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Organic matter in the soil solution and absorption of micro-elements by the plant root. A literature study of the chemistry of solid humus, complexation of organic compounds in the root environment with nutrient elements and uptake of these elements by the plant

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1. Introduction

Roots of plants growing in soils or peat mixtures are in contact with the soil solution, which contains not only inorganic nutrients but also a large number of organic compounds derived from the organic matter of the medium in which the plants grow. Solved organic compounds may also be exuded by the plant roots themselves.

Organic compounds in the solution will influence the growth and nutrition of the plant. Thus it is possible that the organic compounds have growth-regulating properties. Complex formation can take place with nutrients such as micro-elements. Complexes may be very stable and absorbed as such, but also relatively unstable and translocate only the ion to the root surface.

In this report a review will be given of literature results as a start of a project about organic compounds in the soil solution and plant nutrition. Much research has already been done about the chemistry of the solid part of organic matter of soils and peat. To a lesser degree this is also the case for the soil solution. The main problem with humus research is that the organic matter of the soil is a very complex mixture of organic compounds. Based on different principles it was tried in the past to divide these compounds into a number of groups of still a large number of compounds. Criteria for the classification are molecular weight, charge of the molecules and polarity.

For the review, emphasis will be put on the compounds which can play a role in the soil solution. Problems to be discussed concern compounds which stimulate or inhibit root growth and nutrient absorption. In the last case special attention will be given to micro-elements. More knowledge about these problems is needed for a better characterization of potting soils.

2. Chemistry of humic substances

Plant roots grow in a number of chemically very different substrates, for instance peat and potting soils. The humic substances they contain are very diverse and only general information on their structure can be found in the literature. What can be given are the principal chemical entities that occur such as aromatic nuclei and aliphatic parts. For the different group - e.g. hydroxyl group and carboxylic group - the percentage of occurrence can be given. Especially important for the characterization of humus are data about polar groups and molecular weight, which influence ion binding capacity and solubility.

Basic materials for the formation of humic compounds are carbohydrates and lignin. In Figure 1 some basic building blocks



Figure 1. Building blocks of lignin (Streitweiser and Heatcock, 1985)

of lignins are given. Compounds with similar structure or their products can be expected in the humic substances. Via processes of oxidation and via enzymatic conversions polymeric products are formed, in which for example alcoholic, keto and acid groups are found.

The methods of analysis of humic substances consist of extraction, purification and identification. All extraction methods have the disadvantage that they change the compounds originally present. The mixtures can be purified on the basis of differences in the molecular weights and the charges of the molecules. For identification, methods such as visible, ultraviolet and infrared spectroscopy are used. Other methods which give information about the structure are titrations and NMR techniques.

A system for the determination of the composition of humic compounds is given in Figure 2.

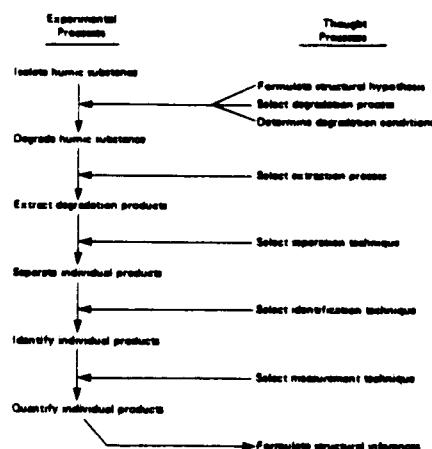


Figure 2. Flow diagram for the application of the degradative approach to humic structural analysis (Norwood, 1988)

A hypothetical formula of structures which may occur in the humic substances is given in Figure 3. The connections in this hypothetical formula of Schnitzer (1978) are hydrogen bonding.

Others, however, assume that real chemical bonding exists because sometimes fairly radical methods as oxidation with permanganate are necessary for the destruction. An example of a structure given by Christman is shown in Figure 4. The formula refers to aquatic humic substances, but comparable structures, are valid for soil humic substances.

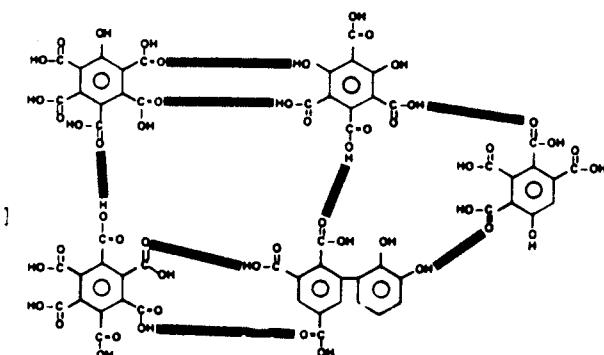


Figure 3. "Building block" model for soil humic macromolecular structure as proposed

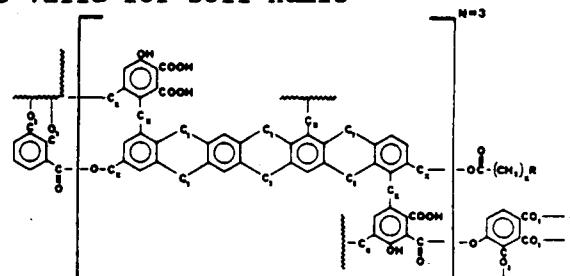


Figure 4. "Aromatic matrix" model for aquatic humic macromolecular structure proposed by Christman et al. (Norwood, 1988)

Table 1. Analytical characteristics of humic and fulvic acid of a soil (Burns et al., 1986)

Element (%)	HA	FA
C	56.4	50.9
H	5.5	3.3
N	4.1	0.7
S	1.1	0.3
O	33.9	44.8
Functional groups (meq/g)		
Total acidity	6.6	12.4
COOH	4.5	9.1
Phenolic OH	2.1	3.3
Alcoholic OH	2.8	3.6
Quinonoid C=O	2.5	0.6
Ketonic C=O	1.9	2.5
OCH ₃	0.3	0.1

More exact information can be found in data about functional groups as in Table 1 from Burns et al. (1986). For these groups, the contents and the elemental composition are given for humic acids and fulvic acids of a certain soil. The former compounds are soluble only in alkaline extractants, the latter both in acid and alkaline extractants.

3. Methods of extraction and separation of humic compounds

Humic compounds are mixtures of a large number of compounds with different molecular weights. A distinction should be made between liquid (water or soil solution) and solid sample (sediment or soil). Different methods have been developed for both cases, especially for the first steps of the procedure. A problem is, however, that the methods which are used to convert humus to a form that can be analyzed produce chemical alterations compared with the original state.

When the humic compounds are in a concentrated watery solution, it is possible to divide them into groups by physico-chemical methods. These can be based, for instance, on differences in molecular weight and molecular form, on the presence and distribution of charged groups, and so on. Also hydrophobic or hydrophylic behaviour is important in determining, the properties in the system. Therefore molecular sieves and ion exchangers can so be used.

After fractionation detection is carried out by spectrophotometric methods. Special attention will be given to the detection methods in a following section.

In Figures 5 and 6 schemes for isolation and preliminary fractionation of humic substances are given. Fractionation into humic acids and fulvic acids is often described in the literature. The fractionation with alkali (NaOH solution) is based on the fact that the fulvic acids are stronger acids than the humic acids. The further steps in Figures 5 are less clear from a chemical viewpoint. Other extractants which were used are sodium pyrophosphate, NN-dimethylformamide and chelating agents as EDTA and acetylacetone. Eloff and Pauli (1975) use other solvents, such as acetone, dioxane, ethanol, anhydrous formic acid, a mixture of sodium hydroxide and stannous chloride in water and buffer media. Frimmel et al. (1988) used supercritical fluids as extractants.

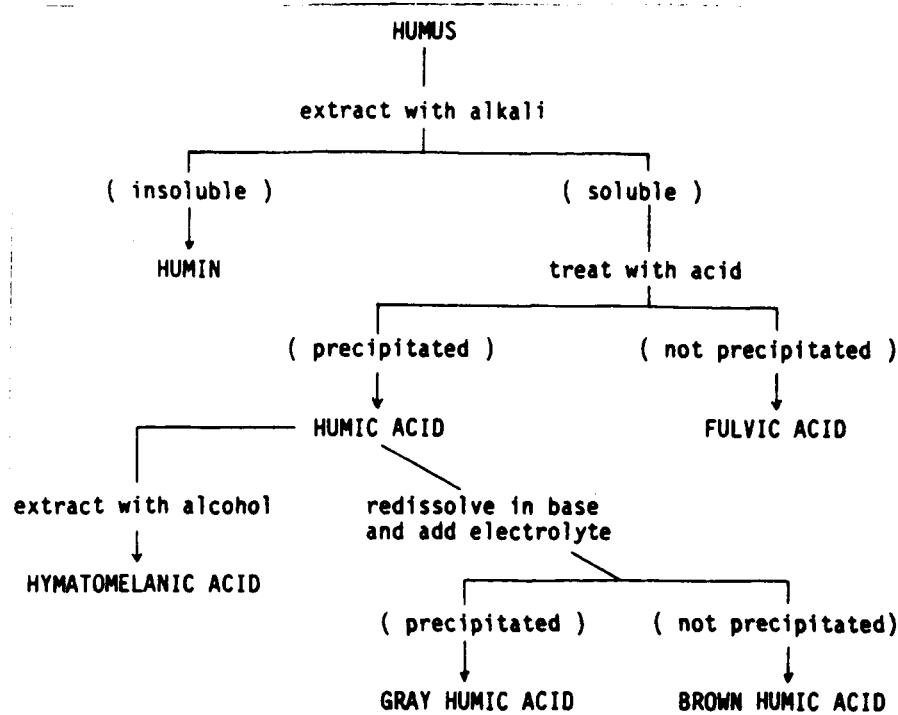


Figure 5. Scheme for the fractionation of humic substances by alkali extraction, after Stevenson (1982)

Group Report; E.M. Thurman et al.

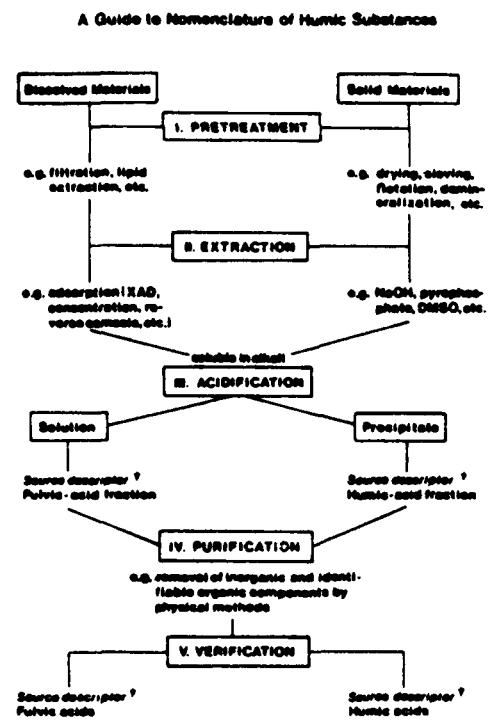


Figure 6. Extraction and purification of humic substances from a solid and liquid matrix. From Parsons (1988)

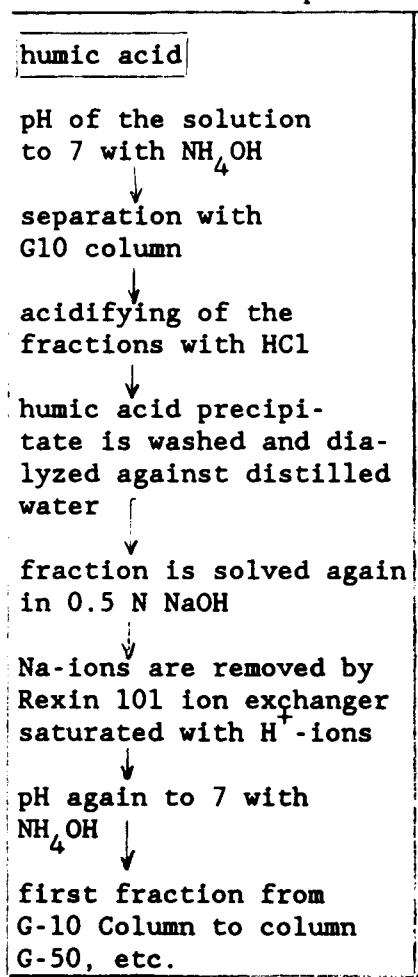
Pretreatment

Isolates such as humic acids are pretreated for further fractionation by desalting and removing small molecules. Thus cation exchangers in the H⁺ form and dialysis are used. Dialysis is used with precipitated humic acid (Rashid, 1971). The isolates can be stored after freeze-drying.

Fractionation

A further fractionation can take now place based on different properties of the fractions as molecular weight, charge, and charge dependence of pH.

Molecular sieves are used by many researchers. Rashid (1971) separated humic acid from aquatic sediments on Sephadex columns G-10, G-50



and G-100. The method which was used is given in Figure 7. The method results in a division into four molecular weight groups: smaller than 700; 700-10000; 10000-100000 and greater than 100.000. For separation of organic matter in the soil solution, Gregson and Alloway (1984) used Sephadex G-50 and G-100 and as an eluant 0.01 N KCl. The molecular ranges of G-50 and G-100 are 500-30000 and 1000-150000, respectively. Plechanov (1983) used Sephadex LH 60 and a mixture of dimethylformide and acetic acid (99-1 v/v) as the eluant for humic and fulvic acids.

Water was also used in different experiments as an eluant.

Reuter and Perdue (1977) found that the separations were dependent on the spatial conformation of the molecules and the ionic strength of the solution when the concentration was below 2000 mg/l organic matter.

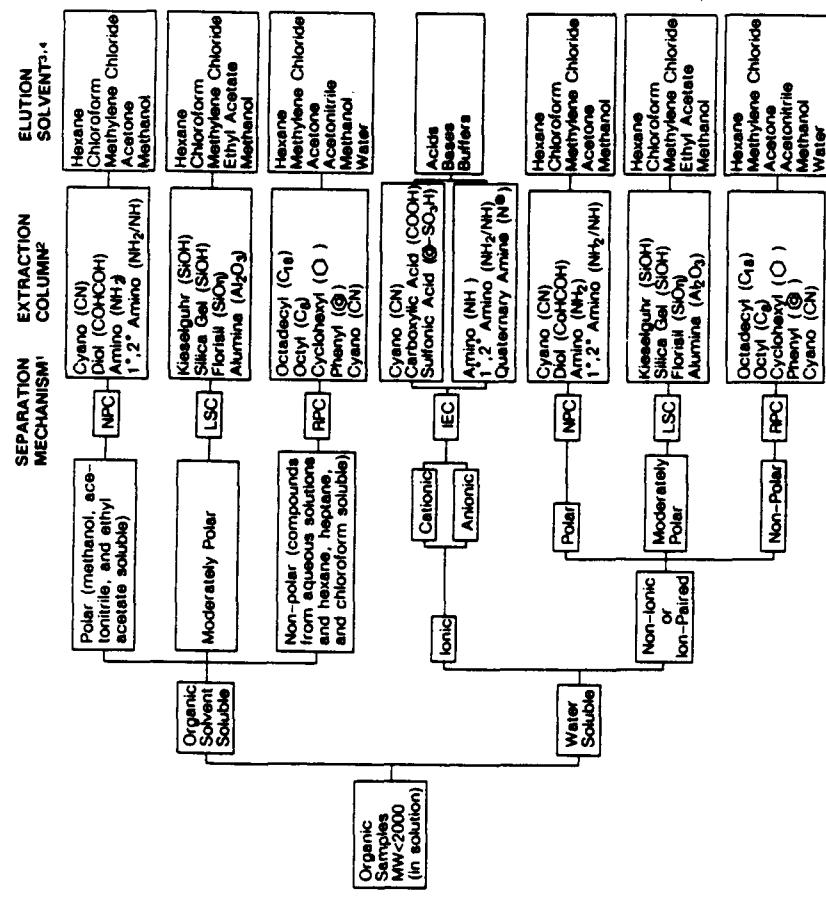
Ceccanti et al. (1986) used ultrafiltration with Amicon diaflo-membranes and in this way obtained a separation between molecular weights higher than 100000, between 10000 and 100000, and smaller than 10000. The behaviour of the fractions may depend as mentioned above on spatial structure of the humic compounds.

Schnitzer (1986) described flexible linear polyelectrolytes and spheroids as being dependent on pH and electrolyte concentration.

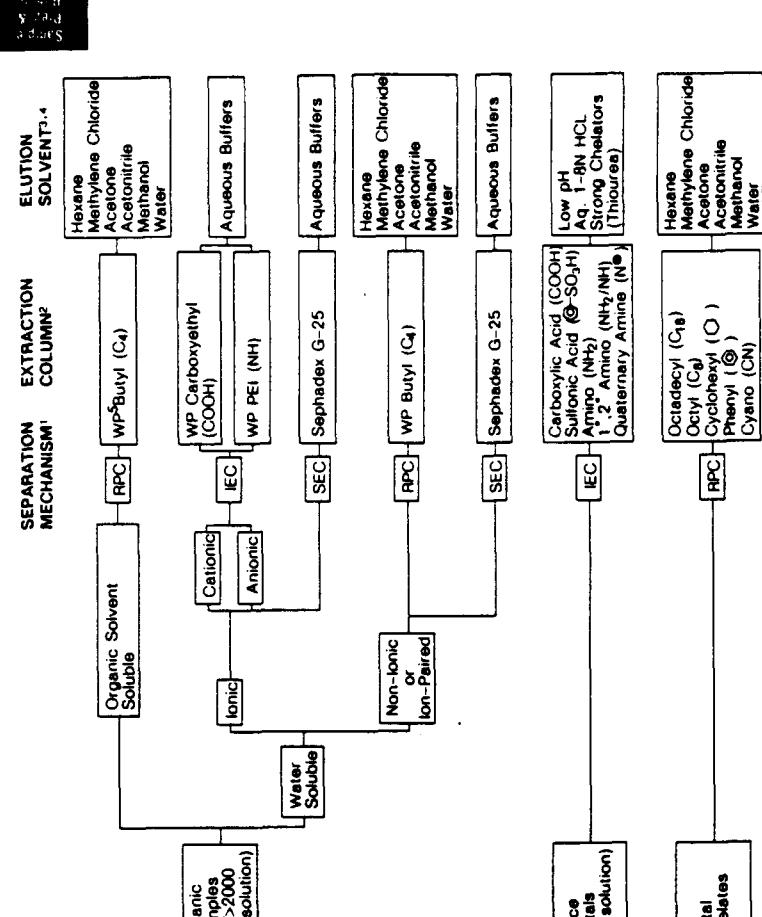
Figure 7. Separation scheme of Rashid (1971)

Sample Preparation Introduction (cont.)

Sample Preparation Introduction (cont.)



Sample
Prep &
Robots



LSC: Liquid/Solid Chromatography (Adsorption)
NPC: Normal Phase Chromatography (Bonded Phase Partition)
RPC: Reversed Phase Chromatography (Bonded Phase Partition)
IEC: Ion Exchange Chromatography (Bonded Phase Ion Exchange)
SEC: Size Exclusion Chromatography

1 Separation Mechanisms

2 Extraction columns listed in degree of increasing polarity

3 Eluting solvents listed in degree of increasing polarity

4 Selective elution can be performed by combining two or more miscible solvents to achieve various degrees of polarity
5 WP=Wide-Pore, 275 Å

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Table 2. Different possibilities of using molecular properties for separations. From Hewlett Packard Analytical Supplies Catalog and Chromatography Reference Guide 1989

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Methods based on charge and polarity

Tills and Alloway (1983) used the ion exchanger Amberlite Resin IR-120. The same authors separated compounds on the basis of their hydrophilic or hydrophobic nature (high solubility in polar or apolar solvents respectively). In reversed phase chromatography on Bondapak C18 the eluant was changed during the procedure from diluted phosphoric acid (polar) to a mixture of this acid with methanol (less polar). Ceccanti (1986) used isoelectric focusing. This is a technique in which the molecules are placed in a pH-gradient in a tube where they move under influence of a potential difference. The molecules reach the place where they are uncharged.

Many methods of separation are possible as becomes apparent in the scheme of Hewlett-Packard in Table 2.

The base is molecular weight, charge and polarity.

4. Quantitative and qualitative determination of humic compounds

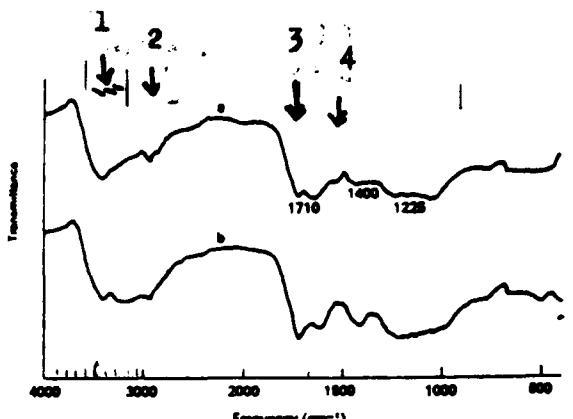
For the analysis of the crude fractions of humic compounds and further purified fractions a large number of techniques are available. For us the characteristics which are related to the effect on the crop are the most important. Properties related to solubility in the soil solution and with the binding of nutrient-ions are such characteristics.

Molecular weight can be determined by cryoscopy and vapour pressure measurements (Reuter and Perdue, 1981).

Elemental analysis on carbon, hydrogen, oxygen, nitrogen and sulphur gives a first impression of the composition (Andreux, 1981). Titrations with alkali can give an idea of the different acid groups such as carboxylic acid groups and phenolic groups (Piccolo, 1988). Further, carbohydrates, amino acids and amino sugars can be determined (Arshad and Schnitzer, 1987). Kögel-Knabner, Zech and Hatcher (1988) report on methods for cellulose and oxidation with CuO.

Very frequently spectrophotometric methods are used. Ultraviolet spectra give rough information on differences between fractions of humic compounds. Sometimes the ratio between the absorption at 465 and 665 nm in the visible region is used. Arshad and Schnitzer call this ratio E4/E6.

More specific information about the different group is given by infrared spectrophotometry as is illustrated by Figure 8. The technique can be used in solution but also in the solid phase on KBr plates.



- 1 - O-H "stretch" wavelength
- 2 - aliphatic C-H "stretch"
- 3 - C = O "stretch" from carboxylic acid group
- 4 - aromates

FIG. 8. Infrared spectra of the acidic form of humic matter with apparent molecular weight (a) more and (b) less than 100,000.

Figure 8. Infrared spectrum of humic matter
After Agrola and Ferrari (1971)

Another technique which gives much information about certain groups in the molecules is nuclear magnetic resonance (NMR). In ^{13}C -NMR the energy after backfall in a lower energy level after directing in a magnetic field is a measure for the molecular environment in which the ^{13}C is situated. In Figure 9 an example of a ^{13}C -NMR-spectrum is given, and a number of specific groups are indicated. In Table 3 and 4 the chemical shift in ppm is given. Burns et al. (1986) calculated

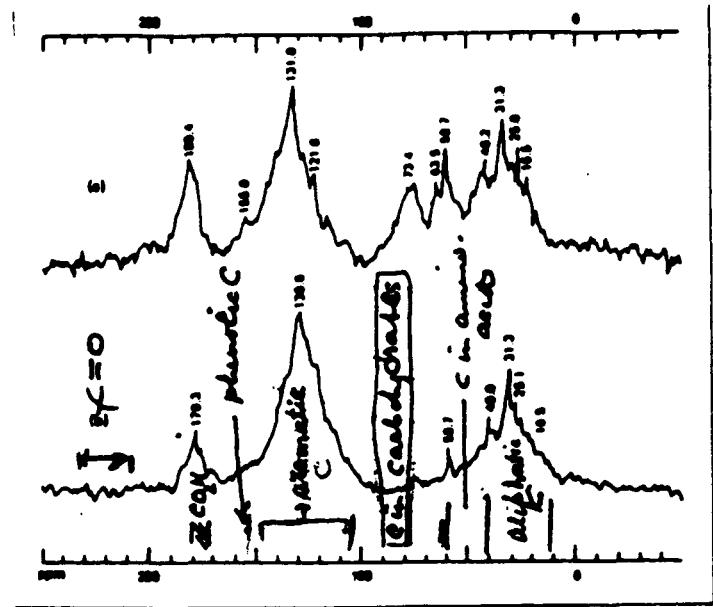


Fig. 3 Liquid-state ^{13}C NMR spectrum of: HA extracted from Mollisol Ab horizons (upper curve); same HA after hot 6M HCl hydrolysis (lower curve).

Figure 9. ^{13}C -NMR-spectrum of humic acid. Burns et al. (1986)

Table 2. Chemical shift integrative limits and assignments

Nucleus	Chemical shift (ppm) Range	Centre	Assignment
^{13}C	191-160	174	Carbonyl, ester, amide
	160-142	152	Oxygenated aromatics
	142-119	129	Protonated and carbosubstituted aromatics
	119-94	106	Acetyl, ketol
	94-45	76	Oxygenated aliphatics, alkyl-amino
	45-12	32	Alkyl

Table 3. Chemical shift (Oades et al., (1987)

Table 2. Types of C which can be determined by ^{13}C NMR in humic materials

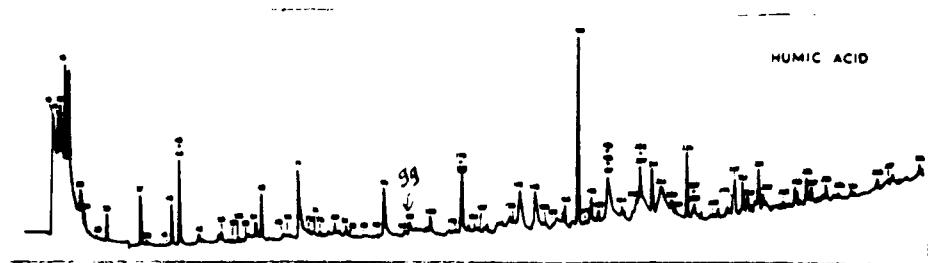
(a) aliphatic C (Alkanes + fatty acids)	10-40 ppm
(b) protein C, peptide C, amino acid C	40-60 ppm
(c) carbohydrate C	60-105 ppm
(d) aromatic C	105-150 ppm
(e) phenolic C	150-160 ppm
(f) CO_2H C (total acidity)	170-190 ppm
(g) C = O C	210-230 ppm
(h) aliphaticity	$\left(\frac{(10-105) \text{ ppm}}{(10-160) \text{ ppm}} \right) \times 100$
(i) aromaticity	$\left(\frac{(105-160) \text{ ppm}}{(10-160) \text{ ppm}} \right) \times 100$

Table 4. Chemical shift in ^{13}C -NMR Burns et al. (1986)

from the chemical shift the parts of the organic matter which are aliphatic and aromatic. Also Kögel-Knabner et al. (1988) used ^{13}C -NMR.

Still another promising technique was used by Saiz-Jimenez and De Leeuw (1988), viz., pyrolysis-gas chromatography-mass spectrometry. Via this technique pyrolysis-products of the humic substances are generated by heating. After ionisation these fractions are identified in a mass spectrometer. The fractions are separated on the basis of ionic weight and charge. In Figure 10 a spectrum is given. Peaks can be specific, as peak 99 for phenol.

Fluorescence spectrometry is a technique which also offers prospects (Miano, Sposito and Marten, 1988).



5. Chemistry of humic compounds in the soil solution

The organic compounds in the soil solution can influence root growth and nutrition. Soluble organic compounds which have their origin in humic substances may influence the root system directly or during transport and uptake.

It will become apparent that it is difficult to give a systematic review of the organic compounds in the rhizosphere and the relationship with tangible chemical data on humus. Important information are molecular weights and polarity of the humic compounds because these determine the nature of the organic compounds which are present in the soil solution.

Humic compounds in the soil solution

A large number of different compounds of humic origin occurring in the soil solution or the aquatic environment have been described. Aliphatic acids (e.g. propionic acid), aromatic acids (benzoic acid) and aminoacids have been reported by Candler, Zech and Alt (1988) and by McKeague et al. (1986). Also carbohydrates (Candler et al., 1988), sugar acids (Stevenson and Alanah Fitch, 1986), aldehydes, hydroxamate compounds, organic phosphates and porphyrines have been described. In Figure 11 a number of formulae of compounds which were found are given.

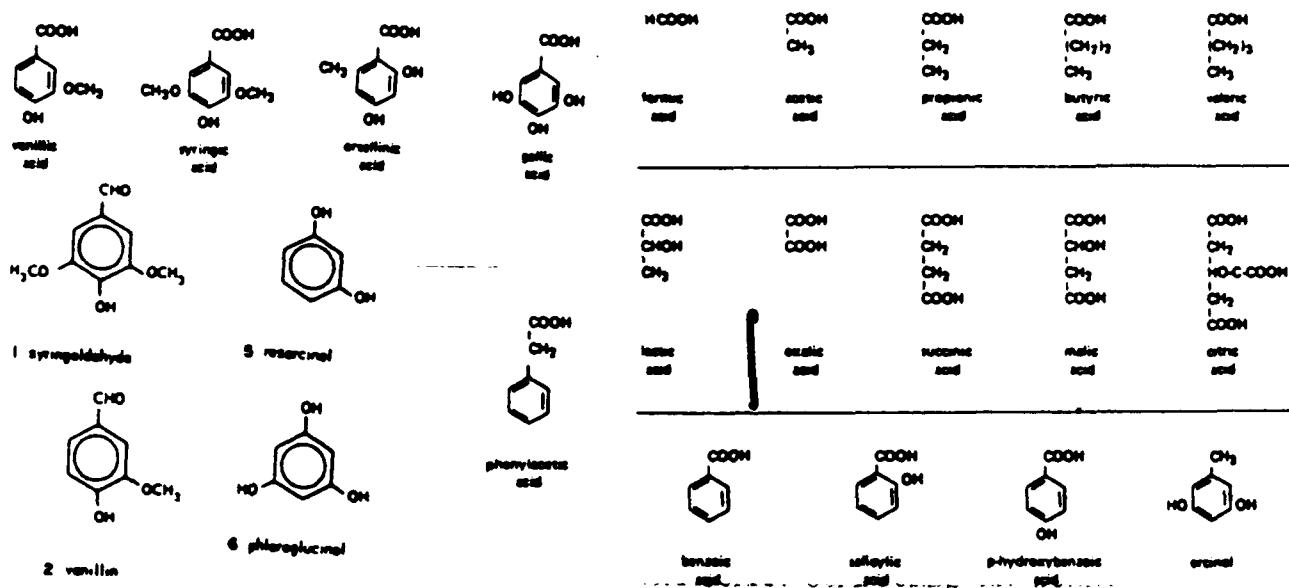


Figure 11. Formulae of some small molecular compounds in soil.
McKeague et al. (1986) and Paul and McLaren (1981)

Table 5. Possible compounds and probable chelators present in the hydrophilic and hydrophobic fractions

Organic fractions	Possible compounds	Probable chelators ^a
Hydrophilic acids	Uronic acids, simple organic acids, polyfunctional acids, or polyhydroxy phenols	Simple aliphatic acids, sugar acids (glucuronic, galacturonic, and gluconic acids), simple organic acids, and hydroxy derivatives (e.g., citric acid)
Hydrophilic neutrals	Carbohydrates, polysaccharides, polyfunctional alcohols, or phosphate salts	Simple sugars (borate complexes), polysaccharides
Hydrophilic bases	Most amino acids, amino sugars, low-molecular-weight amines, and pyridines	Most amino acids
Hydrophobic acids	Certain organic acids, phenols, anionic detergents and aromatic acids	Phenols, oxazine, tithox acids (oxalic or beta-oxalic acids)
Hydrophobic neutrals	Hydrocarbons, fats, waxes, oils, resins, amides, phosphate esters, chlorinated hydrocarbons, high-molecular-weight alcohols, amides, esters, ketones, and aldehydes	Phosphate esters
Hydrophobic bases	Complex polynuclear amines, nucleic acids, quinones, porphyrins, aromatic amines, and others	Porphyrins, phytic acid

^a Stevenson (1982).

In Table 5 a summary of data on groups of compounds in humus is given (Stevenson, 1982). Also the behaviour with respect to water is shown. The concentration range of some of the groups is also given by Stevenson:

Simple aliphatic acids	1×10^{-3} - 4×10^{-3} M
amino acids	8×10^{-5} - 6×10^{-4} M
aromatic acids	5×10^{-5} - 3×10^{-4} M

As the humus compounds are also in the solution, a mixture of a large number of different compounds, another starting point can be to give the percentage of different chemical groups, and to determine average elementary composition and molecular weight.

Table 6. Chemical composition of spruce and pine litter (in %) (Zech et al., 1987)

days of decomp- osition	C		nitro- gen		hemi- carbo- lites		water soluble substances		ether soluble substances		total acidity (meq/g of organic substances)	COOH- groups	phenolic OH-groups
	0	1105	C	N	collo- ids	humic substances	water soluble substances	ether soluble substances	collo- ids	humic substances			
spruce litter	0	43.6	0.40	29.3	20.6	11.7	4.1	2.5	0.4	2.1	5.0	0.9	4.1
	1105	44.3	1.36	22.5	20.2	4.2	2.9	5.0	0.9	4.1			
pine litter	0	44.2	0.36	32.4	18.8	14.3	6.9	3.8	0.2	3.6	3.6	0.7	2.9
	1456	49.1	1.19	19.2	18.4	3.0	8.7	3.6	0.7	2.9			

In Table 6 analytical data on very young humus (litter) is given.

Table 7 Low molecular weight compounds extracted from the litter by maceration in water (Jung et al. 1968)

Water-soluble Compounds	Tropical Litter				Temperature Zone Litter		
	Acacia	Guera	Khaya	Pinus	Calluna	Fagus	Festuca
<i>Aliphatic acids</i>							
Acidic C (p.p.m.)	5225	5620	11516	484	2324	1113	2371
Acidic C total hydrol. C	12	27	47	4	8	38	26
<i>Phenolic acids</i>							
Acidic C (p.p.m.)	93	129	357	128	315	53	30
Acidic C total hydrol. C	0.3	0.6	1.5	0.4	1.1	1.8	0.3
<i>Reducing sugars</i>							
Sugar C (p.p.m.)	8778	1711	6245	3805	12207	760	3161
Sugar C total hydrol. C	20	8	26	26	43	26	35
<i>Amino acids</i>							
Amino N (p.p.m.)	326	403	327	15	185	129	475
Amino N total hydrol. N	19	38	53	13	58	54	44

Results expressed as p.p.m. of litter dried at 105°C.

A relatively large part is water-soluble in the early phases of decomposition, later this fraction decreases. In Table 7 data about the soluble part are given.

In the water extract of litter of different origin aliphatic acids, reducing sugars and amino acids occur in relatively high concentration. After further decomposing, however, this can change. In Figure 12 more information about the functional groups in different types of humus is given. In aquatic humus and soil fulvic acid occur relatively many carboxyl groups. Phenolic groups are more abundant in soil humic acid. In Table 8 the first five samples concern the elemental composition of aquatic humus from the Götariver. The nitrogen content is about 1%, while the nitrogen contents of the sediments (FA-4 and FA-5) are higher. These data

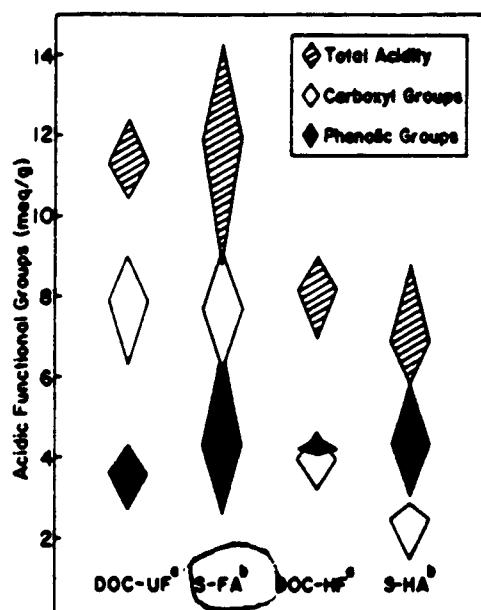


Fig. 12 Distribution of acidic functional groups in soil fulvic acids (S-FA), soil humic acids (S-HA), unfractionated organic matter from river water (DOC-UF), and the high molecular weight fraction of organic matter from river water (DOC-HF). BECK et al. (1974), REUTER et al. (in preparation), SCHMITZ and KHAN (1972), and references cited therein.

Table 8 Elemental analyses* of the fulvic and humic acids

Sample	C%	H%	O%	N%	S%	P%	Ash%
FA-0	51.96	4.32	39.80	1.04	3.05	n.d.	<0.02
FA-1	53.32	4.51	38.90	1.20	1.77	n.d.	0.03
HA-1	56.00	4.51	37.25	1.36	0.72	n.d.	0.37
FA-2	53.15	4.76	37.59	1.04	2.60	n.d.	1.02
HA-2	55.29	4.53	37.08	1.26	1.78	n.d.	0.25
FA-4	39.93	4.93	41.21	3.19	6.34	0.85	2.28
FA-5	44.89	5.46	35.40	4.04	4.25	0.17	4.50

From Reuter and Perdue (1977)

Plechanov (1983)

Table 9 CHEMICAL PARAMETERS OF AQUATIC HUMUS FROM THE SATILLA RIVER (SRHS) AND ITS MOLECULAR SIZE FRACTIONS.

Sample	^a	C	H	N	O	Ash	TA ^b
		Wt. %	(ash-free)		Wt. %		Meq/g
SRHS	1.000	52.1	3.6	0.8	43.5	2.3	10.4
G50E	0.036	54.3	4.7	1.4	39.6	10.8	7.3
G25E	0.470	51.4	3.6	0.7	44.3	0.9	10.6
G15E	0.143	50.3	3.7	0.8	45.2	0.9	10.1
G10E	0.145	50.4	3.7	1.1	44.8	1.1	12.0
G10R	0.206	51.8	4.2	0.8	43.2	0.8	9.9

^a weight fraction of molecular size fraction

^b total acidity--barium hydroxide method

Table 10 NUMBER AVERAGE MOLECULAR WEIGHTS (\bar{A}_n) OF AQUATIC HUMUS FROM THE SATILLA RIVER (SRHS) AND ITS MOLECULAR SIZE FRACTIONS.

Sample	N ^a	Linear Fit			Quadratic Fit		
		I ^b	\bar{A}_n	C ^c	I ^b	\bar{A}_n	C ^c
SRHS	5	8	556	8	8	614	41
G50E	4 ^d	14	1952	746	12	1231	4894
G25E	6	11	1306	75	6	878	11
G15E	4	9	725	46	5	555	120
G10E	6	10	577	27	5	427	3
G10R	6	8	429	16	5	340	2
BPCA ^d	6	8	255	4	8	293	15

agree with those given for another river in Table 9.

Another parameter of the humic fractions is the mean molecular weight. This can be distinguished as number and weight mean molecular weight. In Table 10 molecular weights of aquatic humus determined by Reuter and Perdue are presented. The molecular weights vary from 500 to 2000.

Table 11 Humic acid in river waters

Sample ^a	Concentration (mg/l)	Molecular weight distribution (%)		
		> 5 x 10 ⁴	5 x 10 ⁴ - 1 x 10 ⁵	< 1 x 10 ⁴
River water 1	0.06, 0.07, (av. 0.07)	36, 48 (av. 42)	9, 15 (av. 12)	37, 55 (av. 46)
River water 2	0.04, 0.05, (av. 0.06)	41, 53 (av. 47)	10, 15 (av. 13)	37, 44 (av. 41)

^a Suspended matter was removed by flotation

Reuter and Perdue (1977)

Reuter and Perdue (1977) gave as a mean molecular weight of humus extracted with distilled water $M = 1270$. Table 11 also shows that the smaller molecular fraction is present in a high percentage. In soils and waters this is probably the fraction which is transported and taken up most easily by plants and other organisms.

The separation techniques and detection methods are comparable with those used for solid humus. For fractionation molecular sieves and ion exchangers can be used. For detection UV and visible light spectrophotometry, infrared spectra and nuclear magnetic resonance (NMR) can be used.

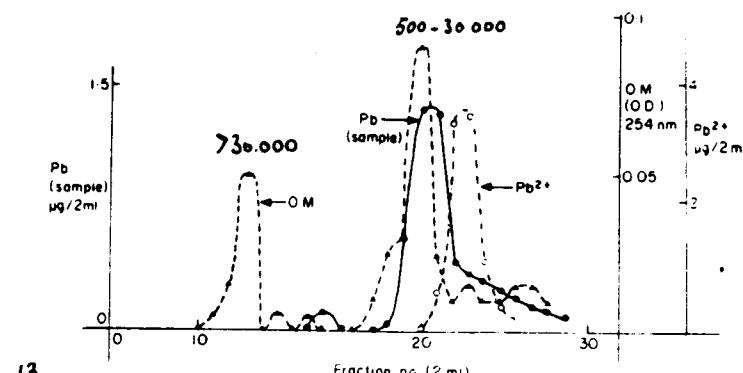


Fig. 13. Elution of solution from soil Pen Craig-ddu through Sephadex G-50 (eluent = 0.01 M KCl; pH = 3.8).

Gregson and Alloway (1984)

In Figure 13 from Gregson and Alloway the dotted line represents a fraction in the soil solution and the absorption in the UV-region. The column is G50. The peak at about 20 represents a molecular weight of between 500 and 30000. Examples of detection techniques are given in the following

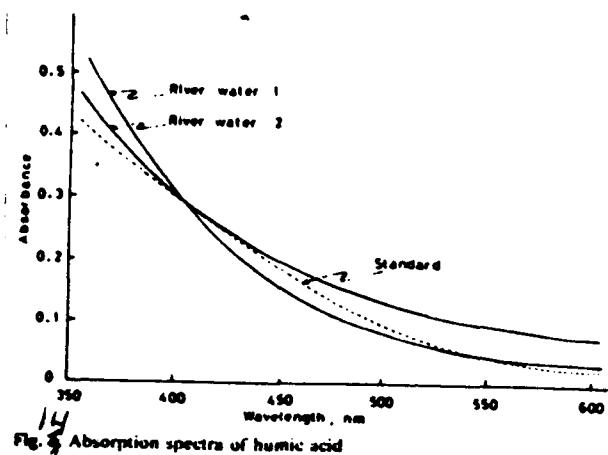


Fig. 14 Absorption spectra of humic acid

Hiraide et al. (1987)

figures. In Figure 14 a spectrum for river humus is given in the visible and a small part of the UV-region. Figures 15-17 present spectra reported by Candler et al. (1989). They used watery extracts of forest soils and litter. Separation took place on Sephadex G25, which makes separations in the region of the smaller molecular weights. In the infrared spectrum (Figure 15) the strong absorptions at 3400 cm^{-1} have their origin in -OH stretch vibrations. Carboxylate groups give bands at 1630 and 1400

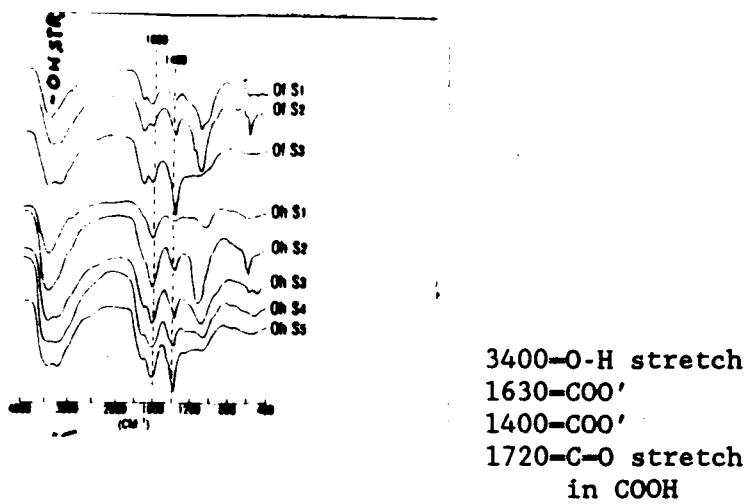


Figure 15. IR-spectra of fractions of separation on G25 column of watery extract of forest soil. Candler et al. (1989)

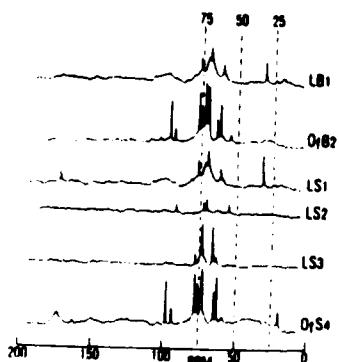


Figure 16. ^{13}C -NMR spectra of the fractions in Figure 15

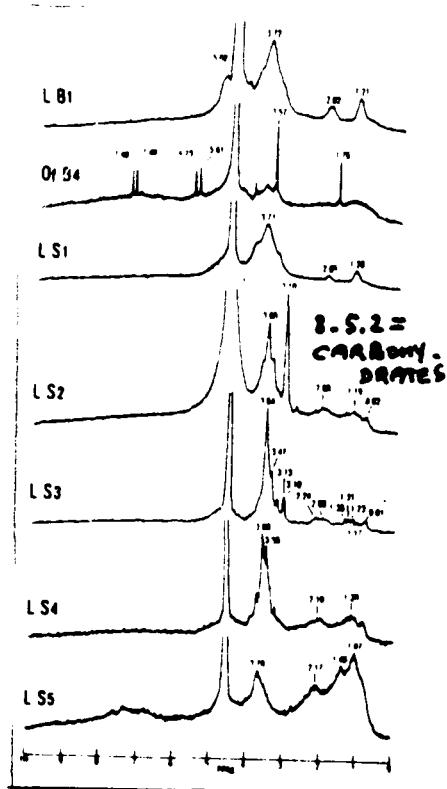


Figure 17. ^1H -NMR spectra of the fractions in Figure 15

It can be concluded that information about the relation between compounds in the soil solution and the type of humus is still incomplete. Compounds must be hydrophilic for dissolution. Carboxylic groups and phenolic groups play a role here. The same groups, together with for instance amino-groups, are responsible for nutrient complexation. Information about nutrient complexation will be given in the next sections.

6. Complexes of humic compounds with nutrient elements in the soil solution

In the following sections attention will be given to the complexation of especially micro-elements in the soil solution, to dissolved humic compounds and the consequences for plant nutrition. Especially in the case of micro-elements complexation is important for the plant because their concentration is so low that they are largely present in the soil solution as complexed compounds. This is in contrast with macro-elements as calcium and magnesium.

Micro-elements as copper and iron with incomplete atomic shells will easily form complexes with compounds which can donate electron groups. Such groups are present in the soluble humus compounds in the oxygen and nitrogen atoms in carboxylic acids, phenols and amino compounds. Oxygen and nitrogen have free electron pairs available which they can donate.

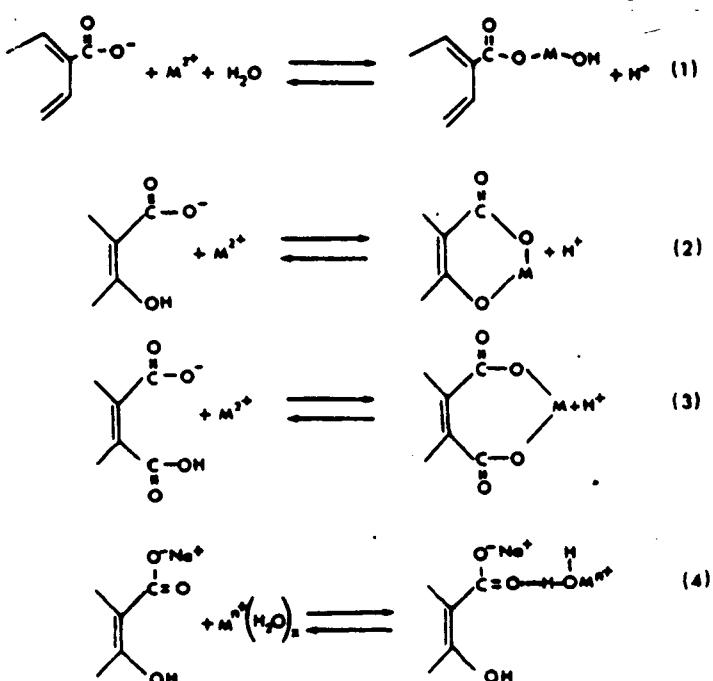
Some data will be given now about the type of complexation in the soil solution, and about the different techniques which are used to determine complexation. The techniques can show if the micro-element is in the free ionic form or in complexed form and give information about the identity of

cm^{-1} . The shoulder at 1720 has its origin in carbonyl groups of the undissociated COOH . In the ^{13}C -NMR spectrum (Figure 16) bands between 60 and 100 have their origin in carbohydrate groups. Carboxylic groups have a band at 175 ppm and alkyl groups at 30 ppm. In the ^1H -NMR spectrum (Figure 17) between 3 and 5.2 ppm, bands of carbohydrates are present. These examples only serve to demonstrate how it is possible to characterize the compounds in the watery phase by a combination of separations and detection techniques.

the complexing compound. Further, it is possible to determine the binding-constant (stability) of the complexes, which determines the behaviour of the complex in the system soil solution-plant root. Much of this information has its origin in aquatic research, but it is probably also applied to the soil solution. The strength of the complexation is dependent on factors as pH and ionic strength in the soil solution and further on the type of compounds of humic origin. In section 6.6 attention will be given to what complex-formation means for nutrient absorption by the plant root; for example, it will be discussed if the complex ion-humic compound is taken up in this form or if the complex only transports the ion to the root surface.

6.1. Bonding of solved humic compounds and nutrient ions.

A number of possibilities are given in Figure 18. In equation (2)



a "salicylate" binding is given where the ion is bound to a carboxylate group and a phenolic group. In equation (3) a "phtalate" binding where the ion is bound to two carboxylate groups. Binding as in equation (4) is a hydrogen bonding which is weaker. In equation (1) only one molecule group has been bound. Another possibility is a mixed ligand complex (Huang and Schnitzer, 1986). The second complex-forming compound can be among others citrate, phosphate, bicarbonate.

From Huang and Schnitzer (1986)

Other examples of possible complexes are shown in figures

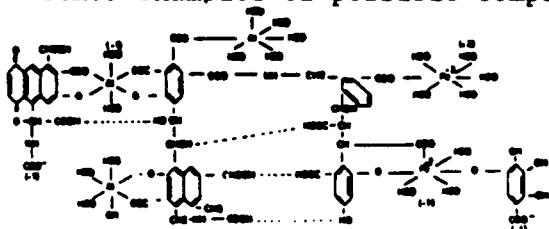
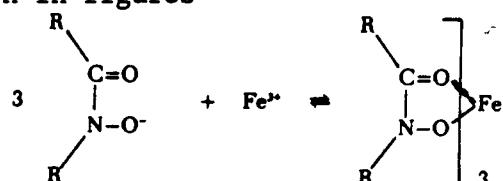


Fig. 19 Hypothetical polymerized compound with Al^{3+} , Fe^{3+} , and Fe^{2+} in 6-coordination with protonic bridges (dotted lines). The net charge is zero. From De Coninck (45). From Stevenson and Fitch (1986)



where R is often a peptide.
Figure 20. Binding of iron on hydroxamate. Stevenson and Fitch (1986)

19 and 20. Figure 19 shows how oxides of iron and aluminium can be mobilized by complexation. In Figure 20 a complex with a nitrogen-containing microbiological hydroxamate with iron is given.

6.2. Techniques used to determine complexation of micro-elements in the soil solution with humic compounds

To be able to calculate the equilibria between nutrients such as micro-elements (M^{b+}) with ligands from the organic matter (L^{a-}) to the complexes $M^{a-}L^{b+}$ it is necessary that M^{b+} , L^{a-} and $M^{a-}L^{b+}$ can be measured. A number of techniques have been used for that purpose (Table 22). All have advantages and disadvantages, the latter mostly caused by the fact that the number of different compounds in the system is large. Thus, Kerven et al. (1984) used AAS to determine total copper and ion-specific electrodes to determine the free ion Cu^{2+} . Qualitatively the complex formation can be followed via the infrared spectra; there is a shift from 1710 cm^{-1} (COOH) to 1615 cm^{-1} (COO'). Gregor and Powell (1988) used anode stripping voltammetry. Peak areas were determined at pH 1.7 (non-complexing) and pH 4.8 (with complexing). Sposito et al. (1988) used fluorescence quenching to calculate the proportion of copper that is complexed. At pH 7 the complexation was maximal. A formula is given for calculation of the proportion of the copper that has been complexed. Dietze and König (1988) use the difference in diffusion velocity through dialysis membranes over a gradient created by a cation exchanger to determine the ratio between complexed and not-complexed. Rashid (1971) first separated humic acid on G10, G50 and G100 Sephadex molecular sieves, then tested the complexation ability by saturation with metal at pH=7. Hydrolysis and solubility limits may have been interfering factors in this determination. Iminodiacetate resins absorb the uncomplexed ions in very small pores, but not the complexes. Murray and Linder (1984) distinguish a number of metal binding sites in the humic compounds from which they calculate the percentage of metal bound.

Table 22. Methods used to determine the complexation of the ion M with a ligand from the humus

M^{b+} ion	Determination of: L^{a-} ligand	$M^{a-}L_b$ complex
ion-specific electrodes		
	electron spin resonance x)1) infrared spec- troscopy 1)	electron spin resonance x)1) infrared spec- troscopy x)1)
anode stripping voltammetry (ASV) 2) pulse voltammetry 8)		anode stripping voltammetry (ASV) 2)* pulse voltammetry 8)
		gel permeation chromatography + detection technique 3)4)6)
		UV-radiation + detec- tion technique +)3)
	fluorescence 1)	fluorescence quenching 1)
dialysis + cation- exchanger + detection +)5)		dialysis + cation- exchanger + detection +)5)
		divinyl benzene resins + detection
iminodiacetate resins + detection +)	HPLC or GC detection- technique +)6) Millipore filters + detection technique 7)	HPLC or GC + detection- technique +)6)

x) only qualitatively

+) this can be AAS (atomic absorption), ICP, etc. for the inorganic part
and spectroscopy, etc. for the organic part.

*) the unstable complex

Literature: 1) Sposito et al. (1988); 2) Gregor and Powell (1988); 3) quoted by Stevenson and Fitch (1986); 4) Gregson and Alloway (1984); 5) Dietze und König (1988); 6) Rashid (1971); 7) Ogiwara and Kodaira (1989); 8) Cleven, del Castilho, Wolfs (1988).

A combination of gas chromatography (GC) and high performance liquid chromatography (HPLC) with a detection technique is described by Landner (1986).

6.3. Differences in complexation capacity between different ions

Some ions form complexes easily, such as copper and iron, others, e.g. zinc, form much less stable complexes.

Table 23. Metal species in a soil extract (SE) as determined by ion exchange procedures (batch-mode: ion exchange resin in its protonated and its sodium form, after 24 h equilibration pH 5.6 (Na^+)) and 2.8 (H^+); column-mode: ion exchange resin in its sodium-form; effluent pH: 4.9)

Element	Metallspuren-Anteile (%) / 100 in der Lösung		
	ionisch, monomer		stark komplexiert, Ionen
	pH 5.6	pH 2.8	
Ca	97	0	0
Mg	97	0	0
Mn	97	0	0
Zn	97	0	0
Cd	90	1	1
Pb	67	3	1
Cu	18	33	7
Al	38	6	5
Fe	15	20	14
Cr	10	63	43

From Dieter and König (1988)

This becomes apparent from Table 23 where results of the complex forming abilities of the soil solution are given. Strongly complexed (high M/M_0) are chromium, copper and iron.

Stevenson and Fitch (1986) gave as results for peat samples 50% of the copper as complexes and for sand 25-30%. Further results are given in Table 24.

Stevenson and Fitch (1986) report that the stability of the complexes approximately follows the Irving-

Table 24. Percentage of organically bound forms in soils solution of different soils

Iron	cobalt	nikkel	copper	zinc	lead
0.69			75-99	5-90	
99 ')	99 ')	99 ')	99 ')	65 ')	
			75 x)	2 x)	74 x)

') with sewage-sludge x) surface water
Stevenson and Fitch (1986)

Williams sequence Cu> Ni-Zn>Co>Mn-Cd>Ca>Mg. Murray and Linder (1984) assume that different chemical sites are to a certain degree specific for certain ions. Phthalates would bind all metals except iron (III), salicylate would bind iron (III) and acetylacetone copper.

6.4. Effect of factors such as type of organic matter, pH and ionic strength on complexation

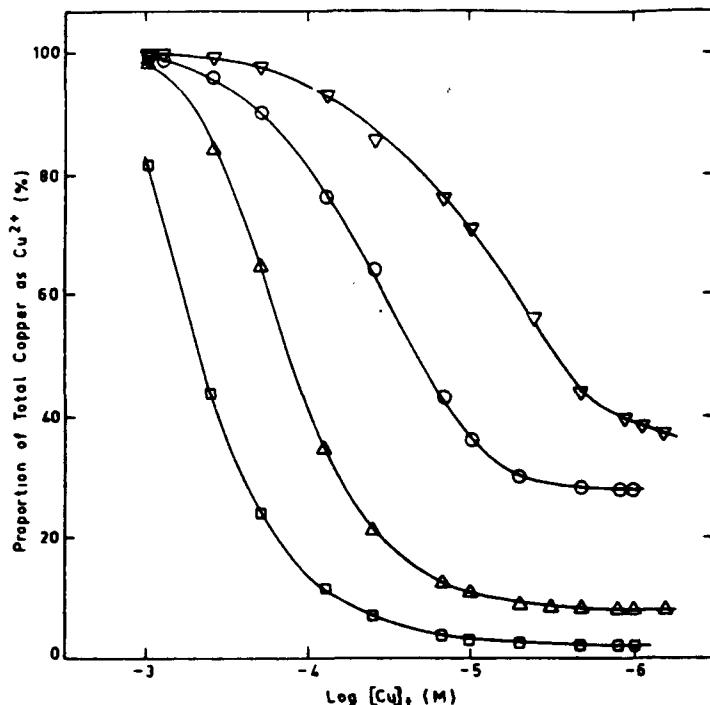


FIG. 21 Relationship of copper species distribution to total solution copper concentration. □ Canadian peat pH 5.4; △ Canadian peat pH 3.4; ○ Malaysian peat pH 3.6; ▽ West German peat pH 3.4.

TABLE 25 Kerven et al.
Chemical composition of peat soil extracts (1984)

Determination	Sample		
	Malaysian peat	Canadian Sphagnum peat moss	West German peat
Extract pH	3.5	3.4	3.4
Element, μM			
Na ⁺	478	2261	957
K ⁺	154	231	359
Ca ²⁺	800	115	228
Mg ²⁺	1365	773	1102
Fe ³⁺	11.6	7.3	9.0
Zn ²⁺	24.9	4.0	7.2
Mn ²⁺	< 1	< 1	< 1
Cu ²⁺	0.20	0.17	0.14
Cl ⁻	617	2282	811
S ²⁻	1094	1906	1000
P ³⁺	2	< 1	25
Soluble organic carbon, $\mu\text{g ml}^{-1}$	130	560	166

From Kerven, Edwards and Asher (1984)

The effect of type of organic matter (peat) is shown in Figure 21 and Table 25. The lower content of free Cu²⁺ in the solution of the Canadian peat can probably be accounted for by the higher organic matter content. Perhaps different peats can be characterized on the basis of their behaviour with respect to copper. The number of complexing sites is an important factor, as is shown for fulvic acid in Figure 22.

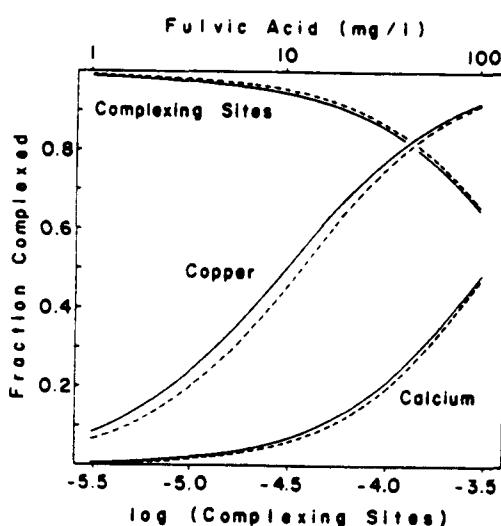


Fig. 22 Degree of complexation of Cu(II) and Ca²⁺ as a function of fulvic acid concentration. Total Cu(II) concentrations $1 \times 10^{-8} \text{ M}$ (—) and $1 \times 10^{-5} \text{ M}$ (---).

Reuter and Perdue (1977)

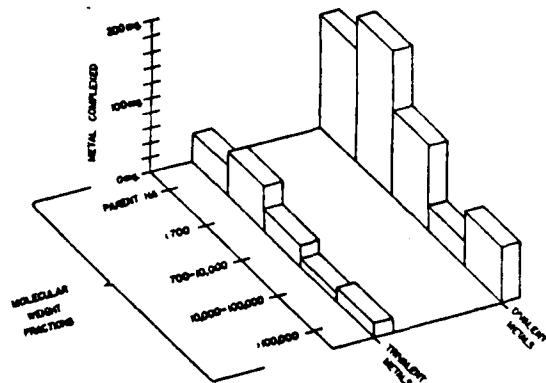


Fig. 23. A comparison of the average metal holding capacities of the parent and fractionated samples of marine humic acids in relation to their molecular weight distribution.
RASHID (1971)

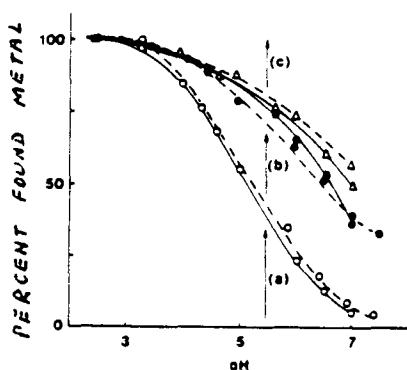
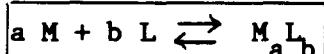


Fig. 24. Binding curves for copper(II)/fulvic acid solutions containing 4×10^{-5} M copper(II) and 1.3×10^{-4} M ligand-COOH: (—) FA2; (---) FA1. Percent labile and/or free metal determined by sampled-d.c. ASV (●), sampled-d.c. polarography (△) and ion-selective electrode (○). Height (a) represents labile metal (ISE), (a)+(b) represents ASV-labile metal, and (c) represents non-labile metal.

From Gregor and Powell (1988)

6.5. The equilibrium constant K for the complexation of ions with humic substances

The equilibrium equation given by Stevenson and Fitch (1986) is



The equilibrium constant is then: $K = (M_a L_b) / (M)^a (L)^b$

In Tables 26-28 estimates of K are given. Differences for one element in different determinations are partly caused by the different origins of the humic substances as soil fulvic acids, peat fulvic acid and also aquatic humic acid. The strong complexing properties of copper, referred to in section 6.3, are reflected in the relatively high values for this element.

Certain molecular fractions form stronger complexes than others. According to Rashid (1971) these are the smaller molecules with molecular weights lower than 700 (Figure 23). At higher pH-values the copper complexes become more stable (Figure 24). Also an effect of ionic strength is assumed (Murray and Linder, 1984).

Table 2^a. Conditional and overall stability constants (log K) for the metal complexes of fulvic acid (FA) and water-soluble humic matter.^t

Metal ion	Source	Supporting electrolyte	pH				Reference
			5	6	7	8	
Ca	SFA ^t	0.1 M KCl	3.40				Schnitzer & Hansen, 1970
	PFA	0.02 M Tris		3.65			Mantoura et al., 1978
	LHC(4) [§]	0.02 M Tris		3.56-4.09			Mantoura et al., 1978
	MHC(4)	0.02 M Tris		3.60-4.12			Mantoura et al., 1978
	SDHC	0.02 M Tris		4.65			Mantoura et al., 1978
Mg	SFA	0.1 M KCl	2.20				Schnitzer & Hansen, 1970
	PFA	0.02 M Tris		3.81			Mantoura et al., 1978
	LHC(4)	0.02 M Tris		3.26-4.00			Mantoura et al., 1978
	MHC(4)	0.02 M Tris		3.41-3.98			Mantoura et al., 1978
	SDHC(2)	0.02 M Tris		3.92-4.09			Mantoura et al., 1978
Cd	SFA	0.1 M KCl	4.10				Schnitzer & Hansen, 1970
	PFA	0.02 M Tris		4.57			Mantoura et al., 1978
	LHC(2)	0.02 M Tris		4.57, 4.70			Mantoura et al., 1978
	MHC(4)	0.02 M Tris		4.69-4.96			Mantoura et al., 1978
	SDHC	0.02 M Tris		4.91			Mantoura et al., 1978
Co	SFA	0.1 M KCl	4.10				Schnitzer & Hansen, 1970
	PFA	0.02 M Tris		4.51			Mantoura et al., 1978
	LHC(4)	0.02 M Tris		4.67-4.90			Mantoura et al., 1978
	MHC(4)	0.02 M Tris		4.29-4.83			Mantoura et al., 1978
	SDHC	0.02 M Tris					Mantoura et al., 1978
Cu	SFA	0.1 M KCl	4.00				Schnitzer & Hansen, 1970
	SFA	0.1 M NaClO ₄	4.35				Cheam & Gamble, 1974
	SFA	0.1 M NaNO ₃	4.00				Buffie et al., 1977
	SFA	0.1 M KNO ₃	4.68	5.03	5.45		Ryan & Weber, 1982
	WFA	0.01 M KNO ₃		7.82			van den Berg & Kramer, 1979
	LHC	-		4.99	5.20		Shuman & Woodward, 1977
	PFA	0.02 M Tris		7.85			Mantoura et al., 1978
	RHC(2)	0.02 M Tris		8.42-9.83			Mantoura et al., 1978
	LHC(4)	0.02 M Tris		9.48-9.58			Mantoura et al., 1978
	MHC(4)	0.02 M Tris		8.89-10.21			Mantoura et al., 1978
Mn	SDHC(4)	0.02 M Tris		9.91-11.37			Mantoura et al., 1978
	SFA	0.1 M KCl	3.70				Schnitzer & Hansen, 1970
	PFA	0.02 M Tris		4.17			Mantoura et al., 1978
	LHC(2)	0.02 M Tris		4.30-4.85			Mantoura et al., 1978
	MHC(2)	0.02 M Tris		4.46, 4.61			Mantoura et al., 1978
Ni	SFA	0.1 M KCl	4.20				Schnitzer & Hansen, 1970
	PFA	0.02 M Tris		4.98			Mantoura et al., 1978
	LHC(2)	0.02 M Tris		5.14, 5.27			Mantoura et al., 1978
	MHC(4)	0.02 M Tris		5.19-5.51			Mantoura et al., 1978
	Pb	0.1 M KCl	4.10				Schnitzer & Hansen, 1970
Zn	SFA	0.1 M NaNO ₃	4.10				Buffie et al., 1977
	SFA	0.1 M KCl	3.70				Schnitzer & Hansen, 1970
	PFA	0.02 M Tris		4.83			Mantoura et al., 1978
	RHC(2)	0.02 M Tris		5.36, 5.41			Mantoura et al., 1978
	LHC(4)	0.02 M Tris		5.03-5.31			Mantoura et al., 1978
	MHC(2)	0.02 M Tris		5.27, 5.31			Mantoura et al., 1978
	SDHC(2)	0.02 M Tris		4.99, 5.87			Mantoura et al., 1978

^t Values at other pHs can be found in references cited.

^t SFA = soil fulvic acid, PFA = peat fulvic acid, LHC = lake humic colloid, RHC = river humic colloid, MHC = marine humic colloid, and SDHC = sediment humic colloid.

[§] The values inside the parentheses indicate the number of samples used.

Table 2^b. Conditional stability constants for the binding of Cu by organic matter in natural waters using the Scatchard plot method (Alberts & Giesey, 1982). Log K₁ = binding at "strongest" site; log K_n = binding at "weakest" site.

Source ^t	Supporting electrolyte	pH	Log K ₁	Log K _n	Reference
SFA	0.1 M KNO ₃	4.0	5.60	3.95	Bresnahan et al., 1978
WFA	0.1 M KNO ₃	4.0	5.48	4.00	Bresnahan et al., 1978
WFA	0.1 M KNO ₃	4.7	6.00	3.85	Bresnahan et al., 1978
SFA	0.1 M KNO ₃	5.0	6.00	4.08	Bresnahan et al., 1978
WFA	0.1 M KNO ₃	5.0	5.95	3.70	Bresnahan et al., 1978
SEW	0.1 M KCIO ₄	5.0	3.85	2.09	Sposito et al., 1979
SFA	0.1 M KNO ₃	6.0	6.30	3.77	Bresnahan et al., 1978
WFA	0.1 M KNO ₃	6.0	6.11	3.86	Bresnahan et al., 1978
WFA ^t	--	6.25	8.11	5.34	Tuschall & Brezonik, 1983
WFA ^t	--	6.25	7.82	5.26	Tuschall & Brezonik, 1983
WFA	0.01 M NaCl	8.0	8.80	8.05	Mantoura & Riley, 1975
PFA	0.01 M NaCl	8.0	8.51	7.16	Mantoura & Riley, 1975

^t SFA = soil fulvic acid, WFA = water fulvic acid, SEW = sewage fulvic acid, and PFA = peat fulvic acid.

^t Constants obtained for binding at three "sites": log K₁ = 7.82-8.11; log K_n = 6.72-6.86; log K₂ = 5.26-5.34.

Table 2^c. Conditional stability constants (logs K₁ and K_n) for the binding of Cu, Pb, and Cd by organic matter in natural waters, using the Scatchard plot method (Alberts & Giesey, 1983).

Metal ion ^t	Log K ₁	Log K _n
Cu ⁺⁺	6.52 ± 0.45	4.89 ± 0.82
Pb ⁺⁺	5.09 ± 0.46	2.00 ± 3.36
Cd ⁺⁺	5.83 ± 0.35	3.13 ± 2.45

^t Number of water samples ranged from 6 to 8.

From Stevenson and Fitch (1986)

From Stevenson and Fitch (1986)

6.6. Effects of complexation on plant growth and ion absorption

Stimulating effects of organic matter on yield of plants as tomato and cucumber have been reported (Burns, 1986).

Complexation can influence uptake by plant roots both positively and negatively. Thus Burns (1986) found a stimulation of uptake of calcium,

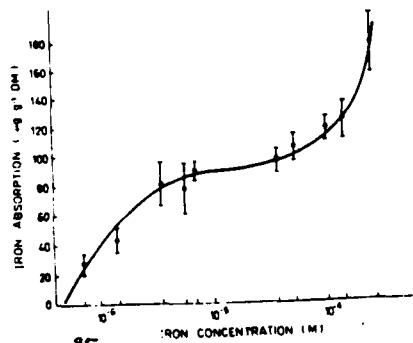


Fig. 25. Iron absorption by excised barley roots from Fe-humate solutions of different concentrations. Absorption period 180 minutes at 25 C.

potassium, magnesium, copper and iron by cucumber grown in a solution containing 100-300 mg fulvo-acid per l. Scotti and Silva (1988) found an increase in manganese uptake with increasing humic content by lettuce and radish from soil. Sonneveld (1983) found stimulation in nutrient culture by humic compounds of calcium and magnesium uptake by cucumber. Lobartini and Orioli (1988) described the uptake of iron from iron humate by excised barley roots as affected by iron concentration (Figure 25). Organic compounds as smaller humic compounds can be taken up by plants (Hargrove, 1986, Burns, et al. 1986).

From Lobartini and Orioli (1988)

Besides complex-forming agents originating in humus, specific compounds released by the roots can also solubilize micro-elements in the rhizosphere. Experiments about this type of compounds - phytosiderophores - were described by Römhild and Marschner (1986).

The more exact relation between type of compounds present in humus and root exudates on the one hand, and uptake of micro-elements by plants on the other, will require further research.

7. Conclusions

- In this review, properties of humus are summarized especially from a viewpoint of characterization of potting soils and peats. This characterization is intended to supply a link between chemical and physical properties of the humus on the one hand and plant growth and root absorption on the other.
- In this context the concentrations and the chemical forms in the soil solution should be known.
- Humic compounds partly consist of aromatic substances with very different molecular weights. As functional groups, carboxyl acid groups and phenolic groups are present. The soluble humus is small in molecular terms (a few thousands or less).
- Separation can be done by molecular sieves, liquid chromatography, ultrafiltration and other separation methods.
- The product can be identified by spectroscopic methods as UV and infrared spectroscopy. Also mass spectrometry is a possibility.
- In the soil solution a large number of smaller and larger fragments occur which can complex micro-elements.
- The binding constant K for different micro-elements with humus or humus components can be measured by different methods. Especially copper forms stable complexes.
- The binding constant is influenced by type of humic compounds, pH and ionic strength.
- For characterization of humus in, for instance, a peat sample a standardized procedure for obtaining soil solution is desirable. It will then be possible to compare different samples.

- Parameters which can be easily measured and appear to be important for characterization are: elemental analysis, functional groups (such as phenolic and carboxyl acid groups), mean molecular weight, titration curves, and in the solutions, pH and ionic strength.
- Characteristic of the functional groups is particularly the infrared absorption. The phenol group absorbs at 3400 cm^{-1} , the dissociated carboxylate group at between 1400 and 1630 cm^{-1} and the undissociated carboxylacidgroup at 1720 cm^{-1} .
- Absorption by plants of micro-elements from complexes with humic compounds has been demonstrated.
- Further research is needed to determine the importance of fractions in the soil solution obtained by separation for soil fertility.
- Humus components can be further characterized by determining ionic binding constants at different pH-values and ionic strengths. It appears to be especially desirable to determine the amounts of free and complexed copper because the stability of copper/humus complexes strongly depends on the Type of humus.

8. References

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