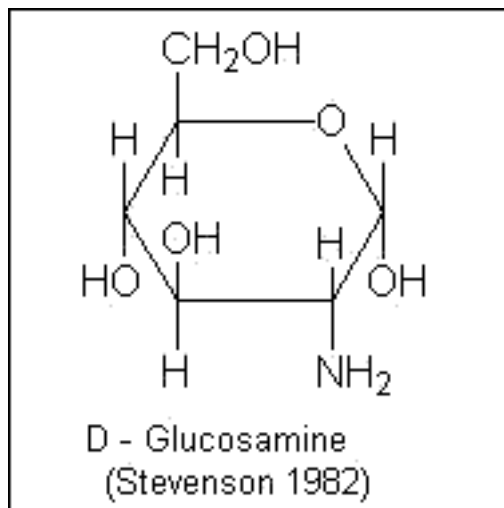
1. As free amino acids
 - in the soil solution

- in soil microspores
2. As amino acids, peptides or proteins bound to clay minerals
 - on external surfaces
 - on internal surfaces
 3. As amino acids, peptides or proteins bound to humic colloids
 - H-bonding and van der Waals' forces
 - in covalent linkage as quinoid-amino acid complexes
 4. As mucoproteins
 5. As a muramic acid

Amino acids, being readily decomposed by microorganisms, have only an ephemeral existence in soil. Thus the amounts present in the soil solution at any one time represent a balance between synthesis and destruction by microorganisms. The free amino acids content of the soil is strongly influenced by weather conditions, moisture status of the soil, type of plant and stage of growth, additions of organic residues, and cultural conditions.

Amino sugars

Amino sugars occur as structural components of a broad group of substances, the mucopolysaccharides and they have been found in combination with mucopeptides and mucoproteins. Some of the amino sugar material in soil may exist in the form of an alkali-insoluble polysaccharide referred to as chitin. Generally the amino sugars in soil are of microbial origin. From 5 to 10% of the N in the surface layer of most soils can be accounted for in N-containing carbohydrates or amino sugars.

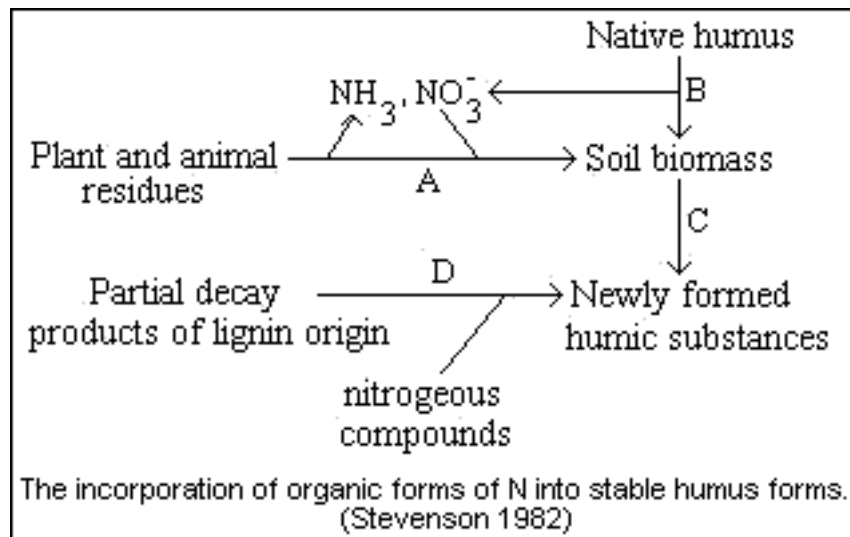


Nucleic Acids

Nucleic acids, which occur in the cells of all living organisms, consist of individual mononucleotide units (base-sugar-phosphate) joined by a phosphoric acid ester linkage through the sugar. Two types: ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). They have pentose sugar (ribose or deoxyribose), the purine: adenine, guanine and the pyrimidine: cytosine, thymine. RNA contains also the uracil. The N in purine and pyrimidine bases is usually considered to account for less than 1% of the total soil N. Small amounts of N are extracted from soil in the form of glycerophosphatides, amines, vitamins, pesticide and pesticide degradation products.

Nitrogen transformation

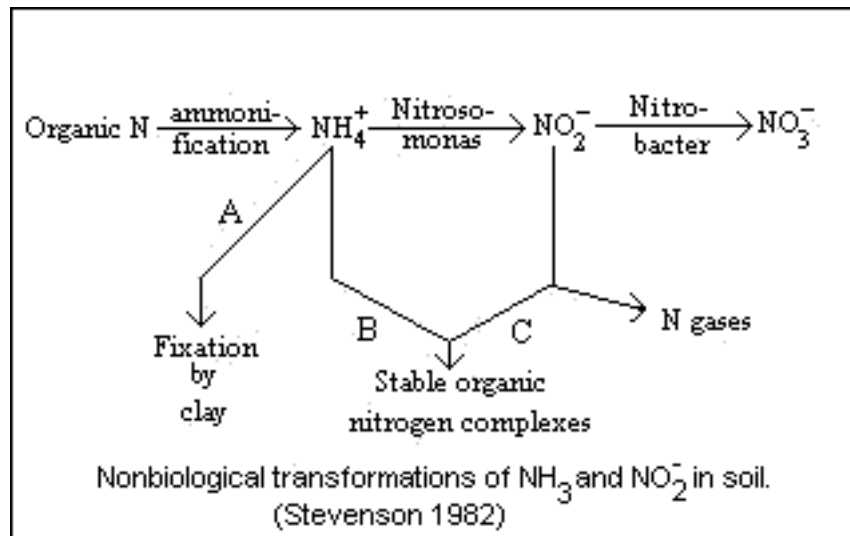
A key feature of the internal cycle is the biological turnover of N between mineral and organic forms through the opposing processes of mineralization and immobilization. The latter leads to incorporation of N into microbial tissues. Whereas much of this newly immobilized N is recycled through mineralization, some is converted to stable humus forms. The overall reaction leading to incorporation of inorganic forms of N into stable humus forms is depicted on the picture. Thus the decay of plant and animal residues by microorganisms results in the formation of mineral forms of N (NH_4^+ and NO_3^-) and assimilation of part of the C into microbial tissue (reaction A). Part of the native humus undergoes a similar fate (reaction B). Subsequent turnover through mineralization-immobilization leads to incorporation of N into stable humus forms (reaction C). Stabilization of N may also occur through the reaction of partial decay products of lignin with nitrogenous constituents (reaction D). Except under unusual circumstances, both mineralization and immobilization always function in soil, but in opposite direction.



Chemical reaction of ammonia and nitrite with organic matter

The fate of mineral forms of N in soil is determined to some extent by nonbiological reactions involving NH_4^+ , NH_3 and NO_2^- as depicted in fig.

In addition to NH_4^+ fixation by clay minerals (reaction A), NH_3 and NO_2^- react chemically with organic matter to form stable organic N complexes (reaction B and C). The chemical interaction of NO_2^- with organic matter may lead to the generation of N gases. Although both types of reactions can proceed over a wide pH range, fixation of NH_3 is favored by a high pH (>7.0). In contrast, NO_2^- -organic matter interactions occur most readily under highly acidic conditions (pH of 5.0 to 5.5 or below).



Stability of soil organic nitrogen

1. Proteinaceous constituents are stabilized through their reaction with other organic constituents, such as lignins, tanins, quinons.
2. Biologically resistant complexes are formed in soil by chemical reactions involving NH_3^+ or NO_2^- with lignins or humic substances.
3. Adsorption of organic N compounds by clay minerals (particularly montmorillinitic types) protects the molecule from decomposition.
4. Complexes formed between organic N compounds and polyvalent cations, such as Fe, are biologically stable.
5. Some of the organic N occurs in small pores or voids and is physically inaccessible to microorganisms.

C/N ratio

For surface soils, and for the top layer of lake and marine sediments, the ratio generally falls within well-defined limits, usually from about 10 to 12. In most soils, the C/N ratio decreases with increasing depth, often attaining values less than 5.0. Native humus would be expected to have a lower C/N ratio than most undecayed plant residues for following reasons. The decay of organic residues by soil organisms leads to incorporation of part of the C into microbial tissue with the remainder being liberated as CO_2 . As a general rule, about one-third of the applied C in fresh residues will remain in the soil after the first few months of decomposition. The decay process is accompanied by conversion of organic form of N to NH_3 and NO_3^- and soil microorganisms utilize part of this N for synthesis of new cells. The gradual transformation of plant raw material into stable organic matter (humus) leads to the establishment of reasonably consistent relationship between C and N. Other factors which may be involved in narrowing of the C/N ratio include chemical fixation of NH_3 or amines by lignin like substances.

The C/N ratio of virgin soils formed under grass vegetation is normally lower than for soils formed under forest vegetation, and for the latter, the C/N ratio of the humus layers is usually higher than for the mineral soil proper. Also the C/N ratio of a well-decomposed muck soil is lower than for a fibrous peat.

As a general rule it can be said that conditions which encourage decomposition of organic matter result in narrowing of the C/N ratio. The ratio nearly always narrows sharply with depth in the profile; for certain subsurface soils C/N ratios lower than 5 are not uncommon.

Organic forms of soil phosphorus

Organic phosphorus

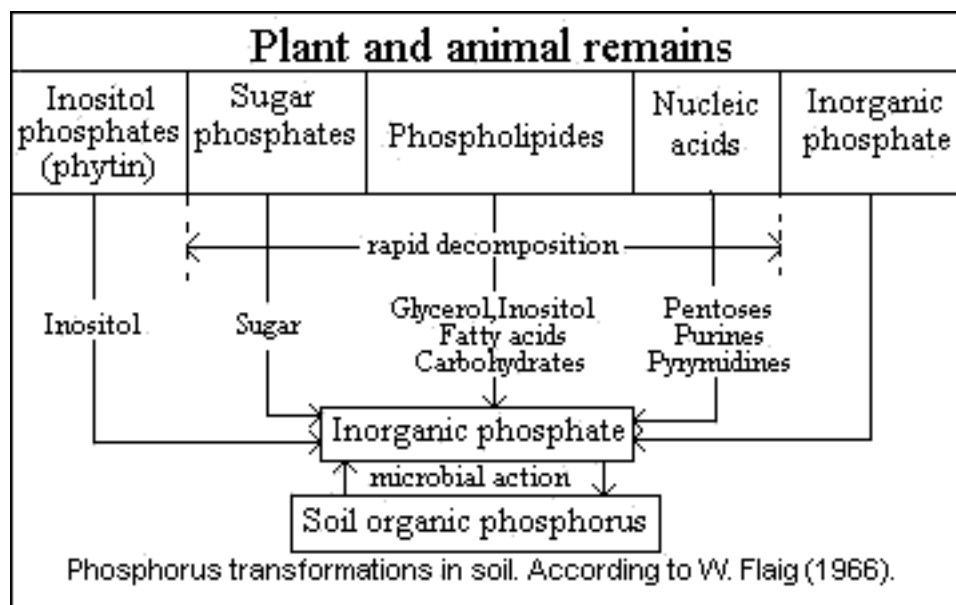
Phosphorus rank in importance with N and K as major plant nutrients. Phosphorus compounds in soil can be placed into the following three classes:

1. organic compound of the soil humus,
2. inorganic compounds in which the P is combined with Ca, Mg, Fe, Al and with clay minerals,
3. organic and inorganic P compounds associated with the cells of living matter. Microorganisms are involved in transformations of phosphorus between organic and mineral forms.

From 15 to 80% of the phosphorus in soils occurs in organic forms, the exact amount being dependent upon the nature of the soil and its composition. The higher percentages are typical of peats and uncultivated forest soils. From the standpoint of plant nutrition, phosphorus is adsorbed by plants largely as the negatively charged primary and secondary orthophosphate ions (H_2PO_4^- and HPO_4^{2-}), which are present in the soil solution. Small quantities of soluble organic P compounds are also present in water extracts of soil.

The phosphorus cycle

In a broad sense, the phosphorus cycle in soil involves the uptake of phosphorus by plants and its return to the soil in plant and animal residues.



As can be seen from picture three general types of compounds make up the bulk of the organic phosphorus in plants, namely: phytin, phospholipids, and nucleic acids. Approximate recoveries of organic phosphorus in these forms are as follows:

Inositol phosphates	2-50%
Phospholipids	1-5%
Nucleic acids	0.2-0.5%
Phosphoproteins	trace
Metabolic phosphates	trace

When crop residues are returned to the soil, net immobilization of P will occur when the C/organic-P ratio is 300 or more; net mineralization will result when the ratio is 200 or less.

Types of humus in soils

Types of humus in soils

Humus occurs in soils in many types, differentiates in regard to morphology and fractional composition. A type of humus is its morphological form of natural accumulation of humic substances in profile or on the surface of soil, conditioned by general direction of soil-forming process and humification of organic matter. A types of humus in terrestrial environment are following:

1. **mor**
2. **moder**
3. **mull**

Mor is a type of humus, which occurs largely in coniferous forest soils and the moorlands soils. This humus arises under conditions of low-biological activity in soil. The mineralization of organic matter proceed slowly and create layers, which maintain a structure of vegetable material. Acidophilic fungi and low active invertebrates participates in transformations of plant residues. Under these circumstances forms a litter of large thickness. C/N ratio of mor humus is always more than 20, or even 30-40, whereas pH is acid.

Moder is a transitional form of humus between mull and moder, characteristic for sod-podzolic soils, loesses and mountain grassland soils. The organic horizons with moder humus consist of low-thickened litter (2-3 cm), which gradually, without bounds, pass on to humus-accumulative horizons. Moder is a type of medium humified humus. Acidophilic fungi and arthropodan participates in transformations of plant residues. C/N ratio equal 15-25. Produced mineral-organic complexes are labile and weakly bounded with mineral portion of soil.

Mull is a type of humus characteristic for chestnut soils, phaeozems, rendzinas and others soils. This type of humus arise under grass vegetation. Mull is a well-humified organic matter, which is produce in very biologically active habitat. This type of humus is characterized by neutral pH, C/N ratio nearing to 10 and ability to creation stable mineral-organic complexes. Mull is a type of humus which occurs in soils under cultivation.

According to Kononova, the types of humus are divide as follows:

First type of humus is characteristic for podzolic soils, grey brown soils and lateric soils under forest communities. In this humus predominate humic acids, thus humic acid/fulvic acid ratio is below 1. Humic acid indicate small extent of aromatic rings condensation and they are approximate to fulvic acids. Considerable hydrophilic properties of humic acids favor to creation of chelates with polyvalent cations and ability to displacement deep into profile of soil. Considerable mobility of this humus favor process of podsolization.

Second type of humus is characteristic for phaeozems, rendzinas, black earths and brown soils. Humic acid/fulvic acid ratio is upper than 1, Extent of aromatic rings condensation is high in humic acids, which cause their hydrophobic properties and inability to creation of chelates. Humic acids are strongly connected with mineral portion of soil in this type of humus.

Third type of humus is characteristic for semi desert soils. In this humus predominate fulvic acids fraction, whereas arise of humic acids is limited. Beyond this, humic acids are largely bounded with mineral portion of soil.

Humus content in soil

Humus content of soil

Humus content in soils fluctuating in broad range. On humus content have influence the following factors:

- amount and quality of humus, which get at soil in given bio ecological zone
- tempo of humification process of organic matter
- tempo of mineralization of humus, which is contain in soil
- chemical, physic-chemical and physical soil properties
- amount and quality of mineral compounds contained in soil

Humus content in accumulation horizons of the main soil units in Poland (Turski 1996)		
Division order	and Type, genera and kind	Humus content %
Calcisols:	Calcarious Rendzinas	3.4
		2.1 - 6.3
		4.4
	Jurassic Rendzinas	1.5 - 7.0
Phaeozems:	Haplic Phaeozems	2.8
		1.8 - 4.0
Cambisols:	Brown soils formed from sands	1.5
		0.9 - 2.2
	Brown soils formed from light and medium loams	1.8
		1.1 - 3.0
	Brown soils formed from heavy loam	2.5
		1.6 - 3.7
Brown soils formed from silt formations	1.7	

		1.3 - 1.9
	Brown soils formed from loess and loess like materials	1.9
		1.4 - 2.6
Luvisols:	Grey brown soils formed from silt formations	1.9
		1.4 - 2.4
	Grey brown soils formed from loess and loess like materials	1.8
		1.0 - 2.5
	Grey brown soils formed from light loam	1.6
		1.0 - 2.6
Podzols:	Podzolic soil formed from sands	1.5
		1.1 - 2.0
Gleysols:	Boggy soils formed from silts	1.6
		1.2 - 2.1
Gleysols:	Black earth formed from sands	2.8
		1.2 - 4.1
	Black earth formed from light and medium loams	2.6
		1.2 - 5.7
Black earth formed from heavy loams and clays	4.9	
	2.5 - 5.6	
Fluvisols:	Alluvial soils formed from sands	2.9
		1.5 - 5.2
	Alluvial soils formed from silts	3.5
		1.7 - 5.8
	Alluvial soils formed from clays	4.2
		2.4 - 6.8

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