Biochemistry of Soil Organic Matter in Relation to Crop Production

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Content

Lecture 4.

Investigations for the Elucidation of the Structure of the Humic Fractions

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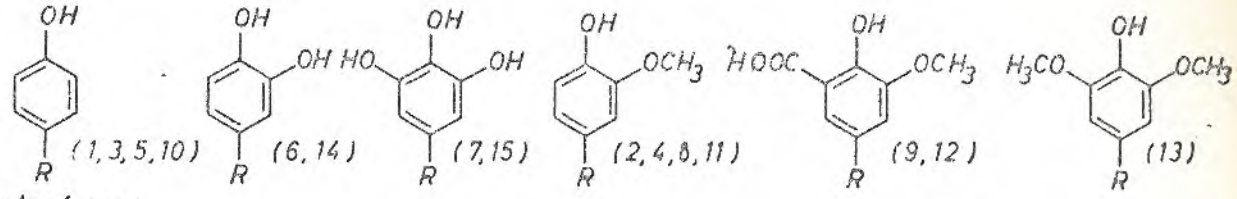
1. Cleavage of humic acids in their constituents by oxidation and reduction (after hydrolysis).

For investigations on the composition of humic acids by oxidation procedures different authors have often used very different methods. Therefore, it is surprising that the isolated oxidation products of humic acids or the structural units found have consisted of chemically similar compounds. The yields of defined oxidation products would not be expected to be high, because the oxidation of very carefully isolated coniferous lignin with nitrobenzene in alkaline solution seldom yields more than 25 % vanillin (BRAUNS and BRAUNS 1960), although this lignin is almost a pure polymer of coniferyl alcohol.

To determine phenolic structure units of humic acids 2 methods mainly are used.

1. Oxidation of humic acids in alkaline solution with nitrobenzene at 120 - 140°C. Yield up to 7 % (MORRISON 1963).
2. Reductive cleavage with sodium-amalgam in oxygen free medium at 100 - 110°C (BURGES, HURST and WALKDEN 1964), yield up to 30 % of mixtures of aldehydes. This method ows the preference. In the following table the identified compounds are mentioned in a summary.

oxidative degradation



components from:

lignin (1) R = -CH=CH-COOH (2) R = -CH=CH-COOH

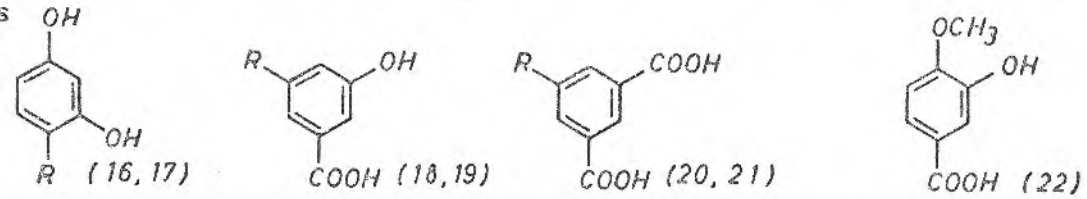
(3) R = -CO-CH3 (4) R = -CO-CH3

(5) R = -COOH (8) R = -COOH (9) R = -COOH

(10) R = -CHO (6) R = -COOH (7) R = -COOH (11) R = -CHO (12) R = -CHO

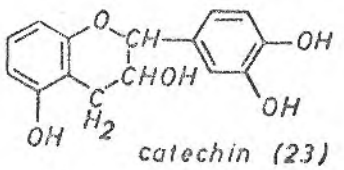
(14) R = -H (15) R = -H (13) R = -CHO

flavonoids or microbial synthesis



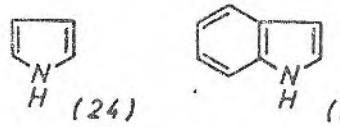
(16) R = -H (18) R = -H (20) R = -H

(17) R = -COOH (19) R = -OH (21) R = -OH



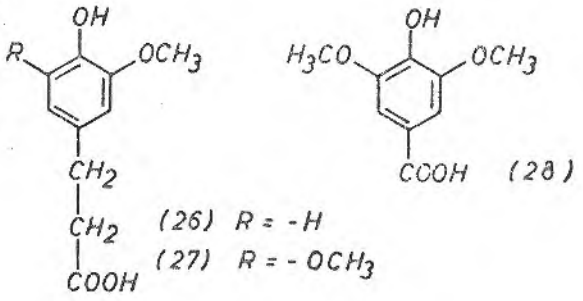
catechin (23)

furthermore derivatives of:



reductive degradation

components from: (5), (6), (8) lignin



flavonoids (16) or microbial synthesis

furthermore: (15, 19)

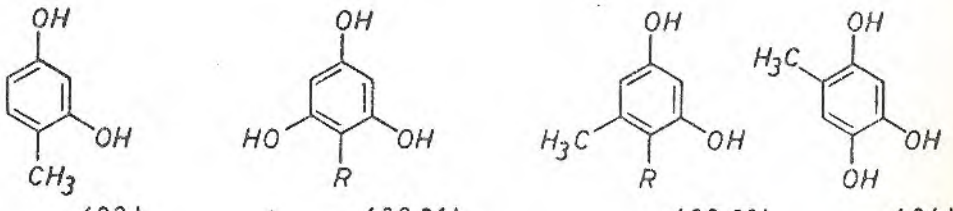


Fig. 1: Degradation products of humic acids.

By oxidative as well as by reductive cleavage mainly two types of degradation products are found.

1. Phenols, which can be derived from lignin.
2. Phenols, which can be derived from flavonoids or are synthesized by microorganisms.

In the first case the phenolic hydroxyl groups are in o-position, in the second case in m-position.

The structure of the degradation products demonstrates, that the humic acids consist of a larger number of phenolic units, which are connected in different manners.

The phenol polycarboxylic acids can be derived from phenolic units, which are bound by side chains or by rings.

Also nitrogenous compounds have been found such as pyrrol- and indol-derivatives; this means, that in humic acids nitrogen is also partly bound in heterocyclic form.

In recent time with zinc dust destillation of humic acids polycyclic aromatic compounds were identified. The numbers given by HANSEN and SCHNITZER (1969) are naphtalene (0,1 %), phenanthrene (0,1 %), anthracene (0,2 %), pyrene (0,1 %), perylene (0,2 %) and benzanthrene. Similar results were obtained by other authors (BLUMER 1961, KUMADA and SUZUKI 1961, CHESHIRE, CRANWELL, FALSHAW, FLOYD and HAWORTH 1967).

It is not yet proved, if these polycyclic aromatic compounds are partly formed by the high temperature over 200°C during zinc dust destillation.

As an example for elemental analysis the data of humic and fulvic acids from different horizons of a podzol and of a gray forest soil are depicted in the next table.

Tab. 1: Analytical characteristics of humic and fulvic acids from a podzol B-horizon.

Element	Humic acid (%)	Fulvic acid (%)
Elementary composition		
C	56,72	50,92
H	5,21	3,34
N	2,37	0,74
S	0,35	0,26
O (by difference)	35,35	44,74
Oxygen containing functional groups (m-equivalent per g dry ash-free material)		
Total acidity	5,7	12,4
Carboxyl	1,5	9,1
Total hydroxyl	6,9	6,9
Phenolic hydroxyl	4,2	3,3
Alcoholic hydroxyl	2,7	3,6
Carbonyl	0,9	3,1
Molecular weight*)	1684	669

*) The average molecular weights were determined by SCHNITZER and DESJARDINS (1962) by lowering of freezing point in sulfolane.

From this table it is to see, that the carbon content of humic acids is higher and the oxygen content is lower than in the case of fulvic acids. This is in accordance with the total acidity and may be mainly caused by the higher carboxyl content of fulvic acids.

The nitrogen content of fulvic acids is generally lower than this of humic acids. This observation will be discussed later in connection with the optical properties (lecture 5).

The aromaticity of the total organic fraction of a podzol A_o - and B_h-horizon was estimated according to the method MAZUMDAR et al. (1957) and CHAKRABARTTY et al. (1960) by WRIGHT and SCHNITZER (1961).

Tab. 2: Distribution of carbon and hydrogen in the organic fraction of two podzol-horizons (WRIGHT and SCHNITZER 1961).

	Carbon in %			Hydrogen in %		
Horizon	aromatic	in COOH-groups	aliphatic and/or alicylic	aromatic	in COOH-groups	aliphatic and/or alicylic
A_o	49	3	48	36	3	61
B_h	48	22	30	21	28	51

As the amount of aliphatic and/or alicyclic carbon and hydrogen is higher in A_o-horizon than in B_h-horizon, the organic fraction in the B_h-horizon has a more aromatic structure than the A_o-horizon. The carboxyl content is higher in the B_h-horizon.

Similar results are obtained by alkaline oxidation with sodium permanganate or with nitric acid of humic acids and determination of the isolated aromatic compounds.

1.1 Distribution of nitrogen.

The nitrogen content of humic acids is between 1 and 5 %.
By hydrolysis with mostly 6 N HCl (BREMNER 1965) can be split off

20-40 % as α-NH_2-N

10-25 % as NH_3-N

1-5 % as amino sugar-N

At least 7-12 % of the α-NH_2-N is in peptide linkage.

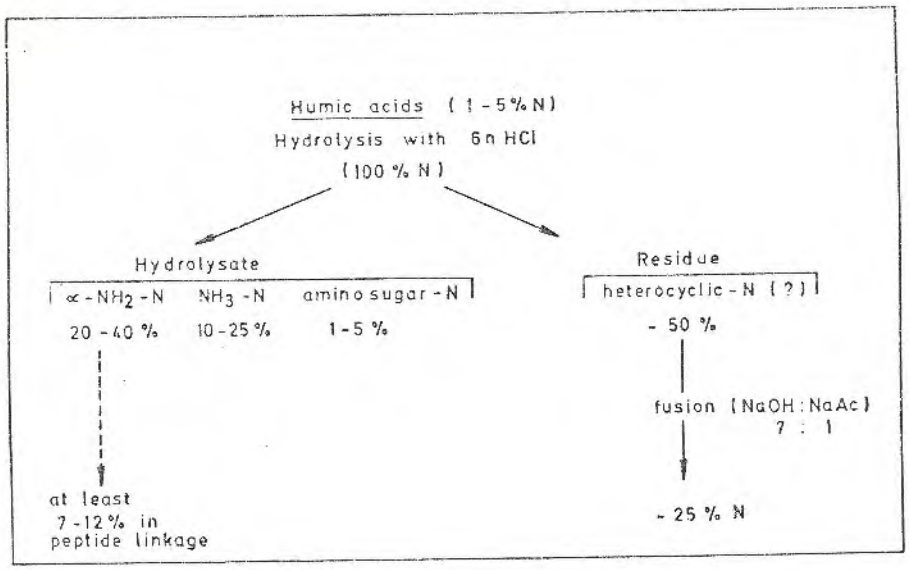


Fig. 2: Distribution of nitrogen in humic acids.

In the residue of hydrolysis about 50 % N remain, which are supposed to be bound to a large extent in heterocyclic form. In a fusion with a mixture of sodium hydroxide and sodium acetate up to 2,5 % N can split off again. According to model experiments with phenyl-glycine a part belongs to amino acids, which are not hydrolized by 6 N HCl. These data are mentioned because they are needed for the discussion of some properties of fractions of humic acids such as light absorption.

2. Problems of isolation of humic substances.

The substances, which are formed from plant material during humification consist of a mixture of various compounds. As these compounds have similar properties, the extraction of substances with a uniform composition is very difficult. Therefore the separation procedures lead to very different results according to the physico-chemical properties of the materials to be extracted and also of the extractants.

A further difficulty is the fact, that the organic substances in the soil are bound by bi- and trivalent cations and possibly by hydrogen bridge linkages of phenolic or aliphatic hydroxyl groups or of carboxylic groups and also, to a smaller extent, of amino groups with silicic clay minerals. A maximum extraction excluding larger chemical alterations is obtained only by the use of polar solvents with high dielectric constants, which increase the dispersity of humic substances or which improve the conditions of solubility by disrupting the hydrogen bonds of the fixed metallic cations and immobilize them (WHITEHEAD and TINSLEY 1964).

Difficulties of separation of humic acids from inorganic soil constituents led mainly to the investigation of humic substances of B-horizons of podzolic soils; the extraction of these is less difficult than of others (BURGES and LATTER 1960, COFFIN and DELONG 1960, DUBACH, MEHTA and DEUEL 1961, JACQUIN 1960, MARTIN and REEVE 1955, MARTIN, DUBACH, MEHTA and DEUEL 1963, SCHNITZER, WRIGHT and DESJARDINS 1958, SOWDEN and DEUEL 1961, STEELINK, BERRY, HO and NORDBY 1960, WRIGHT and SCHNITZER 1960, MARTIN and REEVE 1957 a,b).

Many extractants were used for separation of organic soil substance (see SCHEFFER and ULRICH 1960, TINSLEY and SALAM 1961, DUBACH and MEHTA 1963). With regard to the mentioned point of view, the solvents can be divided according to their chemical and physical efficiency in several groups: 1. acids, 2. strong alkali, 3. weak alkali, 4. complexing agents and 5. organic solvents.

A pretreatment with hydrochloric acid and/or a mixture of hydrochloric acid with hydrofluoric acid is applied for desorption of humic substances which are fixed on sesquioxides, free silicic acid or clay

minerals, and for removal of carbonates or exchangeable bases to increase the yield of extraction (BREMNER and HARADA 1959, CHOUDRI and STEVENSON 1957, DUBACH, MEHTA and DEUEL 1963, POSNER 1966).

The method of isolation of humic fractions is at first of principle importance for their characterization.

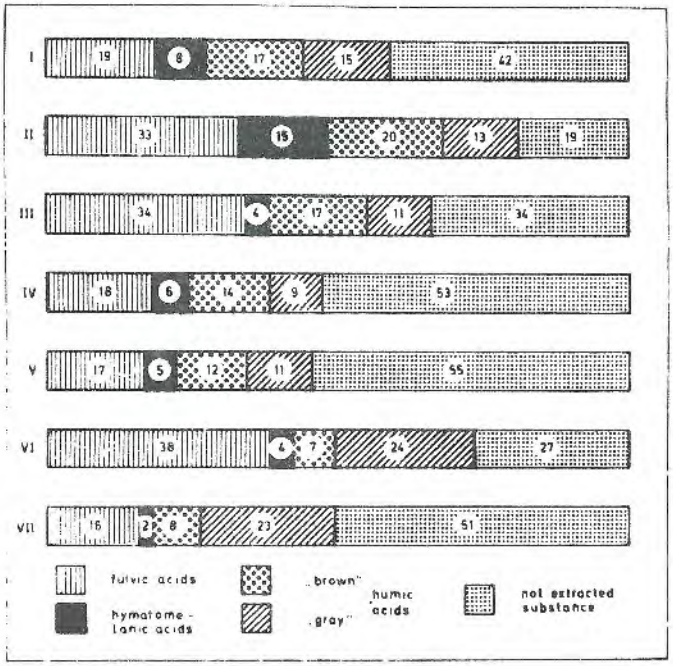
Therefore a summary of extractants, of different efficiencies , is shown for extraction of total organic soil substance of two horizons of a podzol profile (SCHNITZER et al. 1958).

Tab. 3: Efficiency of different extractants for organic soil substance from two horizons of a podzol profile (SCHNITZER, WRIGHT and DESJARDINS 1958)

Solvent	Percentage of carbon extracts from A_0-horizon	B_{21}-horizon
$Na_4P_2O_7$. 1 OH_2O	6.1	91.7
$Na_4P_2O_7$. 1 OH_2O	5.5	82.6
Na_3PO_4 . 12 H_2O	9.1	93.6
$(NaPO_3)_6$	1.2	37.0
$Na_2B_4O_7$. 1 OH_2O	7.7	80.8
NaF	6.6	88.4
NaF	4.4	88.7
NaCl	2.5	2.2
NaBr	T*	2.7
NaI	T*	3.2
Na_2CO_3	8.8	92.3
NaOH	24.8	96.3
HCl	T*	11.2
HF	T*	83.1
EDTA-Na_2	T*	97.0

T* 3 Traces

Only some of them extract soil carbon up to 90 % or more from the B-horizon. The selection of the solvent has a decisive influence on the result of fractionation. In the next figure different procedures of extraction are summarized (WIESENMÜLLER 1965).



I. = 2 % HCl cold, 1 % NaOH, cold: NEHRING (1955), TYURIN (1937)
II. = 5 % HCl, 70°C, 0,5 % NaOH, boiling: SPRINGER(1938)
III. = 5 % HCl, 70°C, 1 % NaOH, cold
IV. = 0,1 M $Na_4P_2O_7$, 0,1 N NaOH, 16 hours, cold: KONONOVA and BELCHIKOVA (1961)
V = 0,1 N $Na_4P_2O_7$, 0,1 N NaOH, 16 hours, cold
VI. = 0,1 N $Na_4P_2O_7$, 8 hours, boiling: WELTE (1956)
VII. = 0,1 N $Na_4P_2O_7$, 5 % hydryzinehydrate, 16 hours, cold

Fig. 3: Comparison of different extractants (data in % of total C from chernozem) (WIESENMÜLLER 1965).

Several facts are remarkable:

1. By the solvents less soil organic carbon is extracted in the case of chernozem in comparison to this of podzol B-horizon. This is due to the quantity and type of inorganic parts of the soil.
2. The ratio of fulvic acids to the sum of "brown" and "gray"

humic acids varies according to the procedure of extraction.

3. The ratio of "brown" to "gray" humic acids depends on the method of extraction.

These facts should lead to the conclusion that all authors should use a standardized, conventional method, that it is possible to compare the results of different investigations. We work according to the method of KONONOVA and BELSCHIKOWA, because with this method the most comparing work of isolation of humic substances from many soils in different climates have been made.

3. Fractionation of humic substances.

The exhaustive extraction of the organic soil constituents is usually followed by a treatment with acid to separate the extracts in the acid soluble fraction (fulvic acids) and in the acid precipitable fraction (humic acids). Usually humic acids are mainly purified by repeated precipitation, dialysis in acid solution and by electrodialysis (COFFIN and DELONG 1960, DUBACH, MEHTA and DEUEL 1963, EVANS 1959, MARTIN and REEVE 1955, SCHEFFER and ULRICH 1960, p. 56-99).

Principally, two different ways were followed for further fractionation of the organic parts of soil extracts:

a) Separation of mostly low molecular weight substituents, the fulvic acids and the byproducts of such as carbohydrates, proteins lignin and its degradation products from the fraction of humic acids extracted with sodium hydroxide for operation of purification.

b) Experiments for specific separation of the main fractions (fulvic and humic acids) by means of different methods of physicochemical and quantitative analysis.

Only some of the methods will be mentioned in the following.

3.1 Isolation and removal of low molecular weight substances and fulvic acids.

As previously mentioned carbohydrates, proteins and lignin are dissolved by extraction with sodium hydroxide. Therefore many attempts have been made to remove these accompanying substances with relatively mild methods. Carbohydrates and proteins have been removed by hydrolysis with acids (FORSYTH 1947 (2), SCHEFFER and KICKUTH 1961 (a,b)),or by enzymatic degradation (HOBSON and PAGE 1932, JENKINSON and TINSLEY 196o), or by precipitation with quarternary ammonium bases (COFFIN and DELONG 1960).

It is especially difficult to remove substituted aromatic constituents, which may be derived from lignin, tannins or metabolic products of microorganisms because they have similar properties. Further details shall not be mentioned. Only some methods are discussed, which seem to have more importance for future work.

3.2 Fractionated precipitation of humic acids with neutral salts ("gray" and "brown" humic acids fractions).

By flocculation with sodium chloride at pH-values between 7 and 8 purified humic acids can be separated in two fractions. The precipitated fraction is called "gray" humic acid, the part, which remains in the solution and is precipitated with hydrochloric acid, is the "brown" humic acids fraction (comp. FLAIG, SCHEFFER and KLAMROTH 1955).

By electrophoresis and by gel filtration similar fractions are obtained.

Tab. 4: Separation of humic acids in "gray" and "brown" humic acids fraction by precipitation with sodium chloride in percent of total humic acids (SCHARPENSEEL and KRAUSE 1962).

Chernozem, Hungary		Podzol, Germany B_h-horizon	
"Gray"-humic acids fraction	"Brown"-humic acids fraction	"Gray"-humic acids fraction	"Brown"-humic acids fraction
68.5 %	31.5 %	28.1 %	71.9 %

The quantity of "gray" and "brown" humic acids fractions is different according to soil type from which the humic acids have been isolated. Both fractions differ in their properties remarkably.

Tab. 5: Differences in the chemical composition of "gray" and "brown" humic acids fractions (values of ash-free substances, oxygen separately determined) (FLAIG, SCHEFFER and KLAMROTH 1955).

Chernozem	C %	H %	O %	N %	Sum	OCH_3	Ash	
Total-h.a.	57,78	3,25	34,32	3,9o	99,52	o,95	9,6o	brown-red
G-Fraction	61,6o	2,79	32,42	2,83	99,64	o,78	1o,18	dark-brown
B-Fraction	56,97	4,25	33,66	4,19	99,o7	1,6o	3,42	carmine-red
Podzol								
G-Fraction	61,62	2,96	33,93	2,18	1oo,89	o,oo	5,26	carmine-red
B-Fraction	59,4o	3,o7	36,34	1,89	1o1,1o	o,27	2,38	gray

In the "gray" humic acids fraction

the carbon and the ash content is higher

the oxygen, the nitrogen and the methoxyl content is lower.

than in the "brown" humic acids fraction.

The distribution of the functions of the nitrogen is different in both fractions.

In this connection it is mentioned, that a methoxyl content of 1,5 % would correspond to a lignin content of about 10 % lignin. This fact is interesting for investigations of separations of humic acids by means of organic solvents. Later some data are given.

The ash content of "gray" humic acids fractions consists mainly of silicic clay minerals, this of "brown" humic acids of oxides of iron and aluminium.

3.3 Gel filtration

It is not possible to discuss here the advantages and disadvantages of all methods for fractionation of humic substances, respectively humic systems or their singular fractions.

One of the most promising methods for separation of humic systems seems to be the separation with molecular sieves.

The fractionation by gel filtration is based on the fact, that those molecules, of which the particle size is larger than the pores of the hydrated gel, go faster through the column by elution with a solvent than those molecules, which are retained by diffusion inside the gel spheres. The exclusion limit is defined as the upper limit of particle size, which can be fractionated effectively. Therefore at first the larger molecules appear as bands during elution of the gel column; these are followed later by one or several bands of smaller molecules. But this sequence is influenced by the charge of the gel matrix, which leads to a specific sorption or a repulsion of analogous charged particles respectively. This fact is very important in the case of separation

of fractions of total humic systems.

The description of gel filtration will be restricted to some work about characterization of fractions of humic systems (SÖCHTIG 1966). Chernozem has been acidified with hydrochloric acid and afterwards washed with water. The alkaline extract - this means the total "humic system" - was deposited on the top of the column and eluted with three different solvents. In the case of water there are two zones on the column, in the case of NaOH one and in the case of mixed elution agent also one zone.

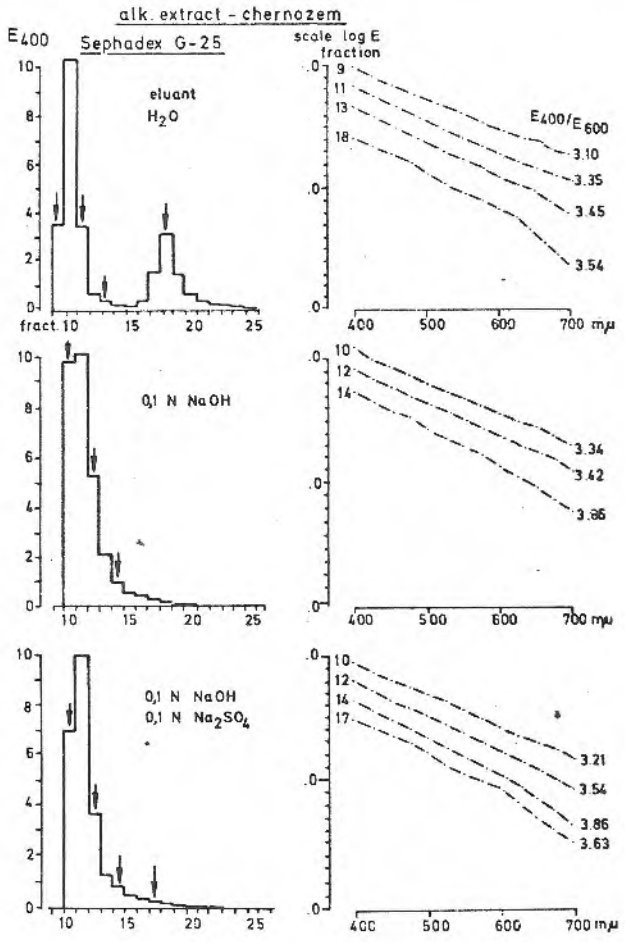


Fig. 4: Fractionation of humic substances from chernozem on Sephadex G 25 after elution with different solvents and light absorption of the fractions (SÖCHTIG 1965).

The amounts of humic substances in the different fractions have been measured by light absorption at a wavelength of 400 nm. The measurement shows the relative distribution of coloured humic substances on the column. The spectra of the fractions 9 - 14 show that these fractions have always nearly the same properties. In the case of the fraction 18 - also to a small amount in the case of fraction 17 - the spectra are slightly different. The spectra are measured from the fractions, which are marked with an arrow.

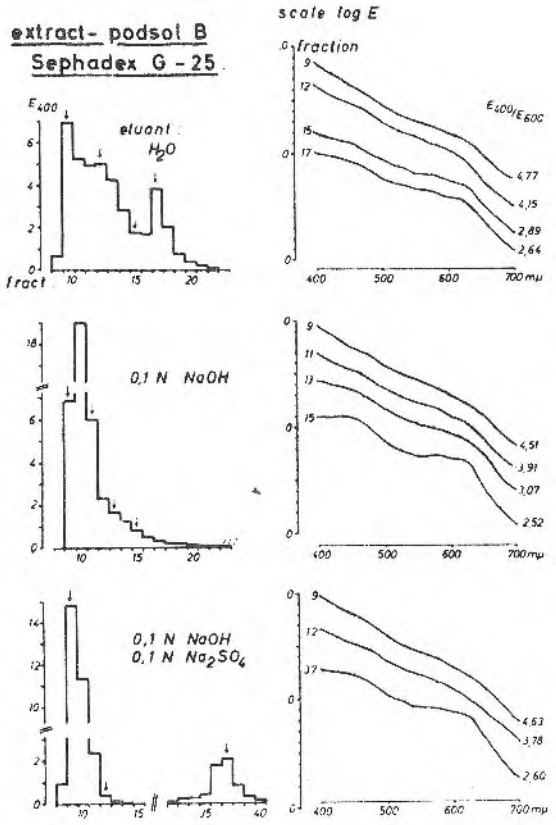


Fig. 5: Fractionation of humic substances from podzol B-horizon on Sephadex G 25 after elution with different solvents and light absorption of the fractions (SÖCHTIG 1965).

In the case of the humic substances of podzol B-horizon more zones could be observed than in the case of chernozem, with water three zones, with 0,1 N NaOH one strong and one weak zone and with the mixed elution agent two zones.

The separation of the humic system from podzol B-horizon occurs in more fractions than in the case of the extract of chernozem.

The spectra of the fractions 9 and 12 of podzol water extract are comparable with the spectra 17 and 18 in the case of the extract of chernozem and have no distinct maxima. The spectra of the fractions between 15 and 17 and especially the spectra of 37 are remarkably different and show several maxima. These substances are more absorbed and possibly of lower molecular weight. The different spectra may be caused by functional groups of the coloured humic substances.

We investigated humic substances of about 15 samples of chernozem and podzol B-horizon. In each case the elution diagrams could always be differentiated into two groups. They were the same for the group of chernozems on the one hand and podzols on the other.

The different eluted fractions have not only been measured by light absorption to determine the content of coloured substances, but also the conductivity of the fractions has been determined. In the case of water as solvent it could be shown, that the fractions 14 and 15 have been eluted, after the highest concentration of salts were washed out.

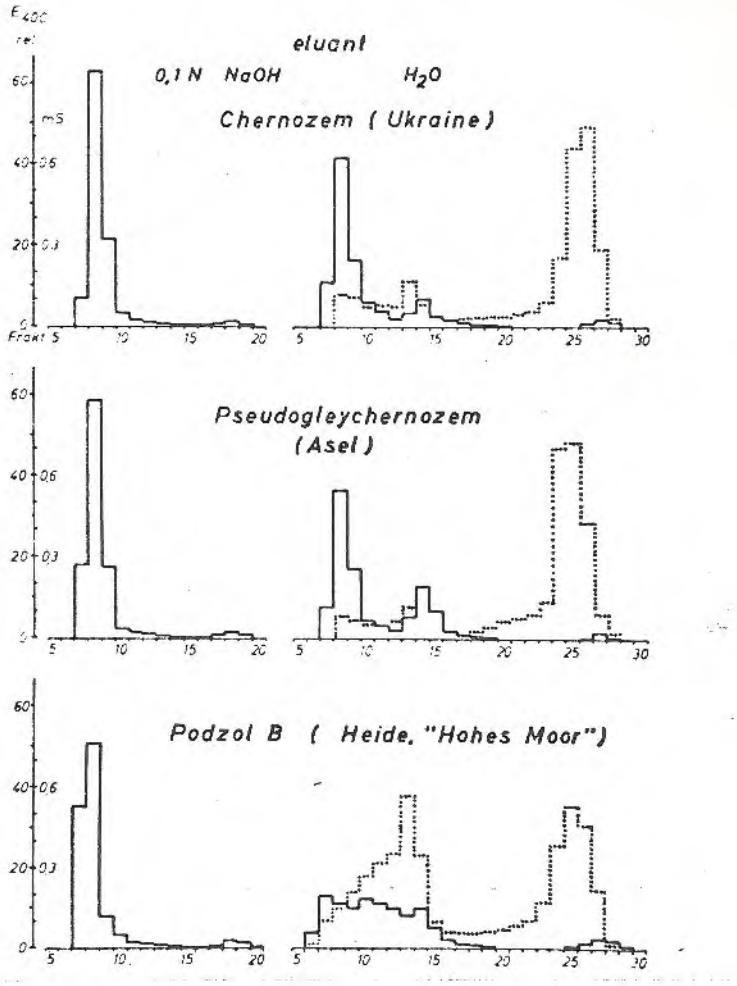


Fig. 6: Distribution of alkaline extracts from two chernozems (Ukraine and Asel) and of podzol B-horizon with 0,1 N NaOH and water on Sephadex G 25 Dotted lines conductivity (SÖCHTIG 1965).

The fractions 25 - 30 occurred, when the NaOH was eluted. These findings show, that a relation exists among the absorption of the coloured substances, the salt concentrations and the elution. The main effect of the Sephadex Gel 25 is caused in this case by adsorption of the humic substances mainly on the surface of the gel and not by the properties of a molecular sieve, by the distribution of particle weight and size. Therefore the determination of particle weights of humic substances is not complete sure and must be proved by other methods in addition. By gel filtration also fractions of humic acids can be obtained.

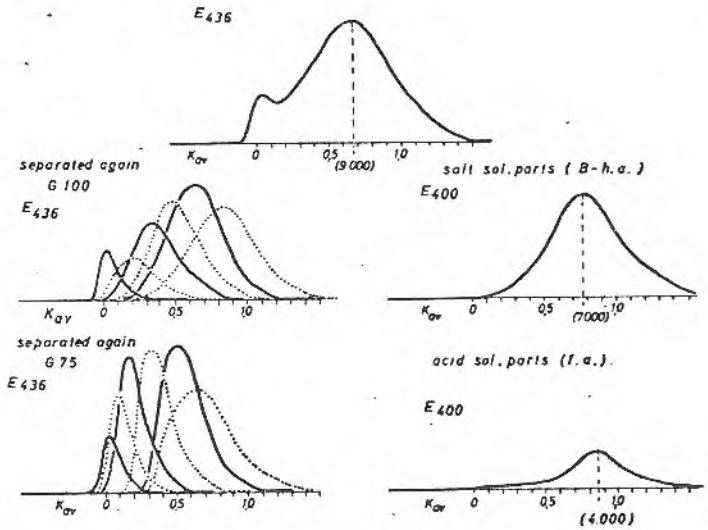


Fig. 7: Separation of humic substances from podzol B with 0,04 N $Na_2B_4O_7$ with Sephadex gel G 100 (SÖCHTIG 1968).

In figure 7 the curve of the distribution of the possible particle weights of the humic substances from podzol B-horizon on Sephadex G 100 eluted with 0,04 N $Na_2B_4O_7$ is shown. When one takes different fractions with always the same parts of volume during the elution procedure and separates them again with the same techniques, then one gets curves of particle weight distribution which correspond to the separated fractions.

When one adds the single curves, the summary curve shows the same course as the original distribution curve of the total extract. This is also the case, when one uses Gel 75 (below left).

When the different parts of the eluted solutions are made 1 N with Na_2SO_4, a part of the coloured substances, the "gray" humic acids fraction, is precipitated with a found particle weight of about 9000.

According to the definition the salt soluble parts are the

"brown" humic acids (B-h.a.) and the fulvic acids (f.a.). The precipitates have been centrifuged and the light absorption of the supernatent has been measured. This part of the "brown" humic substances would have an average particle weight of 7000.

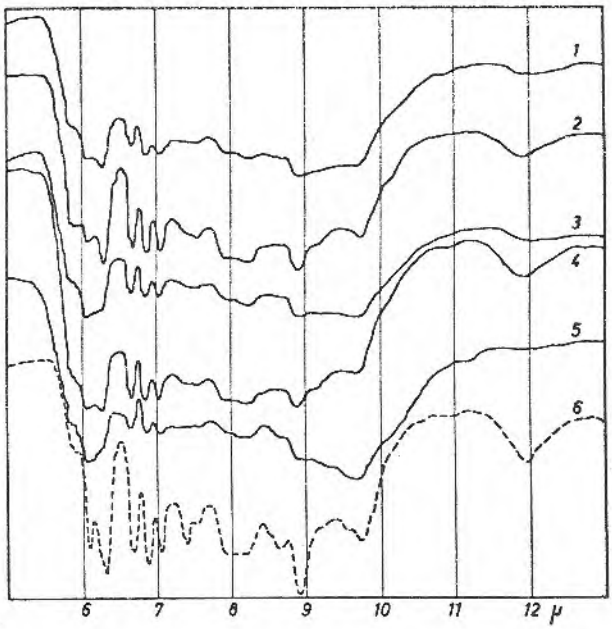
When the different parts of the eluted solutions are acidified with sulphuric acid to pH 1, the acid soluble part, the fulvic acids show an average particle weight of 4000.

These investigations have been undertaken with two aspects. Firstly the method serves for the characterization of soil organic matter in dependence on its formation and degradation under different environmental conditions and soil management. Secondly it will be helpful for the elucidation of the so-called "humus-effect" on plant growth and development, because by this method fractions of humic substances are obtained with the smallest loss of material and without impurities of inorganic salts in the solutions. When one extracts with water (fig. 6) the physiological experiments are not falsified by disturbing salt concentrations.

4. Contribution to the problem of fractionation of humic acids.

A summarizing review of the main methods of fractionation indicates that all so-called "humic substances" prepared by conventional methods in nearly no case lead to substances of chemical uniformity.

So for instance a fraction, defined as "humic acids"was isolated with a mixture of 0,1 N sodium hydroxide and 0,2 N sodium fluoride from decomposed rye straw, which was extracted before with ether and water (FLAIG and TROJANOWSKI, unpublished).



1. Total extract
2. Alcohol soluble fraction
3. Alcohol insoluble fraction
4. Acetone soluble fraction from (3)
5. Acetone insoluble fraction from (3)
6. Björkman lignin from straw

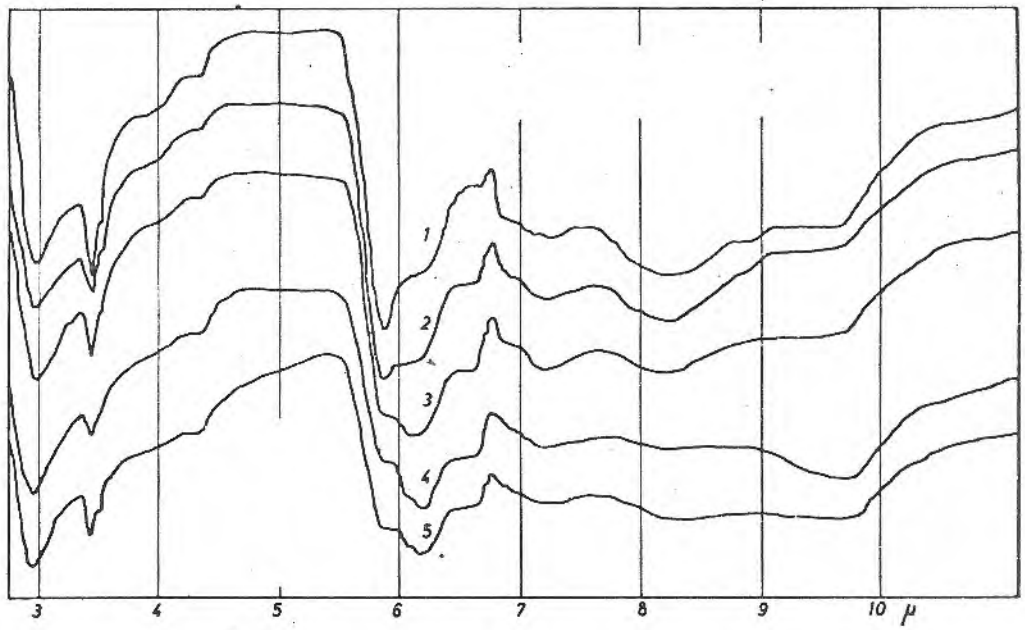
Fig. 8: Infrared spectra of humic acids fractions from decomposed rye straw prepared with different solvents (SALFELD 1964).

After the usual purification by repeated precipitation a product (1) was obtained from which a fraction (2) was extracted with 95 % alcohol. The yield of this fraction decreased with increasing time of decomposition. This fraction was very similar in elemental composition and light absorption properties to the original straw lignin (6), whereas the residue of the extraction (3) became more and more like humic acids isolated from soils. By treatment of the alcohol insoluble residue with aqueous acetone a fraction (4) was obtained whose properties are also

more like lignin than those of the part remaining insoluble in acetone (5) (SALFELD 1964).

A comparison of the infrared spectrum of Björkman lignin (6) (fig.8) with the alcohol (2) and acetone soluble (4) fractions shows, that from the humic acids fraction a relative large portion can be isolated which is nearly unchanged lignin according to its spectrum. The spectra of the insoluble residues are somewhat similar to those of the humic acids.

Similar observations can be made also by separation of fractions of humic acids such as a "brown" humic acids fraction by extraction with tetrahydrofurane (THF) of different water content (SALFELD 1964).



1. 5 % H_2O (39 % soluble of initial material)
2. 10 % " (10 % " " " ")
3. 25 % " (24 % " " " ")
4. Residue (25 % insoluble)
5. Initial material

Fig. 9: Infrared spectra of the subfractions from "brown" humic acids isolated from chernozem and extracted with tetrahydrofurane of different water content (SALFELD 1964).

The infrared spectra show a relative decrease of the carbonyl absorption at 5,9 µ (1695 cm^{-1}) followed by a reduction of the hydroxyl vibration, which means a decrease of the free carboxylic groups. On the other hand the C=C-vibration at 6,2 µ (1610 cm^{-1}) tends to strengthen and may be adjoined to increasing aromatic double bonds. The absorption of the carboxylic group at 7,2 µ (1390 cm^{-1}) decreases with increasing water content similar to the decrease of the carbonyl function.

The elemental composition is in good agreement with the infrared analysis. The oxygen and nitrogen content increases, whilst the methoxyl content decreases.

Not only the elemental analysis changes, but also the slope of the electronic spectra.

KYUMA (1964) obtained similar fractionation series by successive precipitation with alcohols in alkaline solution.

Tab. 6: Elemental composition of fractions of "brown"-humic acids of chernozem isolated with tetrahydrofurane (SALFELD 1964)

	ash in %	C+	O+	N+	OCH_3	log ++ 400-600mµ
1. Fraction 5% H_2O/THF	1.29	58.81	33.20	2.79	3.00	0.82
2. Fraction 25%H_2O/THF	3.72	55.65	33.02	5.89	2.08	0.68
3. Residue	6.73	53.17	34.59	5.50	1.49	0.58
5. Initial material	3.18	56.04	33.64	4.91	2.13	0.66

\+ In % of ashfree substance (H no differences)
++ measured in 0.1 N NaOH

These examples demonstrate, that the chemical and physical properties of the humic acids depend strongly on the methods of isolation.

In spite of this, any different isolated fractions are e.g. called "humic acids". Therefore it should be demanded, that a sample of

used humic acids is isolated by a conventional standard method, especially when other investigations are made with different methods. This is the only way, whereby the various authors can draw conclusions from their results to the materials for comparison (FLAIG 1964 (2), KONONOVA 1964).

Summary of comments on the chemistry of formation of humic substances.

The basic research work about chemical composition of humic substances in regard to their phenolic and nitrogenous structure units is summarized in the following slide.

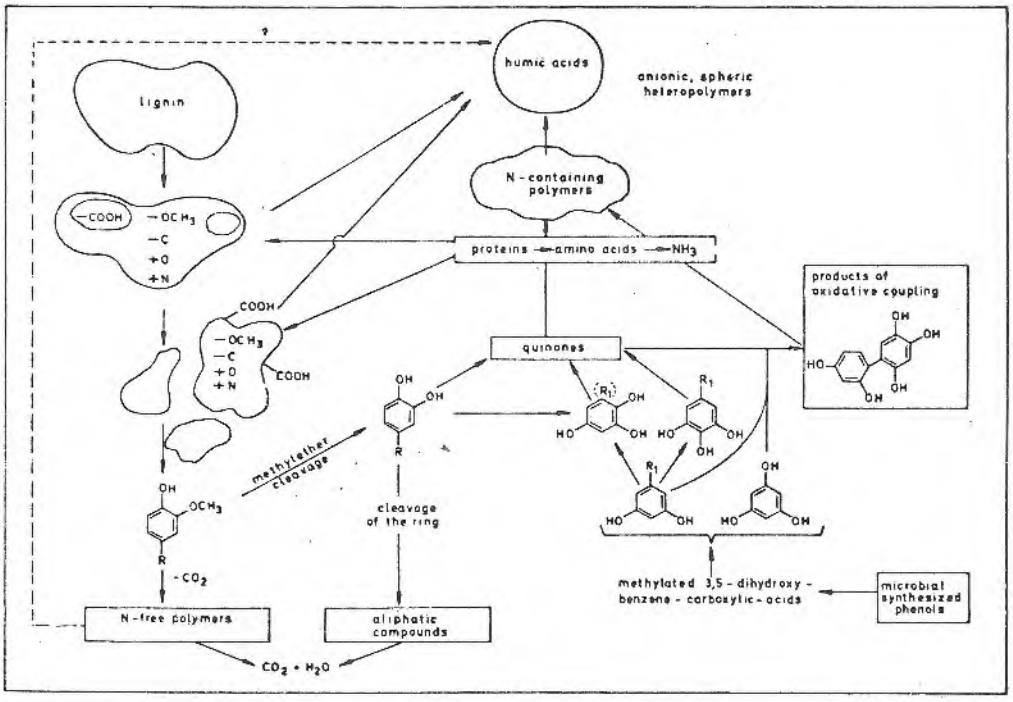


Fig. 10: Contribution of phenolic and nitrogenous structure units to chemical composition properties of humic substances.

The depicted scheme concerns only with the chemical problems of formation of humic substances and therefore does not give complete information about other processes which occur during humification. The scheme shows that there are two essential sources of phenols for the formation

of humic substances in nature.

1) The formation of phenols from lignin occurs mainly by its microbial degradation, whereby the cleavage of C-C bonds, of ether linkages and of the aromatic ring plays an important role. Thereby the structure of the lignin molecule is disrupted. Larger or smaller degradation products are found, which contribute to the composition of humic substances by reactions with nitrogenous compounds in all phases of the degradation. Methylether cleavage plays an important role for nitrogen fixation.
2) The microbial synthesized phenols of mainly resorcinol and phloroglucinol type contribute to the composition of humic acids after transformation by hydroxylation to phenols oxidisable to quinones and after reactions with nitrogenous compounds or by oxidative coupling with quinones.

It is not known how far the nitrogen free polymers participate in the formation of humic substances. They are available for the microorganisms as a carbon source.

The aromatic compounds derived from lignin or synthesized by microorganisms disappear from the equilibrium of compounds which exists during the formation of humic substances by cleavage of the benzene ring and formation of aliphatic acids.

Many questions still remain to be answered; it is not known to which extent the high molecular or the low molecular weight fractions of lignin or its transformation products participate in the formation of various groups of humic substances conventionally defined as fulvic acids, hymatomelanic acids, humic acids and humins. Furthermore

it is not yet clear, which of the condensation products of phenolic and nitrogen containing compounds are the most stable under soil conditions, namely lignins altered in their reactive groups and condensed with nitrogen containing compounds or the nitrogen containing polymers formed by the polymerisation of lignin degradation products and other phenolic compounds. For soil productivity the availability of the organic bound nitrogen is important.

Therefore basic research work about nitrogen fixation and about nitrogen release from soil organic matter is necessary to understand these processes in connection with their importance of crop production.

Now some remarks, which belong to the physical properties of humic substances.

In the course of the mentioned reactions, the formation of heterocyclic compounds also occurs, which may contribute to the colour, an essential property of humic substances.

The chemistry of the mentioned structure units leads to results, in which way higher molecular weight substances are formed.
It will be demonstrated that the humic acids are anionic, spherical shaped heteropolymers.

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