

CONTRIBUTIONS OF SOIL ORGANIC MATTER

TO PRODUCTION POTENTIAL OF SOILS AND

SOME PROPOSALS FOR PRACTICAL APPLICATION

Wolfgang FLAIG

Annex II

to the report of W. FLAIG

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CONTRIBUTIONS OF SOIL ORGANIC MATTER

TO PRODUCTION POTENTIAL OF SOILS AND SOME PROPOSALS

FOR PRACTICAL APPLICATION

Wolfgang FLAIG

1973

Preface

The increase of yield/ha is important for the food production of Indian Population. For this purpose firstly high yielding varieties of rice, wheat and other crops were bred. These demand a higher quantity of mineral fertilizers. Fertilization alone does not allow the realization of the full genetical potential of cereals. The inorganic ions of the fertilizers enhance also microbial activity. The microorganisms use the carbon from soil organic matter as source for energy and reproduction. As a consequence of decrease of soil organic matter unfavourable processes in soil, the substrate for production occur, which cause a reduction in plant production. Therefore an increase of yield by fertilization alone is limited.

Another way to overwhelm the biological border for the use of the genetical potential of cereals is to utilize the effect of bioregulators on plant metabolism, such as CCC, "Cycocel" to increase the resistance against lodging of wheat. There are some other substances in soil organic matter, which have bioregulating effects under unfavourable growth conditions. This influence of soil organic matter on yield is well known by the farmers as "humate-effect".

Therefore, it is evident, that special procedures for maintaining a critical status of soil organic matter should be followed. More research about humus is necessary. An intensive cereal production is then economical only, when yields are always maintained at a relatively high level. One should try to minimize the yield depressions by appropriate procedures.

The lectures are an introduction into the problems. The transfer of results of basic research into practice are mentioned and the experiences about the utilization of new findings are summarized.

I have to thank Mr. M.S. Sachdev (M.Sc.) for his indefatigable help, for the revision of the lectures and for compiling the references.

Wolfgang FLAIG

New Delhi, 29. September 1973

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Lecture - 5

BIOSYNTHESIS OF HUMIC SUBSTANCES- ESPECIALLY THE
PARTICIPATION OF NITROGENOUS COMPOUNDS

1. Principles of formation of humic substances
2. Phenols as building blocks of humic substances
 - 2.1 Lignin degradation products
 - 2.2 Microbial synthesized phenols.
3. Different reactivity of phenols in participating in humic substances formation
 - 3.1 Biochemical degradation of synthetic lignins and lignin containing materials.
 - 3.2 Degradation of straw by different types of microorganisms.
 - 3.3 Influence of clay minerals in microbial synthesis.
4. Participation of nitrogenous substances in the formation of humic systems
 - 4.1 Conditions of formation of N-free polymers.
 - 4.2 Conditions of formation of nitrogenous polymers.
5. About the linkages of amino acids, peptides and proteins in humic acids.
 - 5.1 Nucleophilic addition of nitrogen compounds by phenols during oxidation
 - 5.2 Oxidative deamination of amino acids
 - 5.3 Properties of substances humic acids precipitable with acids
 - 5.4 Distribution of the carbon skeleton and nitrogen constituents of the amino acids in the reaction products and in the solutions
 - 5.5 Mechanism of nucleophilic addition and oxidative deamination of amino acids by phenols.
6. Stabilization of amino sugars

7. Autoxidation of phenol derivatives in the presence of ammonia at pH values about 8.
 - 7.1 1,2- or 1,4-Diphenols
 - 7.2 1,3-Diphenols
8. Random polymerization of tyrosine as a model for the participation of heterocyclic nitrogen in formation of the spheric shaped humic acids
9. Summary of comments on the biochemistry of formation of humic substances.

Biosynthesis of humic substances, especially the participation of nitrogenous components:

1. Principles of formation of humic substances

In the last 10 years, the biochemistry of soil organic matter has made remarkable progress by combined use of improved operatives for physio-chemical measurements and by the use of ^{14}C and ^{15}N and also by ^3H (radioactive and stable isotopes).

Before going in details I recall to the scheme of the synthesis of humic substances which I showed in my introductory lecture. In the scheme the present knowledges are summarized.

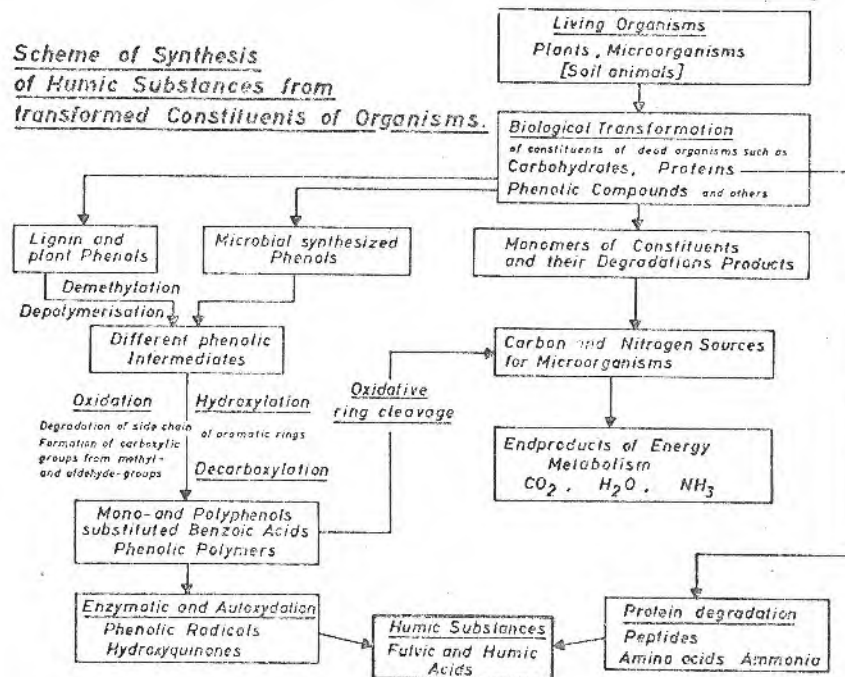


Fig. 1: Scheme of synthesis of humic substances from transformed organic constituents of organisms.

The organic constituents of plants and microorganisms are the most important initial materials for the transformation during the process of humification.

Carbohydrates, proteins and phenolic compounds belong to the most important substances which take part in the reactions. On the one side they are used for microbial metabolism as carbon and nitrogen source, on the other side for the formation of humic substances. Carbondioxide, water and ammonia are the final product of the energy metabolism of microorganisms.

Phenolic compounds are formed from lignin and other plant phenols or are synthesized by microorganisms.

The first step of lignin degradation are cleavage of methylether linkages and degradation of the high molecular weight in more low molecular weight components. On this way, different phenolic intermediate are formed which are mainly transformed by oxidation, hydroxylation and decarboxylation.

In the case of phenol acrylic acids a shortening of the side chain by 2 carbon atoms occurs. In the case of microbial synthesised phenols methyl groups are oxidised to carboxyl group. In both cases decarboxylation occur as the further reaction.

Hydroxylation introduces new hydroxyl groups mainly in ortho- and para-position in aromatic rings.

During all these reactions mono and polyphenols as well as substituted benzoic acids are formed. Some of the phenols are very reactive. Polymers are formed which are different in composition compared with that of lignin.

Diphenols with hydroxyl groups in ortho-position formed by the mentioned reactions are transformed in aliphatic keto acids by the cleavage of the ring. These aliphatic compounds are used by microorganisms as carbon source. In connection with the formation of humic substances this reaction is in so far of interest. As in this way phenolic compounds disappear from the pool of substances, which can participate in the formation of humic substances.

Many reactions occur during the formation of humic substances through phenolic radicals or through hydroxyhydroquinones. These compounds are formed either by phenol oxidising enzymes or by autoxidation.

Finally the humic substances are formed by condensation between phenolic compounds formed during the above mentioned reaction series and protein degradation products such as peptides, amino acids and ammonia. They are separated by different conventional methods in different fractions such as humic acid, fulvic acids and humins.

2. Phenols as building blocks of humic substances:

As just mentioned lignin and its degradation products on one side the microbial synthesized phenols/^{on the other} are the most important sources for phenolic compounds which participate in formation of humic substances.

2.1 Lignin degradation products:

The lignin content is between 10-30% according to species of plant. Therefore, lignin may be supposed as one of the most important initial material for formation of humic substances.

Lignin degradation products with low molecular weight such as phenol acrylic - and phenol carboxylic acids have been identified from humified plant material (MAEDER 1960; FLAIG 1962) from peat (BELAV, 1967; SÖCHTIG and MACIAK, 1971) or from soils (BURCKERT, JAQUIN, and METCHE, 1967; SCHNITZER, 1971 and others).

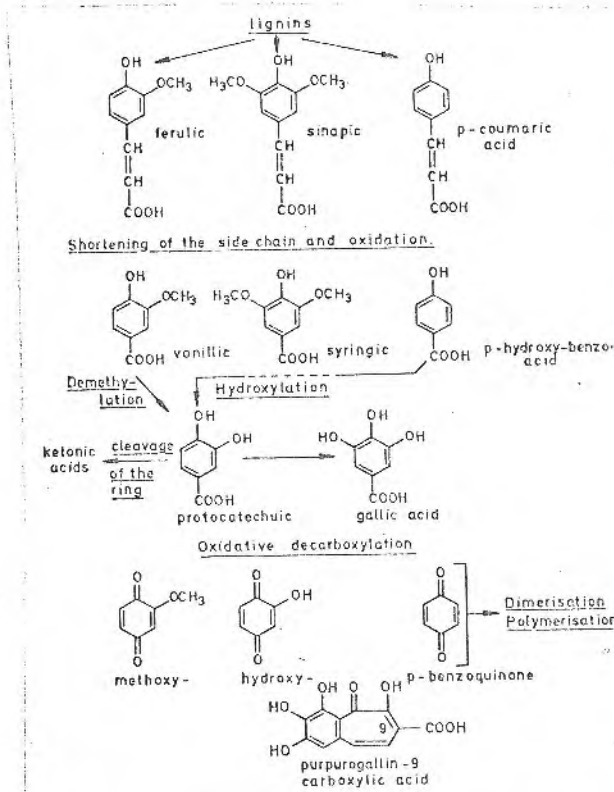


Fig. 2: Transformation of Lignin degradation products.

The most substantial transformations of lignin degradation

products are:

- 1) The shortening of the side chain of phenol acrylic acids and formation of phenol carboxylic acids.
- 2) Demethylation of phenol ether to phenol carboxylic acids with hydroxyl groups in ortho-position (i.e. transformation of vanillic acid to protocatechuic acid)
- 3) Hydroxylation of phenol carboxylic acids to such acids with hydroxyl groups in ortho-positions (i.e. p. hydroxy benzoic - in protocatechuic - and gallic acid).
- 4) The oxidative decarboxylation to hydroquinone derivatives (i.e. transformation of vanillic acid in methoxy-hydroquinone).
- 5) The cleavage of the ring in the case of phenol carboxylic acids with hydroxyl groups in ortho-position to aliphatic keto-acids.
- 6) Dimerization and polymerization reactions.

2.2. Microbial synthesized phenols

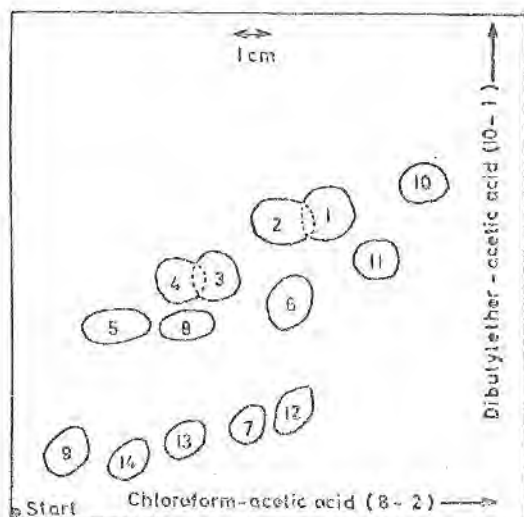
By the work of BURGESS, HURST and WALKDEN (1963, 1964) or MORRISON (1963) and FARMER and MORRISON (1964) it is known that besides the phenolic compounds which can be derived from lignin, also phenols derived from flavonoids or synthesized by microorganisms are important initial material for the formation of humic substances. These authors isolated by reductive or oxidative cleavage of humic acids also compounds

which belong to 1,3-di- or 1,3,5- triphenols, and which cannot be derived from lignin or its degradation products. The participation of phenols of resorcinol type in the formation of humic substances has been demonstrated (HAIDER and MARTIN, 1967, 1968, 1970; MARTIN and HAIDER, 1969, 1971; MARTIN, RICHARDS and HAIDER, 1967). The following reactions occur for instance in the culture of microorganisms such as *Epicoccum nigrum*,

But it should be mentioned that other micro-organisms synthesise still other phenoles (Summary: MARTIN and HAIDER, 1971).

Epicoccum nigrum forms dark coloured substances with properties which are comparable with those of the humic acids.

Different resorcinol derivatives could be identified after reductive cleavage of the microbial synthesized humic acids.



Phenols recovered after Na-amalgam reduction of *E. nigrum* humic acid. Two dimensional thin layer chromatogram on silica gel (GF) plates. Spots located with diazotized sulfanilic acid reagent or with ultra-violet light. Phenols identified as follows: 1 = Orcellinic acid; 2 = 2,4-dihydroxy benzoic acid; 3 = 2,4-dihydroxy toluene; 4 = Orcinol; 5 = 3,5-dihydroxybenzoic acid; 6 = 2,4,5-trihydroxy toluene; 7 = 2,3,5-trihydroxy toluene; 8 = Protocatechuic acid. Found only when caffeic acid was present in culture solution.; 9 = 2,4,6-trihydroxybenzoic acid; 10 to 13 = Unidentified, yellow-orange or pink-violet color with reagent. 14 = Unidentified. Located with ultraviolet light.

Fig. 3: Two dimensional thin layer chromatogram of phenols after reductive cleavage of humic acids from culture of *Epicoccum nigrum* with Na-amalgam (Martin, et al. 1967).

Some more phenolic intermediates could be isolated from the culture media:

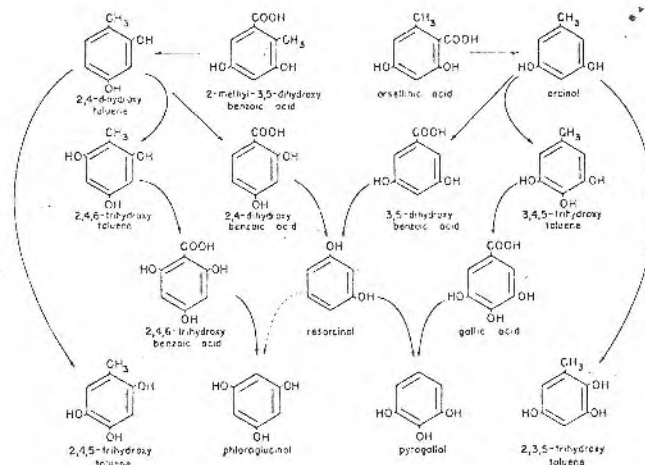


Fig: 4: Synthesis and transformation of phenols by microorganisms (*Epicoccum nigrum*) (Haider and Martin, 1967).

Summarising all results, it can be established orsellinic acid was identified, this if formed through the acetate - malonate metabolism of the organism. 3,5-Dihydroxytoluene (orcinol) is formed by decarboxylation. By oxidation 3,5-dihydroxy-benzoic acid is formed, by decarboxylation resorcinol, but it is seldom found, 3,5-Dihydroxytoluene, 6-methyl-hydroxy-hydroquinone (2,3,5-trihydroxytoluene) and 4-methyl-pyrogallol furthermore gallic acid are formed by hydroxylation. Presumably, pyrogallol is formed by the decarboxylation of gallic acid.

Cresorsellinic acid, which could also be identified, may be formed through the aliphatic acid metabolism. By similar reactions as in the case of orsellinic acid, 2,4-dihydroxytoluene, 2,4-dihydroxybenzoic acid and also resorcinol are formed. The hydroxylation of 2,4-dihydroxytoluene leads to 2,4,6-trihydroxytoluene and 5-methyl-hydroxyhydroquinones (2,4,5-trihydroxytoluene).

In the culture solution of the fungus also substances such as p-hydroxy-benzoic-, protocatechuic-, gallic and p-hydroxy^cinnamic acid could be identified to small amounts. These acids are formed through the carbohydrate metabolism by the reaction of phosphoenolpyruvic acid with erythrose-4-phosphate to shikimic acid and then by some further metabolic pathways. In culture solution no methoxyderivatives such as vanillic- or syringic acid could be found.

The 5- and 6-methyl-hydroxyhydroquinones (2,4,5- and 2,3,5-trihydroxytoluene) as well as pyrogallol and its methyl- and carboxyl derivatives are responsible for the formation of the higher molecular weight humic acids like substances in the culture media of *Epicoccum nigrum*, because resorcinol derivatives do not react with amino acids under these conditions and do not form this type of nitrogenous compounds.

In this connection it must be mentioned that hydroxyhydroquinone is formed after the enzymatic oxidation of different lignin degradation products in microbial cultures from protocatechuic decarboxylation acid and by further oxidation of this acid in the presence of phenol oxidases (FLAIG and HAIDER 1961; HAIDER, LIM and FLAIG 1964).

These derivatives of polyphenols can be oxidised to quinones in the culture media at pH-values of 6 to 8, whilst this is not the case with derivatives of resorcinol and phloroglucinol.

This means, that generally compounds, which are able to form quinones, would be important for the formation of nitrogenous humic substances.

3. Different reactivity of phenols participating in humic substances formations.

Among the identified phenols isolated after oxidative or reductive cleavage of humic acids, or isolated from soils or synthesized by microorganisms, there exist two types of phenolic compounds, which are of interest in studies about the structure and therefore also of the chemical and physical properties of humic acids.

In the following scheme the different reactions are mentioned:

After demethylation of lignin degradation products with side chains of 1 or 3 carbon atoms different phenols are formed which possess two or several hydroxyl groups in o-position. Dimerisation and poly-merisation occur during oxidation. Many microbial synthesized phenols have two or three hydroxyl groups in m-position. By hydroxylation also phenols are formed with OH-groups

in *o*-position.

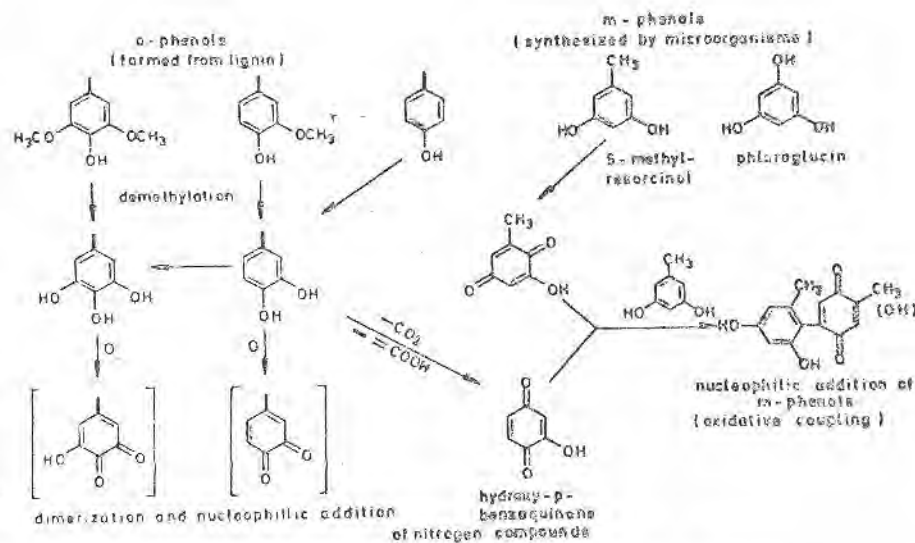


Fig. 5: Transformation of polyphenols under oxidising conditions.

The compounds with OH-groups in *o*- or *m*-position differ widely in their reactivity. Catechol, pyrogallol, hydroxyhydroquinone derivatives add nucleophilically derivatives of resorcinol and phloroglucinol (MUSSO et al. 1965) as well as proteins or their products of hydrolysis during oxidation to quinonoid intermediates. Phenols with OH-groups in *m*-position do not. Phenolcarboxylic acids are transformed by decarboxylation to hydroquinone derivatives and by further oxidation to quinones.

The differences in reactivity are important with respect to the function of nitrogen in the molecule of humic acids. The oxidative coupling leads to ramification and increases aromaticity.

3.1 Biochemical degradation of "synthetic lignins" and lignin containing material.

The degradation of the single carbon atoms in the molecule of lignins can only be investigated, when these are labelled.

For this purpose extensive studies have been made. (HAIDER, LIM and FLAIG, 1964; HAIDER 1965; LIM 1965; FLAIG and HAIDER 1968; HAIDER and MARTIN 1968). Variously labelled lignin monomers have been polymerised by Freudenberg's mushroom phenoloxidase method (1962, 1964a,b). According to FREUDENBERG (1964a,b) and KRATZL et al. (1957) these "synthetic lignins" are identical in their physical properties and chemical composition with those isolated from plants.

Table 1: Carbon dioxide released from variously labelled synthetic lignin by the activity of fungi (according to HAIDER 1965, HAIDER and MARTIN 1968).

Organism	Pleurotus ostreatus			Stachybotrys chartarum
	10 days			28 days
Incubation time				
Polymers from	Coniferylalcohol (labelled)	Coniferyl- (labelled) + p-Coumaryl- + Sinapylalcohol	Sinapyl- (labelled) + Coniferyl- p-Coumarylalcohol	Coniferyl- (labelled) + Coumaryl- + Sinapylalcohol
-O ¹⁴ CH ₃	4,5*)	3,8	7,0	13,2
-CH=CH- ¹⁴ CH ₂ OH	4,4	4,5	8,4	19,2
-CH- ¹⁴ CH-CH ₂ OH	2,6	2,5	-	-
- ¹⁴ C ₁₋₆ -Ring	14	22	-	9,7

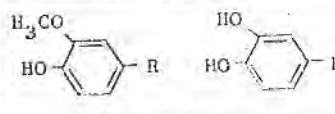
All values in percent of added activity

The carbon atoms of variously labelled synthetic lignins are split off in cultures of *Pleurotus ostreatus* and *Stachybotrys chartarum* with different rate, which can be followed by determination of active carbon dioxide. Generally the carbon atoms of the methoxyl groups and carbinol carbon atoms are split off faster than the other carbon atoms. In the first 10 days a strong cleavage of the aromatic ring occurs in the culture of *Pleurotus ostreatus*. After 14 days however, it decreases and the percentage of the

cleavage of the carbon atoms of the alcoholic and the methoxyl group increases. Phenoloxidases are activated by phenol carboxylic acids. However, in case of *Stachybotrys chartarum* after 28 days the values for released labelled carbondioxide caused by ring cleavage were lower than by degradation of the carbinol group in the side chain.

Some fungi synthesize humic acid-like polymers in the culture solution or in their mycelium. By investigations with labelled compounds it is possible to determine, to which extent the carbon atoms either of labelled "synthetic lignis" or of added phenolic lignin degradation products participate in formation of humic substances.

Tab. 2: Participation of carbon-14 from synthetic lignins or from phenolic degradationproducts in formation of humic acid-like polymers in cultures of fungi (according to HAIDER and MARTIN 1967, 1968).

Stachybotrys chartarum, 4 weeks				Epicoccum nigrum, 6 weeks				
Synthetic lignin Coniferyl (labelled) + p-Coumaryl- + Sinapylalcohol				 R = -CH=CH-COOH				
	¹⁴ CO ₂	Humic acids of Solution	Humic acids of Mycelium		¹⁴ CO ₂	Humic acids	¹⁴ CO ₂	Humic acids
-O ¹⁴ CH ₃	13,2	9,6	4,9	-O ¹⁴ CH ₃	58,4	9,8	-	-
¹ -CH= ² -CH- ¹⁴ ³ CH ₂ OH	19,8	4,6	3,1	³ -CH= ² -CH- ¹⁴ ¹ COOH	-	-	54,8	1,3
				-CH= ¹⁴ CH-COOH	45,6	6,8	52,1	6,2
¹⁴ CH=CH-CH ₂ OH	9,7	16,7	14,6	¹⁴ CH=CH-COOH	14,7	15,5	-	-
- ¹⁴ C ₁₋₆ -Ring	9,6	21,6	17,6	- ¹⁴ C ₁₋₆ -Ring	6,6	41,0	6,5	46,0

All values in percent of added activity.

In the cultures of *Stachybotrys chartarum* humic acids can be precipitated by mineral acids after separation of mycelium or isolated from the mycelium by extraction with dilute sodium hydroxide. In case of *Epiconium nigrum* the humic acids were obtained by precipitation with acid after separation of the mycelium by centrifugation. Both strains form the humic acid-like polymers from aliphatic carbon sources. The dark coloured polymers do not differ very much in their properties from humic acids isolated from soils or peat.

Furthermore, it is known, that both strains synthesize phenols in their metabolism. The metabolic pathways for synthesis of those phenols have been mentioned which must be present for formation of humic acid-like polymers (MARTIN, RICHARDS and HAIDER 1967, HAIDER and MARTIN 1968). Furthermore, it was demonstrated that the presence of phenolic lignin degradation products in the culture solution increased the quantity of humic acids remarkably.

In case of addition of synthetic lignin as well as ferulic acid or caffeic acid the values of the labelled carbon dioxide indicate that the carbon atoms of the methoxyl, carbinol or carboxyl group, take part much more in the release of carbon dioxide than in formation of humic acids.

Furthermore, it could be shown, that the shortening of the side chain of phenolacrylic acids occurs mainly at the double bond. The formed C₂-degradation products, oxalic or glyoxylic acid,

are easily available for microorganisms. For this reason the radioactivity of carbondioxide is high and this of humic acids low, when ferulic acid was labelled at the carbon atom 2. The most important fact for formation of humic substances is the relatively high activity of humic acids after addition of ring labelled compounds. The aromatic parts of lignin or its phenolic degradation products participate in formation of humic acids.

The numbers indicate, that also degradation products with one carbon atom in the side chain may have taken part in the formation of humic substances. This may be concluded by the fact that in the case of added compounds with carbon atoms labelled in 1-position (i.e. coniferyl alcohol respectively 3-position i.e. ferulic acid) the values of activity are higher in the humic acids than by addition of compounds labelled in other carbon atoms of the side chain.

These experiments demonstrate, that lignin or its phenolic degradation products take part in the formation of humic acids after several chemical transformations considerably.

For some further discussions it shall be mentioned, that the total nitrogen content of the microbial synthesized humic acids ranges between 6 and 7%. The quantity of α -NH₂-nitrogen is more than the half of total-N after a short time of cultivation and decreases with time to a fifth.

3.2 Degradation of straw by different types of microorganisms

Further experiments have been made about the decomposition of lignin containing material of such as wheat straw:

Table 3: Phenol synthesizing soil-fungi and Basidiomycetes not phenol synthesizing (Grabbe and Haider, 1971):

Lignin Decomposing, Phenol Synthesizing soil Fungi	Lignin Decomposing Basidiomycetes <u>without</u> Microbial Phenol Synthesis.
Preussia fleischhakeri (A) Phialophora cyclaminis (F.i.)	Lentinus edodes
Pyrenochaeta sp. I (F.i.) Humicola grisea (F.i.)	Hypholoma capnoides
Sordaria verruculosa (A) Hormiactis candida (F.i.)	Polystictus versicolor
Chaetomium piluliferum (A) Stachybotrys chartarum (F.i.)	Agaricus bisporus
Sporarmia aemulans (A) Mammaria echinobotryoides (F.i.)	Cantharellus aurantiaceus
Cladorrhinum sp. III (F.i.) Epicoccum nigrum (F.i.)	Bjerkandera adusta
Doratomyces nanus (F.i.)	

A = Ascomycetes

F.i. = Fungi imperfecti

- a) by pure cultures of soil fungi of different systematic groups, which synthesize phenols in their metabolism, and
- b) by pure cultures of basidiomycetes, which do not synthesize phenols.

In total 19 strains were investigated. The results are:

1. By the action of fungi with and without biosynthesis of phenols the general observations have been made.
 - 1.1 The lignin degradation was after 4 months between 25-45% in both cases.
 - 1.2 The methoxyl content of the isolated lignin fractions decreased between 50-60% afterwards in both cases.

- 1.3 In the high molecular weight fractions of lignin and humic acids a remarkable amount of nitrogen (which was added as asparagine) was fixed, which can be hydrolyzed by 6 n HCl to 30-40%.

2. In the case of not phenol synthesizing strains the production of humic acids was lower.

3. The chemical structure of phenols in the humic acids were different.
 - 3.1 In the case of fungi, which synthesize phenols, lignin derived and microbial synthesized phenols are found by two dimensional thinlayer chromatography with Silica Gel Merck by reductive cleavage with Na-amalgam of humic acids fractions and fractions of rotted lignin. This means, that humic acids are formed not only by condensation of lignin derived but also microbial synthesized phenols. It seems furthermore that also degraded lignin reacts with microbial synthesized phenols.

 - 3.2 In the case of fungi which do not synthesize phenols only lignin derived phenolic compounds are found in the humic acids fractions by thinlayer chromatography.

4. Spectrographic measurements indicate that humic acids formed during rotting of straw in the presence of phenol synthesizing fungi are more similar to those isolated from soils than the others.

3.3. Influences of clay minerals on microbial synthesis of humic substances

The interest for this subject increased in the last few years, the problem is summarised i.e. by Filips et.al. (1971).

Clay minerals are important constituents of soil colloids and exert a pronounced influence on microbial ecology (Alexander, 1964; STOTZKY, 1967). This influence may be related to adsorption phenomena (TSCHAPEK and GARBOSKY, 1950; ZVJAGINCEV, 1962; ZVJAGINCEV and VELIKANOV, 1968; MARSHAL, 1970) or to a more indirect influence, namely, adsorption of organic and inorganic nutrients, of metabolic and autobolic products, and of extracellular enzymes (GREENLAND, 1956; LYANCH ET AL., 1956, 1957; McLAREN and PETERSON, 1967; SKUJINS, 1967; GREAVES and WILSON, 1969; SCHARPENSEEL, 1970).

McCalla (1939, 1940) observed that clay greatly increased the numbers of several bacterial species in liquid culture and fixation of nitrogen by Azotobacter spp. in nitrogen free media. Later, Conn and Conn (1940), Naumova and Gromyko (1953), Filip (1968 a,b) and Kubista (1969) reported an increase in bacterial cells in soil or stand cultures following the addition of clay minerals. The increase in O₂ uptake by bacteria by clays was noted by many investigators (STOTZKY 1966; STOTZKY and REM, 1966; NOVAKOVA, 1969) using respirometer techniques. The respiration of mycelial homogenates of 27 fungal species was generally not affected by montmorillonite or kaolinite at concentrations below 4 per cent (STOTZKY and REM, 1967). The inhibition was

related to viscosity of the system which presumably influenced the rate of O₂ diffusion. Haider et al. (1970) noted that the addition of montmorillonite to shake culture of Saccharomyces cerevisiae, Candida utilis and Aspergillus niger greatly accelerated cell synthesis and glucose utilization.

Clay minerals could presumably influence the synthesis of humic substances, which are predominantly a product of microbial activity, by soil microbes. As reported by Filip (1968 b, 1969) and others (i.e. Novakova, 1967, 1968) the bentonite was found to increase the formation of humic substances by a mixed population of microorganisms in liquid, sand and soil culture media. Novak (1963) expressed the opinion that only a complex soil microflora is able to form humus from organic residues incorporated into the soil. However, synthesis of humic substances by a single microbial species was also reported by several investigators (KUSTER, 1955; PLOTHO, 1951; KANG and FELBECK, 1965). There are number of microscopic soil fungi (MARTIN and HAIDER, 1971) which synthesize phenols from non-aromatic carbon sources and the phenols polymerize to dark coloured humic acid like substances.

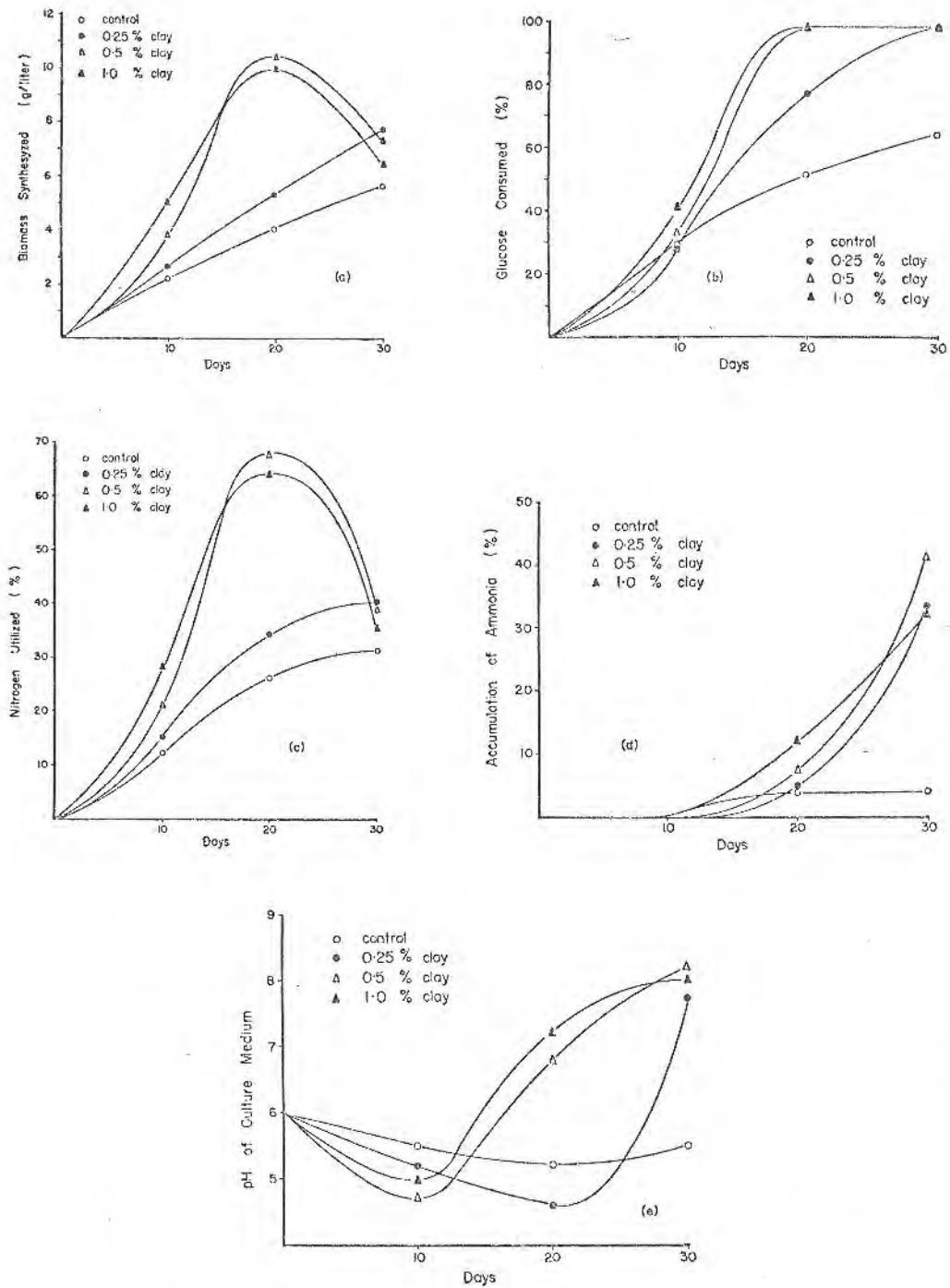


Fig. 6 Effect of different concentrations of montmorillonite on growth and various metabolic activities of *Epicoccum nigrum* in shake cultures. a. synthesis of biomass, b. utilization of glucose (30 g glucose/l), c. utilization of nitrogen (870 mg/l), d. ammonium accumulation during autolysis (per cent of added N), e. pH of the medium (FILIP, HAIDER and MARTIN, 1972).

In the case of Epicoccum nigrum in shake cultures by the addition of different amounts of clay mineral, it was observed;

- 1 Increase in synthesis of bio-mass and later a decrease caused by the autolysis.
- 2 An accelerated utilization of glucose.
- 3 An accelerated utilization of nitrogen
- 4 An accumulation of ammonium during autolysis.
- 5 An increase of the pH in the culture medium.

In the course of the present discussion, we must come back to the observations 4 and 5. The effect of addition of clay minerals to culture solution of fungi have been investigated also in the case of Epicoccum nigrum in connection with humic substances (summary, FILIP et al. 1971 c). These investigations have been made with this fungus, because by former investigations, it was known, which phenolics are formed by this fungus in its metabolisms and which transformations had to occur when humic substances are formed as it was reported above.

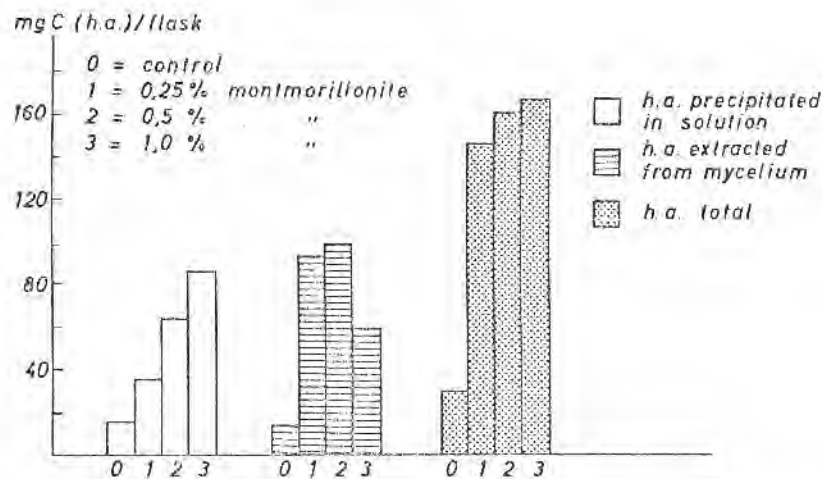


Fig. 7 Formation of humic acids dependent on the quantity of added montmorillonite (FILIP et al. 1972 a)

The addition of montmorillonite has also effect on the quantity of humic acids. The humic acids are taken as g carbon/vessel of culture.

The humic acids which could be precipitated with mineral acids from a nutrient solution, increase with the quantity of montmorillonite. Humic acids which can be extracted from mycelium by alkaline extraction do not follow exactly the same tendency. If one adds different types of humic acids and also those which can be isolated from mycelium with sulphuric acid after hydrolysis, in total an increase of the weight of humic acids can be observed by increasing the quantity of montmorillonite added.

This effect could not be observed by addition of kaolinite or quartz. Only some types of microorganism showed similar behaviour. In some cases the effect was larger in shake cultures than in stand cultures (FILIP et al. 1972 a,b). Some observations let conclude that alterations of the concentrations of the low molecular weight substances causes this effect.

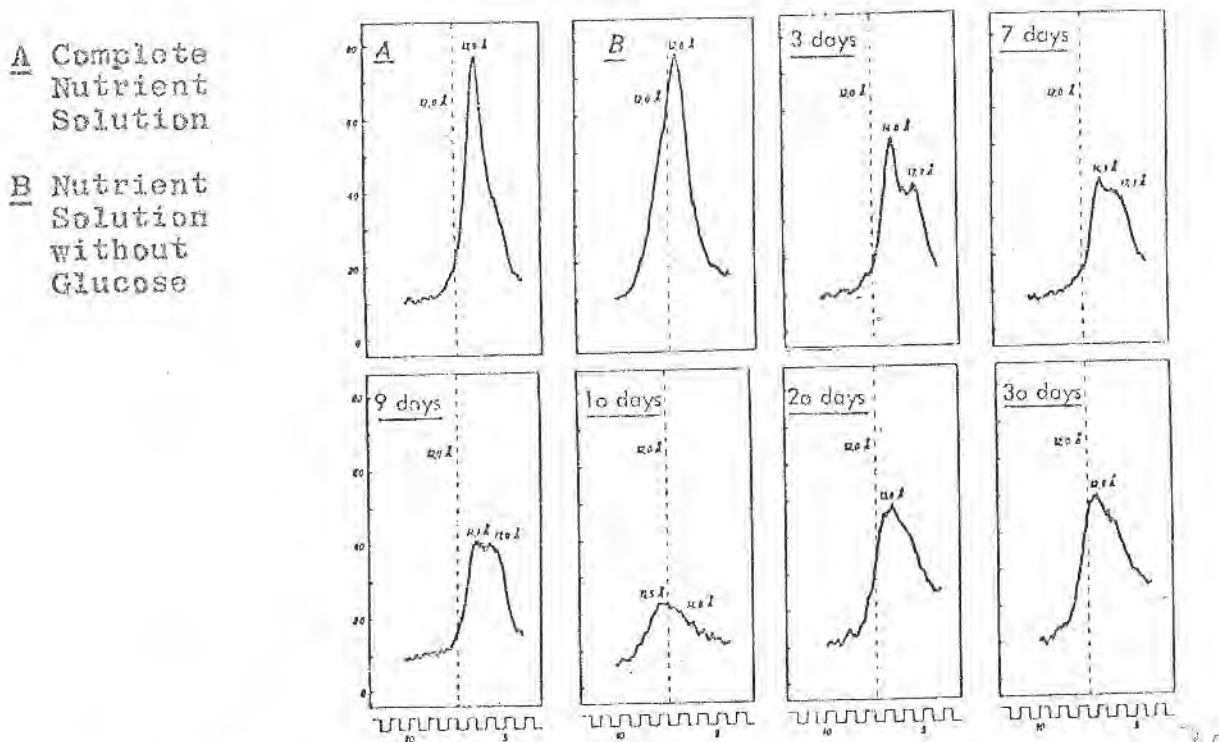


Fig. 8 Alterations of X-ray diffractogram pattern of montmorillonite during culture period (FILIP, FLAIG and BUTELSPACHER-in press)

X-ray investigations showed that during the first 20 days the base reflection is changed. After this time the original pattern is appearing again. After 20 days autolysis occurs and ammonium ions are formed. The ammonium ions exchange, presumably the sorbed organic substances, which affects the spacing of interlayers in montmorillonite. The constitution of the sorbed compounds is not yet known.

At the time being we know only that these substances are low molecular weight moities. This fact could be proved because montmorillonite enclosed in a dialysis tube, gave the same results.

In other investigations, it was established that the autooxidation of phenols synthesized by Epicoccum nigrum are not influenced by montmorillonite, in spite of the fact that this contains less amounts of heavy metals such as iron, copper and manganese.

4. Participation of nitrogenous substances in the formation of humic substances.

Finally the distribution of nitrogen in humic acids was determined mostly by hydrolysis with 6N HCl (Bremner, 1965). Depending on the environmental conditions during the formation, the nitrogen content of humic acids is between 1-5 per cent. The nitrogen content of humic acids was determined by different methods as:

20-40% as α -NH₂-N

10-25% as NH₃-N

1-5% as amino-sugar and about 50% nitrogen remains in residues. At least 7-12% of -NH₂-N is in the peptide bondings.

In cultures of microorganisms the formation of dark coloured nitrogenous humic like substances has been observed several years ago (BREMNER, FLAIG and KUSTER 1955, KUSTER 1952, 1956, LAATSCH, HOOPS and BIENNEK 1952, von PLOTTHO 1950, 1951, SCHEFFER, von PLOTTHO and WELTE, 1950, and others).

The content of hydrolysable α -amino-nitrogen of the lignin fractions decreases during the humification of plant material, but the percentage to total nitrogen increases (see lecture. 2).

This can be explained in two ways. Either nitrogen as amino acids or peptides continues to condense with the lignin during humification, or the peptide chains are increasingly decomposed by the microorganisms. There is the possibility that both of these latter nitrogen reactions occur simultaneously.

Lignin degradation products can always be isolated during the course of humification. Furthermore, it could be shown, that the formation of phenols or quinones is possible during oxidative degradation or by enzymatic dehydrogenation of lignin. In addition, it is known, that dark coloured polymers are formed during the oxidation of 1,2-di- or 1,2,3-triphenols together with amino acids in the presence of phenoloxidases, (STEINMETZ 1956, SWABY 1956, 1958, FLAIG 1956).

4.1 Conditions of the formation of N-free polymers.

Phenolic lignin degradation products are reactive compounds. Partially methylated o-diphenols and the o-diphenols themselves show some differences in the case of oxidative polymerisation.

The methoxyl substituted lignin degradation products such as ferulic and vanillic acid polymerise in the presence of phenoloxydase from microorganisms. The dehydrogenative polymerisation occurs only when the monomers are substituted with a phenolic hydroxyl group in p- position of the side chain. In case of carboxyl-labelled compounds different quantities of active carbon-dioxide are split off from the two acids. No labelled carbondioxide is formed, when the carbon atoms of the methoxyl groups of the two acids or the carbon atoms 2 and 3 of ferulic acid are labelled.

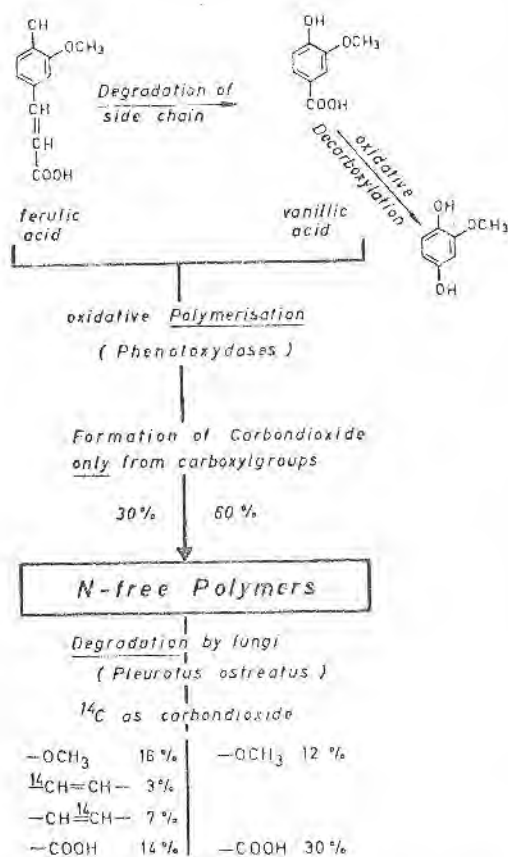


Fig. 9 : Oxidative polymerisation of methoxyl substituted phenol carboxylic acids.

By oxidative polymerisation nitrogen free polymers are formed. No nucleophilic addition of amino acids occurs in the case of hydroxymethoxy-compounds.

For instance ^{they} can be used by microorganisms as carbon source- they are degraded by white rot fungi, such as *Pleurotus ostreatus*. The carbon atoms of the carboxylic and methoxyl groups are more split off by the activity of the microorganisms than the carbon atoms 2 and 3 in the side chain of ferulic acid (HAIDER, LIM and FLAIG 1962, 1964).

Vanillic acid is transformed to methoxy-benzoquinone-1,4 by oxidative decarboxylation (SUNDMAN and HARO 1966) or to its dimer

(FLAIG and HAIDER 1961a). The mentioned reactions are also interesting in connection with the uptake of lignin degradation products by the roots of plants, their translocation and transformation in plants and their physiological effect (summarized in: FLAIG, 1968) (lecture 6).

4.2 Conditions of the formation of nitrogenous polymers.

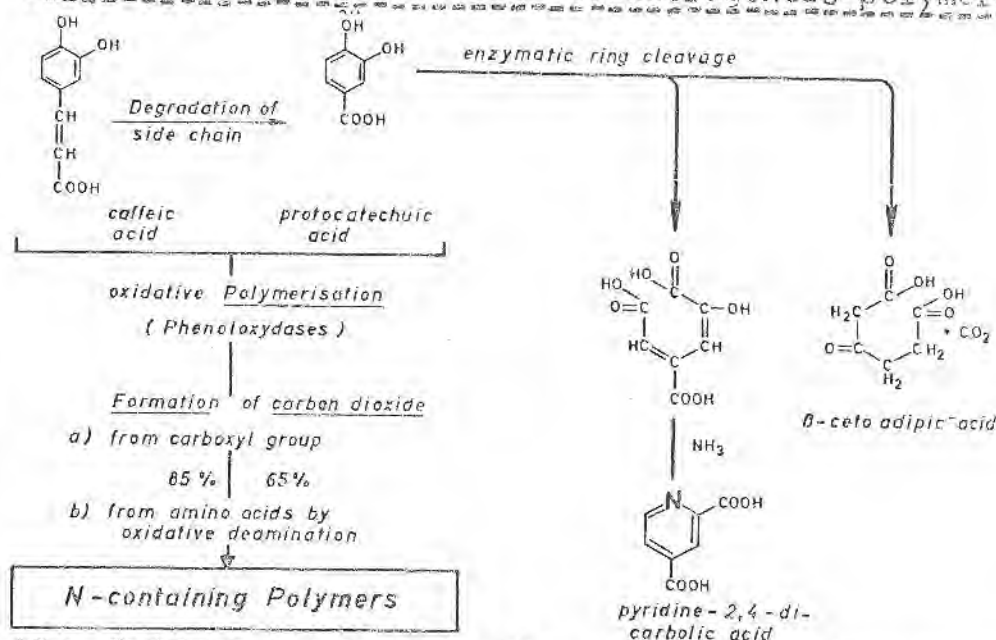


Fig. 10: Oxidative polymerisation of α-diphenolcarboxylic acids and their reaction with nitrogenous compounds.

Dihydroxyphenylcarboxylic acids such as protocatechuic or caffeic acid polymerise also in the presence of phenoloxylases. But the released quantity of labelled carbondioxide is higher than in case of hydroxymethoxy-derivatives. The formed polymers contain nitrogen, whilst the polymers in case of methoxyl substituted compounds had been nitrogen free. A nucleophilic addition of amino acids occurs. In the presence of nitrogenous compounds from proteins the total released carbondioxide is partly derived from the carboxyl groups (case a) or from the amino acids and peptides (case b). The nitrogenous polymers are degraded much more slowly

by microorganisms; this may be one of the reasons that such type of substances are accumulated in the soils. In contrast, the above mentioned polymers of hydro-methoxy-compounds can serve as carbon source for microorganisms.

As further reaction, the cleavage of the aromatic ring of protocatechuic acid occurs and β -ceto adipic acid is formed during enzymatic oxidation (STANIER and INGRAHAM 1954, McDONALDS, STANIER and INGRAHAM 1954, GROSS, GRAFFORD and TATUM 1956, OTTEY and TATUM 1956, FLAIG and HAIDER 1961 a). This aliphatic acid is easily available for the microorganisms. The formation of protocatechuic acid and the cleavage of its ring is an important reaction for the transformation of phenolic compounds from plants and microorganism during humification because some reactions lead by demethylation and hydroxylation to this o-diphenolic acid. By this way the aromatic compounds disappear from the mixture of compounds, which participate in formation of humic substances.

TRIPET, DAGLEY and STOPHER (1960) found an enzymatic cleavage of protocatechuic acid with cell-free extracts from *Pseudomonas* sp., whereby the semialdehyd of muconic acid is formed. This reacts with ammonia on a notenzymatic way to pyridine-2,4-dicarboxylic acid. This reaction may be considered as one example for the fixation of nitrogen in hetrocyclic compounds.

5. About the linkage of amino acids, peptides and proteins in humic acids

5.1 Nucleophilic addition of nitrogen compounds by phenols during oxidation.

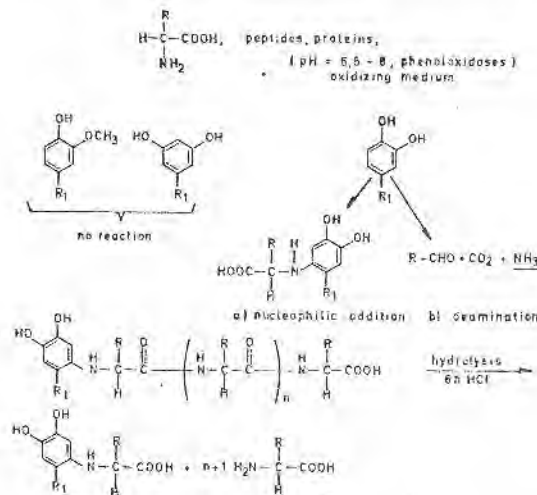


Fig. 11: a) Nucleophilic addition of proteins, peptides and amino acids by phenols during oxidation.
 b) Oxidative deamination.

Extensive studies have been made with labelled amino acids about the possible linkage of proteins and of their products of hydrolysis to phenols during oxidation (HAIDER, FREDERICK and ELAIG 1965). Some lignin degradation products isolated from decomposing lignin from soils or some phenols of microbial origin can add nitrogenous compounds derived from proteins at variable rate and in different amounts during oxidation in the presence of phenoloxidases. Summarizing, we could establish that guajacol and resorcinol derivatives however do not add amino acids in a pH-range of 6.5 to 8.0.

The rate of nucleophilic addition (a) is the highest in the case of catechol and hydroxyquinone derivatives. Addition by pyrogallol derivatives occurs also to a smaller extent, dependent on the chemical constitution of the derivatives. By means of labelled compounds, it could be established that the amino acids are added intact. An addition also occurs with peptides and proteins.

Oxidative deamination (b) which occurs mostly contemporarely, is discussed later in detail.

Furthermore, during oxidation in the presence of phenol oxidase and nitrogen compounds, catechol derivatives polymerise to nitrogenous polymers which have properties comparable to those of humic acids. In contrast, as mentioned before, the polymers of guajacol derivatives do not contain nitrogen (FLAIG and HAIDER 1961) (comp. 2.1 with 2.2).

After nucleophilic addition the amino acids can not be hydrolized from the corresponding addition products by 6N hydrochloric acid. In case of addition products formed with proteins and peptides, only the amino acids could be hydrolized with the exception of the N-terminal amino acids, in which the amino groups has reacted with the oxidised phenol (HAIDER, FREDERICK and FLAIG 1965, HAIDER and MARTIN 1970). Therefore not all non-hydrolizable nitrogen is bound in humic substances in heterocyclic form as it is often supposed.

5.2 Oxidative deamination of amino acids

By oxidative deamination the amino acids are transformed in ammonia, carbondioxide and in a carbonyl compound.

If both reactions, addition and deamination, occur together. More than 1 Mol oxygen is uptaken (HAIDER, FREDERICK and FLAIG 1965). One molecule quinone gives one addition product, but deaminates several Mols of amino acids.

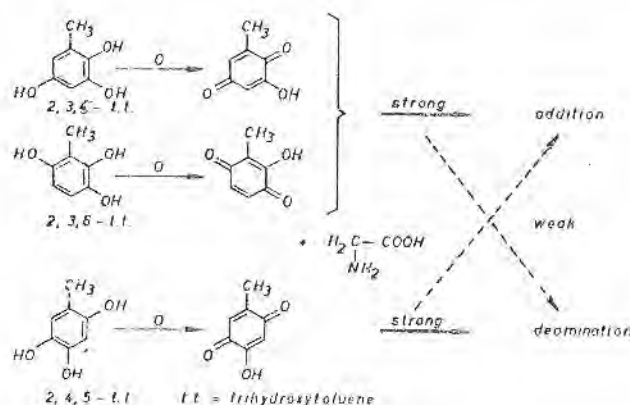


Fig. 12: Addition and oxidative deamination of amino acids by *o*-diphenols during oxidation in the presence of phenol oxidases.

The ratio of the two reactions such as the addition of an amino acid and its deamination depends upon the constitution of the added phenol.

2,3,5- and 2,3,6-Trihydroxytoluene or their oxidation products effect a strong addition but a weak deamination, whilst in the presence of 2,4,5-trihydroxytoluene the deamination occurs more strongly than the addition of an amino acid (HAIDER and MARKIN 1967). Two of these compounds 2,4,5- and 2,3,5-trihydroxytoluene are formed in the culture media of *Epicoccum nigrum* as mentioned above.

5.3 Properties of substances (humic acids), precipitable with acids.

Precipitates can be isolated by addition of mineral acids to the reaction solution of phenols and amino acids. The precipitates are the main part of the addition products.

Tab. 4

Distribution of the nitrogen in the reaction of 2500 μ mole glycine with 500 μ mole catechol (2500 μ atom N; 3 mg mushroom phenoloxydase)

	α -NH ₂ -N	NH ₃ -N	N-diff.*	N-prec.
nitrogen in μ atom in solution and in precipitate	1574	452	170	304

* Kjehldahl-N minus (NH₂-N + NH₃-N)

Precipitate	weight in mg	weight % of total appl. subst.	% N	N in prec. in % of total N	% NH ₃ -N of total N
	75,1	31	5,6	12	18

(According to HAIDER, FREDERICK and FLAIG 1965)

In one example the distribution of the nitrogen in the precipitate and in the solution is depicted. Under the mentioned conditions about one molecule of glycine is condensed with one molecule oxidised catechol, because the sum of the nitrogen in the precipitate plus N-diff. (that is the nitrogen in the solution, which is not α -NH₂-N and not NH₃-N) is nearly 500 μ atom like the amount of the applied catechol. The nitrogen N-diff. belongs to soluble addition products, which cannot be precipitated by mineral acids.

The number for NH₃-N in the solution demonstrates, that a further molecule glycine is deaminated. The value of α -NH₂-N in the solution belongs to the not yet transformed glycine.

The sum of all N-fractions is about 2500 μ Moles - the added amount of nitrogen.

The nitrogen content of the precipitate is 5,6%; 12% of the nitrogen applied in form of glycine are fixed in the precipitate and 18% are released as ammonia. The numbers are representative for the most investigated amino acids.

Some properties of the precipitates are comparable with those of humic acids. The fixed amino acids of the reaction products with the phenols cannot be hydrolyzed with 6 n HCl. After electrophoresis or paper chromatography of hydrolysates no spots are observed, which belong to the applied amino acid. This result means that not all unhydrolysable nitrogen of the humic acids is heterocyclic bound. Some other linkages of nitrogen must have occurred perhaps in form of aromatic amines.

5.4 Distribution of the carbon skeleton and nitrogen as constituents of the amino acids in the reaction products and in the solution.

Some further experiments have been made, to elucidate, how the amino acids are transformed, and where the parts of the transformed amino acids remain during both reactions, during the addition and the deamination. For this purpose different ¹⁴C-labelled amino acids have been used (HAIDER, FREDERICK and FLAIG 1965).

Tab. 5 Oxidative deamination of differently labelled amino acids during the oxidation of catechol in the presence of mushroom phenoloxidase (pH =). Distribution of the carbon¹⁴ and the nitrogen in different fractions.

Amino acid	% added activity in		% added nitrogen in	
	CO ₂	precipitate	NH ₃ in solution	precipitate
$\begin{array}{c} \text{NH}_2 \\ \\ \text{HC}-^{14}\text{COOH} \end{array}$	31	14	20	18
$\begin{array}{c} \text{NH}_2 \\ \\ \text{H}^{14}\text{C}-\text{COOH} \end{array}$	1	15	21	18
$\text{HOOC} - (\text{CH}_2)_2 - \begin{array}{c} \text{NH}_2 \\ \\ \text{CH}-^{14}\text{COOH} \end{array}$	21	6	16	10
$\text{HOOC} - \begin{array}{c} \text{NH}_2 \\ \\ \text{CH}-\text{COOH} \end{array} - (^{14}\text{CH}_2)_2$	0,2	8	16	10

Catechol has been oxidised in the presence of amino acids labelled at different C-atoms and in the presence of phenoloxidase. (HAIDER, FREDRICK and FLAIG, 1965). When the oxidation was finished the reaction solution has been acidified till pH = 1. The released carbon-dioxide was absorbed in sodium hydroxide solution and the precipitation was isolated. The summarized results are the following:

- 1 The larger amount of released labelled carbondioxide in the case of carboxyl labelled amino acids in comparison to those, which are labelled in another carbon atom, is a measure for the deamination, because the activity in the precipitates formed in the presence of the two different labelled amino acids are in every case the same.

Furthermore, the activity in the precipitates formed with differently labelled amino acids demonstrates, that the

amino acids are added as a whole in the oxidation product without a loss of carbondioxide.

2. The quantity of the released carbondioxide in the carboxyl labelled amino acids was larger than the amount of the ammonia nitrogen in the solution.

Furthermore the nitrogen content in the precipitates was larger than their content of labelled carbon. These data indicate, that a part of the nitrogen in the precipitate is not bound in form of an amino acid. Further condensation must have taken place, by which nitrogen is bound in another way.

Experiments with labelled di- or tripeptides demonstrated, that not only nucleophilic addition of a peptide by a quinone but also deamination occurred. When the addition products have been hydrolyzed, all the amino acids were identified except the N-terminal, which is bound at the ring.

5.5 Mechanism of nucleophilic addition and oxidative deamination of amino acids by phenols.

We were interested in mechanism of the influence of molecular structure of the different phenols on addition or deamination of amino acids. The reaction between different substituted trihydroxytoluenes and glycine in oxidative medium was investigated by polarographic methods. (RIEMER 1970; FLAIG and RIEMER 1971).

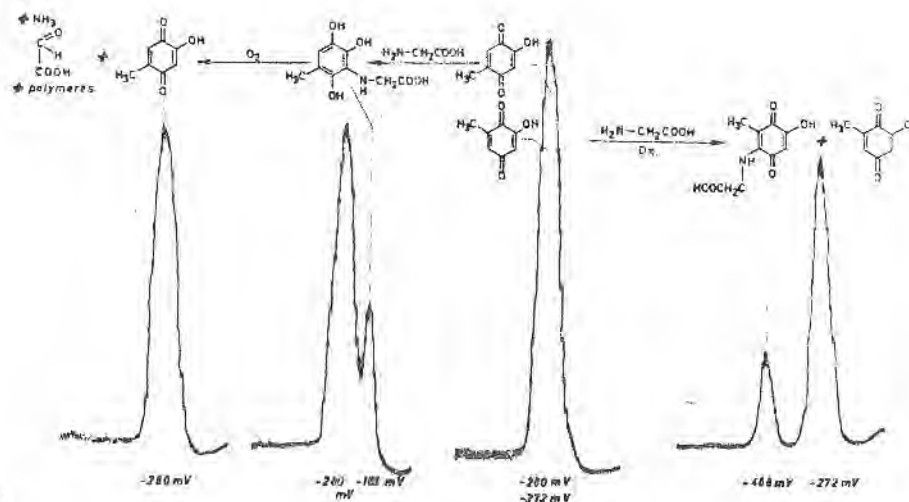


Fig. 13: Reaction of the oxidation products of different substituted trihydroxytoluenes with glycine; polarographic measurements.

The oxidation products of 2,3,5-trihydroxytoluene (right) adds amino acids in p-position to the phenolic hydroxyl group.

The addition product is very stable. Its half-wave potential is 216 mV more than this of the initial compound. Deamination does not occur in this case.

The stability of this addition product is caused by the increase of the p-quinoid part in the resonance hybrids by interaction of the electron donating substituents across the ring system.

In contrary to this, a slow reaction occurs with glycine in the case of 2,4,5-trihydroxytoluene (left) after oxidation to the corresponding quinone. The addition product has a more positive half-wave potential than the initial product. In the addition product with the higher half-wave potential the o-quinoid part of the resonance hybride is increased and decomposes by further addition of oxygen, whereby 2-hydroxy-5-methyl-benzoquinone-1,4 is formed again as well as ammonia, glyoxylic acid and polymers.

According to the polarographic investigations the p reaction mechanism of addition of glycine to 2-hydroxy-5-methyl-benzoquinone-1, 4 and the following oxidative deamination is depicted in the following scheme:

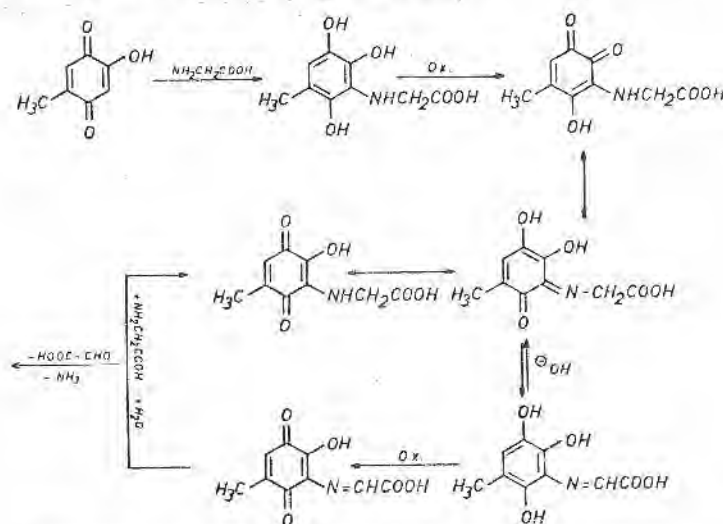


Fig. 14 Mechanism of oxidative deamination of glycine by 2-hydroxy-5-methyl-benzoquinone-1,4.

The first step is the addition of the amino acid on the quinone and oxidation to the corresponding o-quinone with the more positive half-wave potential. A tautomerisation to a pH-dependent equilibrium occurs. Further oxidation leads to oxidative deamination by nucleophilic displacement with a further molecule of an amino acid. The formation of glyoxylic acid has been identified by polarographic measurements.

The catalytic effect of quinones with different molecular structure on nucleophilic addition and on oxidative deamination seems to be not only interesting for the formation of humic substances, but also in the connection with the physiological effect of phenols or quinones on plant metabolism, their uptake,

translocation and transformation in the plant or their fixation as insoluble products in the roots.

6. Stabilization of Amino sugars.

Very recently work has been done with labelled glucosamine, chitosan (polyglucoseamine) to show that these substances are stabilized by nucleophilic addition on phenols in oxidizing medium.

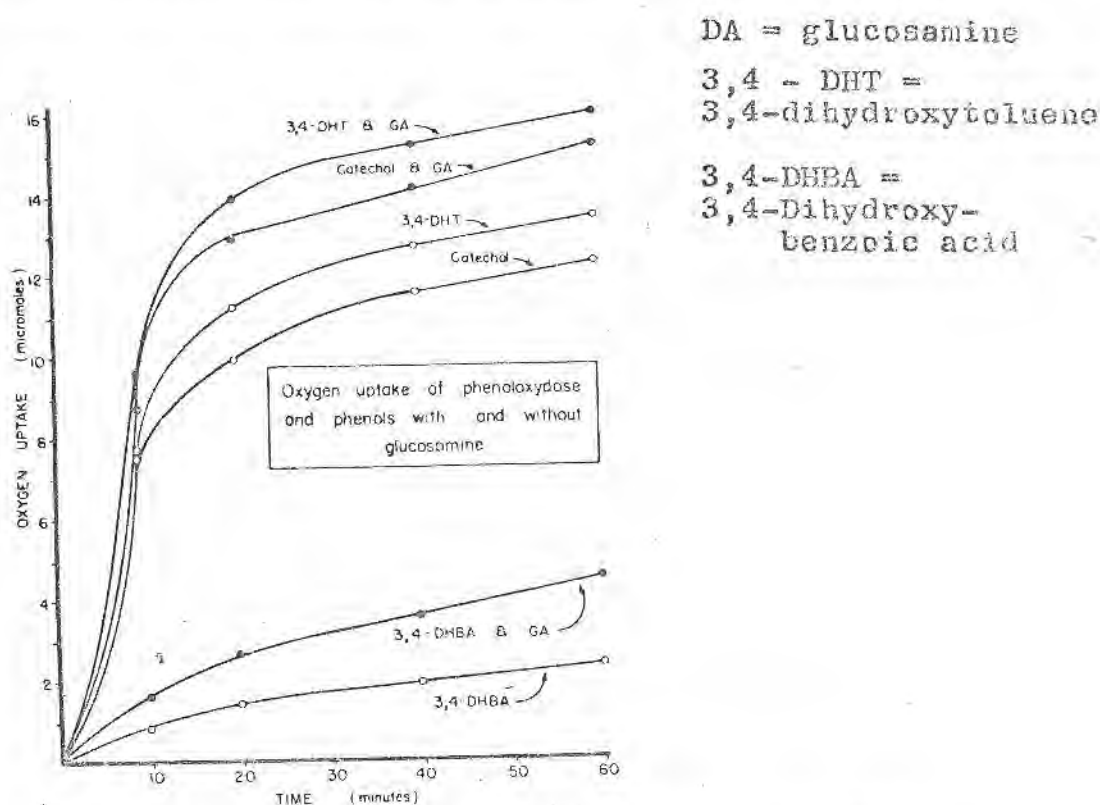


Fig. 15 Oxygen uptake in reaction mixtures of phenol oxidase and phenols with and without glucosamine (Bondietti, Martin and Haider, 1972).

Model substances have been prepared by shaking of phenolics with hydroxy groups in ortho position in the presence and absence of glucosamine at pH 8.0. The O_2 in the presence of glucosamine and chitosan was higher than without glucosamine. This means that reaction between glucosamine and the phenols occur. Experiments

about degraded humic acid like polymers with ^{14}C labelled glucosamine or chitosan have shown that the glucosamine in these polymers is more or less stabilized.

7. Autoxidation of phenol derivatives in the presence of ammonia at pH-values about 8.

7.1. 1,2- or 1,4-Diphenols.

Till now only the addition reactions in oxidising medium have been reported, which were catalysed by phenoloxidases and which occur in a range of pH-value between 6 and 8. At higher pH-values the phenols with at least two hydroxyl groups in o- or p-position react with ammonia to dark coloured nitrogenous polymers:

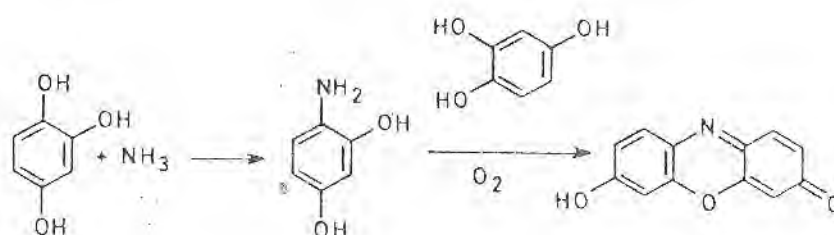


Fig. 16: Formation of phenoxazine derivatives

In the absence of air and in the presence of ammonia a hydroxyl group can be replaced in hydroxyhydroquinone by an amino group (LANTZ and MICHEL 1961). The aminoresorcinol thus formed reacts in the presence of oxygen with a further hydroxyhydroquinone to produce 7-hydroxyphenoxazine.

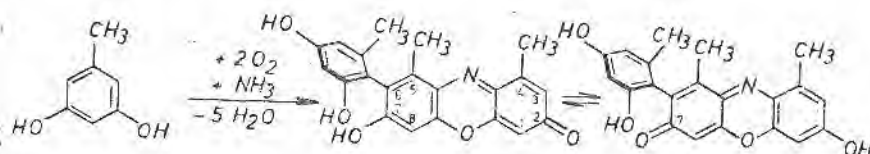
7.2 1,3-Diphenols

Fig. 17 : Reaction of resorcinol derivatives (e.g. orcinol) during oxidation with ammonia. (Formation of phenoxazines; orcein dyestuffs) (MUSSO 1961).

Before it has been mentioned, that 3,5-dihydroxytoluene (orcinol) was found in the culture media of *Epicoecum nigrum* in the humic acids after reductive cleavage. Also, such types of substances react with ammonia in an oxidising medium but only at higher pH-values than 8. MUSSO *et al.* (1961, 1963, 1965) investigated these reactions to elucidate the constitution of the orcein dyestuffs. For instance α -hydroxy-orcein is a tautomeric mixture of the derivatives from phenoxazine-2 and phenoxazine-7, which are also formed through quinonoid intermediate by the addition of ammonia at orcinol.

8. Random polymerisation of tyrosine to melanin as a model for the participation of heterocyclic nitrogen in formation of the spheric shaped humic acids.

A further possibility of the formation of dark coloured humic substances is the participation of aromatic amino acids from the protein of the microorganisms. For example, the oxidation of tyrosine could lead through the dopaquinone (VII) (RAPER 1927) to dark coloured polymers (CROMARTIE and HARLEY-MASON 1953).

The proposed random polymerisation of tyrosine to melanin (BLOIS 1965, MASON 1948, NICOLAUS 1962, RAPER 1928) can be used for the explanation of the formation of humic acids. Both are dark coloured, higher molecular substances with a spherical shape. The polymerisation occurs in each case through quinonoid intermediates, such as semiquinone free radicals.

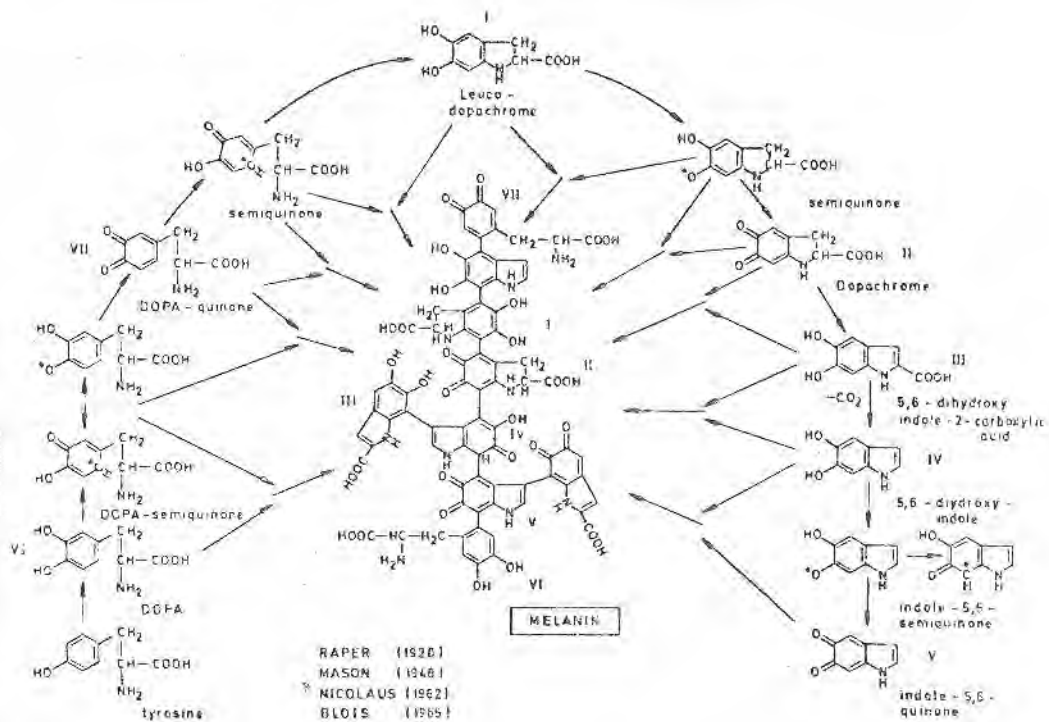


Fig. 18: Hypothetical scheme of melanin synthesis assuming the participation of semiquinone free radicals.

The random polymerisation to melanin starts from tyrosine and all intermediates participate in the formation of the polymer. Melanin is not only formed by the polymerisation of the end product of oxidation of tyrosine, indole-5, 6-quinone.

Similar but much more complicated processes occur during the formation of humic substances, since not one but several phenolic components and various nitrogenous compounds take part in the formation of the polymers.

The formation of dark coloured substances in structurally intact dead plant tissues at the beginning of humification could be explained in a similar way (KONONOVA 1961).

This contribution to the participation of nitrogenous compounds in the formation of humic substances concerned much fundamental research work to this problem.

One of the functions of humus is its ability to contribute to soil productivity as a slow releasing nitrogen source. Many field experiments and experiments with $^{15}\text{-N}$ have been made to find out the dynamics of nitrogen transformations and nitrogen economy in soil. But all the observations, which have been made, cannot be explained without knowledge about their causal connections. We are here only at the beginning of research work. But the fact, that phenolic constituents of humus catalyse the liberation of ammonia from nitrogenous organic compounds such as amino acids, a product of dead soil organisms, seems to be a step for further elucidation of nitrogen economy in soil. Some of the mentioned reactions gave valuable suggestions for synthetic, slow releasing nitrogen fertilizers.

9. Summary of comments on the biochemistry 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.

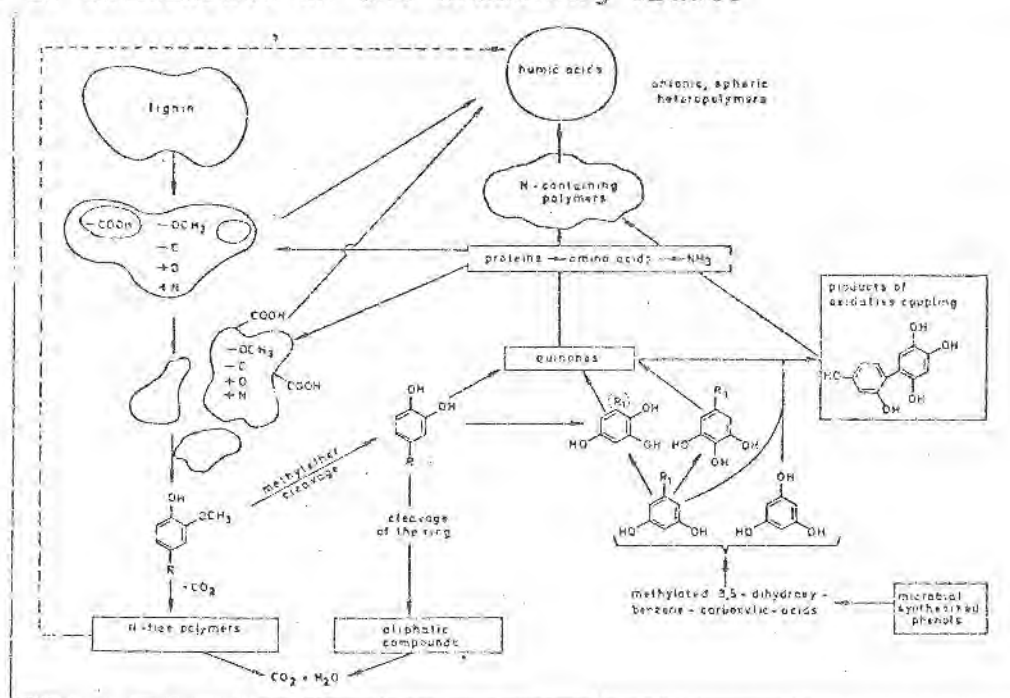


Fig. 19: Contribution of phenolic and nitrogenous structure units to chemical composition properties of humic substances.

The depicted scheme concerns mainly with the biochemical 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. Methylene ether 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 micro-organisms 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, humatmelanic 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. For soil productivity the availability of the organic bound nitrogen is important.

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. This concerns also the experiments with ^{15}N .

In respect to some physical properties of humic substances, it could be mentioned that in the course of the mentioned reactions, the formation of heterocyclic compounds may occur which possibly contribute to the colour, an essential property of humic substances. The reactions have been discussed which lead to the formation of higher molecular weight substances like humic acids, which are anionic, spherical, shaped heteropolymers.

References:

- ACERBO, R., H. KASTORI, H. SOCHTIG, H. HARMS and K. HAIDER: Effect of boron in synthesis and transformation of lignin precursors in Zea Mays. *Zeitschr. f. Pflanzenphysiologie*, 69, 306-317 (19).
- ALEXANDER, M. Biochemical ecology of soil microorganisms. *Ann. Rev. Microbiol.* 18. 217-252 (1964).
- BAILEY, N.T., G.G. BRIGGS, G.J. LAWSON, J.M. SCHWTON and S.G. WARD: Observation on the structure of humic acid. 6. Intern. Kohlenwiss. Tagung, Münster, W., 1.-3.6.1965. Beitrag Nr. 3, 1. (1965).
- BARTLETT, J.B.: The effect of decomposition of the lignin of plant materials. *Iowa State Coll. J. Sci.* 14, 11-13 (1939).
- BARTLETT, J.B., F.P. SMITH and P.E. BROWN: Lignin decomposition in soils. *Proc. Iowa Acad. Sci.* 44, 97-101 (1937).
- BARTLETT, J.B., and A.G. NORMAN: Changes in the lignin of some plant material as a result of decomposition. *Soil Sci. Soc. Amer., Proc.* 3, 210-216 (1938).
- BELAV, L: Chemische Untersuchungen heimischer Torfbildner ein zur Kenntnis der umwandlung von pflanzenstoffen unter natürlichen und künstlichen Bedingungen. Dissertation Rostock (1967).
- BERLING, J., K. BARZ, H. HARMS and K. HAIDER: Degradation of phenolic compounds in plant cell cultures. *FEBS LETTERS*, 16(2), 141-146 (1971).
- BIELAWSKI, J., T.E. THOMPSON and A.L. LEHNINGER: The effect of 2,4-dinitrophenol on the electrical resistance of phospholipid bilayer membranes. *Biochem. and Biophys. Res. Comm.* 24, 948-954(1966).
- BJORKMAN, A.: Isolation of lignin from finely divided wood with neutral solvents. *Nature* 174, 1057 (1954).
- BJORKMAN, A.: Lignin and Lignin-Carbohydrate Complexes. Extraction from wood meal with neutral solvents. *Ind. Eng. Chem.* 49, 9 1395-1398 (1957).
- BLOIS, M.S.: Random polymers as a matrix for chemical evolution. In: *The origins of prebiological systems*, edit. by Sidney W. Fox, Academic Press New York-London 1965, 19-38.
- BONDIETTI, E., J.P. MARTIN and K. HAIDER.: Influence of nitrogen source and clay on growth and phenolic polymer production by *Stachybotrys* species, *Hendersonula toruloidea* and *Aspergillus sydowi*. *Proc. Soil Sci. Soc. Am.* 35(6), 917-922(1971).

- BONDIETTI, E., J.P. MARTIN and K. HAIDER: Stabilization of amino sugar units in humic type polymers. Proc. Soil Sci. Soc. Am. 36, 597-602 (1972).
- BRAUNS, F.E.: Native Lignin. I. Its isolation and methylation. J. Amer. Chem. Soc. 61, 2120-2127 (1939).
- BRAUNS, F.E. and D.A. BRAUNS: The chemistry of lignin; supplement volume: Covering the literature for the years 1949-1958. New York and London, Academic Press 516-518, (1960).
- BREMNER, J.M.: "Organic forms of nitrogen". Ch. 85 Methods of Soil Analysis. (Agron. Monographs 9) ASA Madison 1965.
- BREMNER, J.M., W. FLAIG and E. KÜSTER: Zur Kenntnis der Huminsäuren. IX. Mitteilung. Der Gehalt an Aminosäuren in Streptomyceten-Huminsäuren. Z. Pflanzenernähr., Düng., Bodenkunde 71, 58-63 (1955).
- BREMNER, J.M. and T. HARADA: Release of ammonium and organic matter from soil by hydrofluoric acid and effect of hydrofluoric acid treatment on extraction of soil organic matter by neutral and alkaline reagents. J. Agric. Sci. 52, 2 (1959).
- BREYHAN, Th.: Eine Mikromethode zur Stickstoffbestimmung. Z. analyt. Chem. 152, 412-417 (1956).
- BROADBENT, F.E.: Modification in chemical properties of straw during decomposition. Soil Sci. Soc. Amer., Proc. 18, 165-169 (1954).
- BROCKMANN, H. and E. MEYER: Äquivalent- und Molekulargewichtsbestimmungen durch potentiometrische Mikrotitration in nicht-wässrigen Lösungsmitteln. Chem. Ber. 86, 1514-1523 (1953).
- BRUCKERT, S.F., F. JACQUIN and M. METEHE: Contribution a l'etude des acides phenols presents dans les sols. Bull. de l'Ecole Nationale Supérieure Agronomique de Nancy, IX, 73-92 (1967).
- BURGES, A. and P. LATTER: Decomposition of humic acid by fungi. Nature 186, 4722, 404-405 (1960).
- BURGES, N.A., H.M. HURST and B. WALKDEN: The phenolic constituents of humic acid and their relation to the lignin of the plant cover. Geochim. Cosmochim. Acta 28, 1547-1554 (1964).
- CHAMINADE, R.: Action de l'acide humique sur le developement et la nutrition minerale des vegetaux. VI. Int. Soil Sci. Congr. Vol. D. Paris, 443 (1956).
- CHAMINADE, R.: Effet physiologique des constituants de la matière organique des sols, sur le metabolisme des plantes, la croissance et le rendement. In: The use of isotopes in soil organic matter studies. Report of the FAO/IAEA Technical Meeting. Pergamon Press Ltd. Oxford 1966, 35-47.

- CHOUHRI, M.B. and F.J. STEVENSON: Chemical and physicochemical properties of soil humic colloids. III. Extraction of organic matter from soils. Soil Sci. Soc. Amer., Proc. 21, 508-513 (1957).
- CHRISTEWA, L.A.: Die stimulierende Wirkung der Huminsäuren auf die Lebenstätigkeit höherer Pflanzen und die Effektivität der Humindünger in südlichen Gebieten der Ukraine, SSR. Int. Soil Sci. Congr. Vol. II, Comm. II u. IV. Hamburg 46-55(1958).
- CHRISTEWA, L.A.: Theorie und Praxis der Humindüngeranwendung in der Ukraine. Der Internationale Torfkongress, UdSSR Leningrad (1963) (russ.).
- COFFIN, D.E. and W.A. DELONG: Extraction and characterization of organic matter of a podzol B-horizon. 7th. Int. Congr. of Soil Sci. Madison, Wisc. USA Vol. II, 91-97 (1960).
- CONN, H.J. and J.F. CONN: The stimulative effect of colloides upon the growth of certain bacteria. J. Bact. 32, 99-100 (1940).
- CROMARTIE, R.I.T. and H. MASON: The structure of tyrosine melanin. Chem. Ind., 972-973 (1953).
- DAMBROTH, M.: Einfluß von N-lignin auf Ertrag und wertbestimmende Merkmale von Sommergerste, Z. Pflanzenern., Düng. Bodenk.(1972).
- DEUEL, H. and P. DUBACH: Decarboxylierung der organischen Substanz des Bodens. II. Nachweis von Uronsäuren. Z. Pflanzenernähr., Düng., Bodenkunde 82, 97-106 (1958)a.
- DEUEL, H. and P. DUBACH: Decarboxylierung der organischen Substanz des Bodens. III. Extraktion und Fraktionierung decarboxylierbarer Humusstoffe. Helv. Chim. Acta 41, 1310-1321 (1958 b).
- DEUEL, H., P. DUBACH and R. BACH: Decarboxylierung der organischen Substanz des Bodens. I. Decarboxylierung der gesamten Humusstoffe. Z. Pflanzenernähr., Düng., Bodenkunde 81, 189(1958).
- DUBACH, P. and N.C. MEHTA: The chemistry of soil humic substances. Soils Fertilizers XXVI, 293-300 (1963).
- DUBACH, P., N.C. MEHTA and H. DEUEL: Extraktion von Huminstoffen aus dem B-Horizont eines Podsols mit ADPE. Z. Pflanzenernähr., Düng., Bodenkunde 25, 119-123 (1961).
- DUBACH, P., N.C. MEHTA and H. DEUEL: Schonende Extraktion von Huminstoffen und Isolierung der Fulvosäure-Fraktion aus verschiedenen Bodentypen. Z. Pflanzenernähr., Düng., Bodenkunde 102, 1-7 (1963).

- FARMER, V.C. and R.I. MORRISON: Chemical and infrared studies on phragmites peat and its humic acid. Sci. Proc. Roy, Dublin Soc. Series A 1, 85-104 (1960).
- FARMER, V.C. and R.I. MORRISON: Lignin in Sphagnum and phragmites and in peats derived from these plants. Geochim. Cosmochim. Acta 28, 1537-1546 (1964).
- FILIPS, Z.: The influence of small supplements of bentonite on the development of certain groups of microorganisms in a soil culture. Rostlinna Vyroba (Prague) 14, 209-216 (1968 a).
- FILIPS, Z.: Development of microorganisms and humus substance formation in media with different content of bentonite. Pochvovedeniye (Moscow) 9, 55-61 (1968 b).
- FILIPS, Z.: Characteristic of humic substances in a soil incubated with additions of bentonite. Rastlinna Vyroba (Prague) 15, 377-390 (1969).
- FILIPS, Z., K. HAIDER, W. FLAIG, H. BEUTELSPACHER, E. KUSTER u. J.P. MARTIN: Einfluß der Tonminerale auf die Huminstoffbildung einiger Bodenpilze. Landbauforschung Völkenrode, 21, 97-102 (1971).
- FILIPS, Z., K. HAIDER and J.P. MARTIN: Influence of clay minerals on growth and metabolic activity of *Epicoccum Nigrum* and *Stachybotrys Chartarum*. Soil Bio. Biochem. 4, 135-145 (1972a).
- FILIPS, Z., K. HAIDER and J.P. MARTIN: Influence of clay minerals on the formation of humic substances by *Epicoccum Nigrum* and *Stachybotrys chartarum*. Soil Biol. Biochem. 4, 147-154 (1972 b).
- FLAIG, W.: Zur Bildungsmöglichkeit von Huminsäuren aus Lignin. Holzforschung 9, 1-4 (1955).
- FLAIG, W.: Zur Chemie der Huminsäuren und deren Modellsubstanzen. Vle. Congres Internat. de la Science du Sol. Paris, 2, 471-478 (1956).
- FLAIG, W.: Die Chemie organischer Stoffe im Boden und deren physiologische Wirkung. Verhandl. d. II. und IV. Komm. d. Internat. Bodenkundl. Ges., Hamburg, Vol. II (1958).
- FLAIG, W.: Einige Reaktionen der Benzochinone und ihre Absorptionsspektren. Angew. Chem. 69, 723 (1957).
- FLAIG, W.: Chemie der Humusstoffe. Suomen Kemistilehti A 33, 229-251 (1960 a).

- FLAIG, W.: Comparative chemical investigations on natural humic compounds and their model substances. *Sci. Proc. Roy. Dublin Soc. Ser. A* 1, 4. 149 (1960 b).
- FLAIG, W.: Zur Umwandlung von Lignin in Humusstoffe. *Freiberger Forschungshefte A* 254, 39-56 (1962).
- FLAIG, W.: Über den Einfluß von Humusstoffen auf den Stoffwechsel der Pflanzen. *Der internationale Torfkongress UdSSR Leningrad 1963*.
- FLAIG, W.: Chemische Untersuchungen an Humusstoffen. *Z. Chem.* 4, 253-265 (1964 a).
- FLAIG, W.: c) Humusstoffe. *Handbuch der Pflanzenernährung und Düngung. Bd. II: Boden und Düngemittel 1966*, S. 282-458, Springer-Verlag Wien.
- FLAIG, W.: Uptake of organic substances from soil organic matter by plant and their influence on metabolism. *Pontificiae Academiae Scientiarum Scripta Varia* 32, 723-770 (1968).
- FLAIG, W.: Contribution a la connaissance de la constitution et de la synthese des acides humiques. *Extrait de "Sciences du Sol"*, Supplement au Bulletin de l'Association Francaise pour l'Etude du Sol, No. 2, 39-72 (1970).
- FLAIG, W.: Organic compounds in soil. *Soil Sci.* 111, 19-33 (1971).
- FLAIG, W. and H. BEUTELSPACHER: Investigations of humic acids with the analytical ultracentrifuge. In: *Isotopes and radiation in soil organic matter studies*. International Atomic Energy Agency, Vienna 1968, 23-30.
- FLAIG, W. and Th. BREYHAN: Über das Vorkommen von Indolverbindungen in Schwarzerde-Huminsäuren. *Z. Pflanzenernähr., Düng., Bodenkunde* 75, 132-135 (1956).
- FLAIG, W. and K. HALDER: Reaktionen mit oxydierenden Enzymen aus Mikroorganismen. *Planta Medica, Z.f. Arzneipflanzenforsch.* 9, 123-139 (1961 a).
- FLAIG, W. and K. HALDER: Die Verwertung phenolischer Verbindungen durch Weißfäulepilze. *Arch. Mikrobiol.* 40, 212-223 (1961 b).
- FLAIG, W. and K. HALDER: Über die Beteiligung von Phenolen am Aufbau von Huminsäuren. - 9th International Congress of Soil Science Transactions III, 175-182 (1968).

- FLAIG, W. and W. de JONG: Untersuchungen über die Wirkung von p-Benzochinonderivaten auf den aeroben und anaeroben Stoffwechsel der Hefe (*Saccharomyces cerevisiae*). Arch. f. Mikrobiol. 37, 355-368 (1960 a).
- FLAIG, W. and W. de JONG: Vergleich der Wirkung von Thymochinon und Thymohydrochinon mit der von 2,4-Dinitrophenol auf den Stoffwechsel der Hefe (*Saccharomyces cerevisiae*). Arch. f. Mikrobiol. 37, 369-378 (1960 b).
- FLAIG, W. and H. RIEMER: Polarographische Untersuchungen zum Verhalten von Trihydroxytoluolen bei der Reaktion mit Glycin unter Oxydierenden Bedingungen. Justus Liebigs ANNALEN DER CHEMIE 246, 81-85 (1971).
- FLAIG, W. and E. SAALBACH: Zur Kenntnis der Huminsäure. XIII. Untersuchungen über die Beeinflussung der Anfangs-entwicklung von Getreide in Neubauerschalen durch Thymohydrochinon als Modellsubstanz von Vorstufen bzw. Abbauprodukten von Huminsäuren. Z. Pflanzenernähr. Düng. Bodenk. 72, 7-15 (1956)-Beziehungen zwischen Kalium, Wasser und Boden, Kalium-Symposium (1958).
- FLAIG, W. and Chr. SALFELD: UV-Spektren und Konstitution von p-Benzochinonen. Liebigs Ann. Chem. 618, 117-139 (1958).
- FLAIG, W., K. SCHARNER and G. SCHOLL: Humic acids XVI. The effect of Thymohydroquinone as model substance of humic matter on the activity of various enzymes in rye. Z. Pflanzenernähr. Düng. 76, 201-209 (1957 a).
- FLAIG, W., K. SCHARNER and G. SCHOLL: Humic acids XVII. The effect of Thymohydroquinone as model substance of humic matter on the phosphorus uptake by rye seedlings. Z. Pflanzenernähr. Düng. 76, 210-212 (1957 b).
- FLAIG, W. and G. SCHMID: Über den Wirkungsmechanismus stoffwechsellaktiver Substanzen. In: Eigenschaften und Wirkungen der Gibberelline, Symposium der Oberhessischen Gesellschaft für Natur- und Heilkunde, Naturwissenschaft. Abt. zu Giessen vom 1.-3. Dez. 1960, S. 25-27 (Herausg. R. Knapp). Springer-Verlag Berlin-Göttingen-Heidelberg 1962.
- FLAIG, W., U. SCHÖBLINGER and H. DEWEL: Umwandlung von Lignin in Huminsäuren bei der Verrottung von Weizenstroh. Chem. Ber. 92, 1973-1982 (1959).
- FLAIG, W. and G. SCHOLL: Hacia el conocimiento de los ácidos húmicos. XVIII. Comunicación "Influencias de la timohidroquinona sobre la economía del agua en plantas de interés agrícola. Anales de Edafología y Agrobiología XIX, 251-259 (1960).

- FLAIG, W. and H. SÖCHTIG: Einfluß organischer Stoffe auf die Aufnahme anorganischer Ionen.- *Agrochimica* 6, 251-264 (1962).
- FLAIG, W., F. SCHEFFER and B. KLAMROTH: Zur Kenntnis der Huminsäuren. VIII. Zur Charakterisierung der Huminsäuren des Bodens. *Z. Pflanzenernähr., Düng., Bodenkunde* 71, 33-37 (1955).
- FREUDENBERG, K.: Forschung am Lignin, *Fortschr. Chem. org. Naturstoffe* 20, 41 (1962).
- FREUDENBERG, K.: Entwurf eines Konstitutionsschemas für das Lignin der Fichte, *Holzforsch.* 18, 3-9 (1964 a).
- FREUDENBERG, K.: Ergänzung des Konstitutionsschemas für das Lignin der Fichte, *Holzforschung* 18, 166 (1964 b).
- FREUDENBERG, K. and J.M. HARKIN: Ergänzung des Konstitutionsschemas für das Lignin der Fichte. -*Holzforsch.* 18, 166-168 (1964).
- FREUDENBERG, K. and A.C. NELSH: Constitution and biosynthesis of lignin. Springer-Verlag Berlin-Heidelberg, New York 1968.
- FRÖMEL, W.: Über Absorptionsspektren von Huminsäuren in Lösungen. *Bodenkunde und Pflanzenernähr.* 6, 93-119 (1938 a).
- FRÖMEL, W.: Über UV-Absorptionsspektren von Huminsäuren in Lösungen. *Bodenkunde und Pflanzenernähr.* 11, 129-144 (1938 b).
- FRÖMEL, W.: Über Fulvosäuren. *Bodenkunde und Pflanzenernähr.* 25, 345-358 (1941).
- FÜHR, F.: Untersuchungen zur Aufnahme von Kohlendioxid und Strohabbauprodukten durch die Pflanzenwurzel. Dissertation Bonn 1962.
- FÜHR, F. and D. SAUERBECK: Über die Aufnahme und Translokation ¹⁴C-markierter organischer Rotteprodukte bei *Raphanus sativus*. *Z. Pflanzenernähr., Düng., Bodenkunde* 105, 136 (1964).
- FÜHR, F. and D. SAUERBECK: Die räumliche und chemische Verteilung durch die Wurzel aufgenommener organischer Rotteprodukte bei *Daucus carota*. - *Z. Landw. Forsch.* 19, Sonderheft 153-163 (1965).
- FÜHR, F. and D. SAUERBECK: The uptake of straw decomposition products by plant roots. In: "The use of isotopes in soil organic matter studies". Report of the FAO/IAEA Technical Meeting. Oxford Pergamon Press Ltd., 73-83 (1966).
- FÜHR, F. and D. SAUERBECK: The uptake of colloidal organic substances by plant roots as shown by experiments with ¹⁴C-labelled humus compounds. *Isotopes in plant nutrition and physiology*. International Atomic Energy Agency, Vienna 1967, 317-328.

- GALE, E.F.: The bacterial amino acid decarboxylases. *Advan. Enzymol* 6, 1-32 (1946).
- GRABBE, K. und K. HAIDER: Die Huminstoffbildung und die Stickstoffverteilung bei der Strohrotte in Beziehung zur mikrobiellen Phenolbildung. *Z. Pflanzenern., Bodenkunde* 129, 202-216 (1971).
- GRABBE, K. and K. HAIDER: *Z. Pflanzenernähr., Bodenkunde* (1971) in press.
- GREAVES, M.P. and M.J. WILSON: The adsorption of nucleic acids by montmorillonite. *Soil Bio. Biochem.* 1, 317-323 (1969).
- GREENLAND, D.J.: The adsorption of sugar by montmorillonite II. Chemical studies. *J. Soil-Sci.* 2, 329-334 (1956).
- GROSS, S.R., R.D. GAFFORD and E.L. TATUM: The metabolism of protocatechuic acid by *Neurospora*. *J. Bio. Chem.* 210, 781-796 (1956).
- GUMINSKA, Z. and J. SULEJ: The effect of sodium humate and sodium versenate upon sprouting of seed. *Biuletyn Instytutu Howowli i Aklimatyzacji. Roslin* Nr. 3 29 (1964).
- HAIDER, K.: Untersuchungen über den mikrobiellen Abbau von Lignin. *Zbl. Bakteriol. Parasitenkunde Infektionskrankh. und Hygiene*, 198, 308-316 (1965).
- HAIDER, K. and J.P. MARTIN: Synthesis and transformation of phenolic compounds by *Epicoccum nigrum* in relation to humic acid formation. *Proc. Soil Sci. Soc. Am.* 31(6), 766-772 (1967).
- HAIDER, K., J.P. MARTIN: The role microorganisms in the formation of humic acids. - In: *Isotopes and radiation in soil organic matter studies. Proceedings of the Symposium on the use of isotopes and radiation in soil organic matter studies.* Intern. Atomic Energy Agency, Vienna 1968, 188-195.
- HAIDER, K., Z. FILIPS, and J.P. MARTIN: Einfluss von Montmorillonit auf die Bildung von Biomasse und Stoffwechselzwischenprodukten durch einige Mikroorganismen. *Arch. Mikrob.* 73, 201-215 (1970).
- HAIDER, K. and J.P. MARTIN: Humic acid-type phenolic polymers from *Aspergillus sydowi* culture medium. *Stachybotrys* spp. cells and autoxidized phenol mixtures. - *Soil Biol. Biochem.* 2, 145-156 (1970).
- HAIDER, K., L.R. FREDERICK and W. FLAIG: Reactions between amino acid compounds and phenols during oxidation. - *Plant and Soil* XXII, 49-64 (1965).
- HAIDER, K., S. LIM and W. FLAIG: Untersuchungen über die Einwirkung von Mikroorganismen auf ¹⁴C-markierte phenolische Verbindungen, *Landw. Forschung* 15, 3/4, 1-9 (1962).

- HAIDER, K., S. LIM and W. FLAIG: Experimente und Theorien über den Ligninabbau bei der Weißfäule des Holzes und bei der Verrottung pflanzlicher Substanz im Boden. *Holzforsch.* 18, 81-88 (1964).
- HAMILTON, G.L.: Mechanismen of two- and four-electron oxidations catalysed by some metalloenzymes in "Adv. in Enzymology", 32, 55-96 (1969) Intersciences Publishers, New York, London, Sydney, Toronto (1969).
- HANF, M.: Pflanzenschutzentwicklung i. J. 1946-1971. BASF-Mitteilg. f. d. Landbau, Pflanzenschutz März 1972.
- HANSEN, E.H. and M. SCHNITZER: The alkaline permanganate oxidation of Danish illuvial organic matter. *Proc. Soil Sci. Soc. Am.* 30, 745-748 (1966).
- HANSEN, E.H. and M. SCHNITZER: Zn-dust distillation and fusion of a soil humic and fulvic acid. *Soil Sci. Soc. Amer. Proc.* 33, 29-36 (1969).
- HARMS, H.: Untersuchungen zur Aufnahme von phenolischen Ligninspaltstücken durch die Wurzeln von Weizenkeimpflanzen. Dissert. Justus-Liebig-Universität Giessen (1967).
- HARMS, H. und I. PRIESS: Positionsspezifische O-Demethylierung von Benzoesäuren in Weizenkeimpflanzen. *Planta* 109, 307-315 (1973)
- HARMS, H., H. SÖCHTIG and K. HAIDER: Untersuchungen zur Aufnahme und Umwandlung C^{14} -markierter Phenole durch die Pflanze. I. Aufnahme von C^{14} -carboxylmarkierter p-Hydroxy-benzoe-, Vanillin- und Syringasäure durch die Wurzeln von Weizenkeimpflanzen und Verteilung der Aktivität über die Pflanze. *Plant and Soil* XXXI, No. 1 129-142 (1969 a).
- HARMS, H., H. SÖCHTIG and K. HAIDER: Untersuchungen zur Aufnahme und Umwandlung C^{14} -markierter Phenole durch die Pflanze. II. Die Umwandlung von p-Hydroxy-benzoesäure, Vanillinsäure, sowie Syringasäure nach der Aufnahme durch die Wurzeln von Weizenkeimpflanzen.- *Plant and Soil* XXXI, No. 2, 257-272 (1969 b).
- HARMS, H., H. SÖCHTIG and K. HAIDER: Aufnahme und Umwandlung von unterschiedlichen Stellungen C^{14} -markierter Phenolcarbonsäuren in Weizenkeimpflanzen.- *Z. Pflanzenphysiologie* 64, Heft 5, S. 437-445 (1971).
- HARMS, H., K. HAIDER, J. BERLIN, P. KISS and W. BARZ: Über O-demethylierung und Decarboxylierung von Benzoesäuren in pflanzlichen Zellsuspensions-kulturen. *Planta* 105, 342-351 (1972).

- HOLLECK, L. and D. BECHER: Untersuchungen über den Einfluß der Leit-salitionen auf die polarographische Reduktion aromatischer Nitroverbindungen in Acetonitril und Dimethylformamid. *J. electroanal. Chem.* 4, 321-331 (1962).
- HOLMBERG, B: Thioglykolsäure als Ligninreagenz. *Ing. Ventenskaps Akad. Handl. No.* 131, 5-15 (1934).
- HOLMBERG, B. : Hypobromite lignin. -- *Ber.* 75, 1760-1764 (1942).
- ISHIWATARI, R. : An estimation of aromaticity of lake sediment humic acid by air oxidation and evaluation of it. *Soil Sci.* 107, 53-57 (1969).
- JACQUIN, F. : Chromatographic study of various types of humic acids. *C. R. hebd. Seances Acad. Sci.* 250, 1892-1893 (1960).
- JACQUIN, F. : Contribution a l'étude des processus de formation et d' evolution des divers composés humiques. Thèse Docteur des Sciences. *Bull. ENSAN V*, 1-156 (1963).
- JANSSON, S.L. and J. PERSON: Co-Ordination of humus chemistry and soil organic matter biology by isotope techniques. In: *Isotopes and radiation in soil organic matter studies.* International Atomic Energy Agency, Vienna 1968, 111-123.
- JENKINSON, D.S.: The turnover of organic matter in soil. In: *The use of isotopes in soil organic matter studies.* Report of the FAO/IAEA Technical Meeting. Pergamon Press. Ltd. Oxford 1966 a, 187-197.
- JENKINSON, D.S.: The priming action. In: *the use of isotopes in soil organic matter studies.* Report of the FAO/IAEA Technical Meeting. Pergamon Press, Ltd., Oxford 1966 b, 199-208.
- JENKINSON, D.S.: Experimental techniques for using carbon-14 in studies of soil organic matter. In: *The use of isotopes in soil organic matter studies.* Report of the FAO/IAEA Technical Meeting. Pergamon Press. Ltd. Oxford 1966 c, 365-369.
- JENKINSON, D.S.: Studies on the decomposition of plant materials in soil I-III. *J. Soil Sci.* 16, 104-115 (1965; 17, 280-302 (1966 b); 19, 25-39 (1968).
- KAILA, A. : Humification of straw at various temperatures. *Acta agral. fenn. Helsinki* 28, 3-32 (1952).
- KANG, K.S. and G.T. FELBACK: A comparison of the alkaline extracts of tissues of *Aspergillus niger* with humic acids from soils. *Soil Sci.* 99, 175-181 (1965).
- KASATOCHKIN, V.I. and O.I. ZILBERBRAND: X-ray and infrared spectroscopy applied to the study of the structure of humic substances. *Pochvovedenie No.* 5, 80-85 (1956).

- KASATOCHKIN, V.I., M.M. KONONOVA and G.I. ZILBERBRAND: Infrarotspektren von Huminsäuren des Bodens. Dokl. Akad. Nauk 119, 785-788 (1958). (russ.)
- KASTORI, R., H. HANAS, H. SÖCHTIG and K. HAIDER: Untersuchungen zur Aufnahme und Umwandlung C^{14} -markierter Phenole durch die Pflanze. III. Die Aufnahme, der Transport und die Umwandlung von C^{14} -markiertem Thymohydrochinon durch Weizenkeimpflanzen. *Plant and Soil* 33, 597-611 (1970).
- KHAN, S.U. : Distribution and characteristics of organic matter extracted from the black solonchic and black chernozemic soils of Alberta; the humic acid fraction. *Soil Sci.* 112, 401-409 (1971).
- KHAN, S.U. and F.J. SOWDEN: Distribution of nitrogen in the black solonchic and black chernozemic soils of Alberta. *J. Soil Sci.* 51, 185-193 (1971).
- KHAN, S.U. and F.J. SOWDEN: Distribution of nitrogen in fulvic acid fraction extracted from the black solonchic and black chernozemic soils of Alberta. *Can. J. Soil Sci.* 52, 116-118 (1972).
- KLEIST, H. and D. MUCKE: Stabile freie Radikale in Huminsäuren. *Experientia* 22, 136-137 (1966).
- KLEIST, H. : 4. Torf-Kolloquium DDR-VR Polen 1/4 Rostock (1967).
- KOLENBRANDER, G.J.: Die Verluste an organischer Substanz im Stalldünger. *Z. Pflanzenernähr., Düng., Bodenkunde* 69, 125-134 (1955).
- KONONOVA, M.M.: Humus der Hauptbodentypen der UdSSR, seine Natur und Bildungsweisen. *Rapports VI. Congr. Internat. de la Science du Sol, Chemie du Sol*, S. 5, Moskau (1956) (russ.)
- KONONOVA, M.M.: Soil organic matter. Its nature, its role in soil formation and in soil fertility. Pergamon Press Oxford, London, New York, Paris (1961).
- KONONOVA, M.M.: Gedanken zur Nomenklatur der im Boden vorhandenen organischen Stoffe. 8th Internat. Congr. of Soil Sci. Transaction, Vol. III, Bucharest-Romania, 401-404 (1964). Discussions regarding the terminology in the field of humus, 405-413.
- KONONOVA, M.M.: Soil organic matter, its nature, its role in soil formation and in soil fertility. Pergamon Press, Oxford, London, Edinburgh, New York, Toronto, Sydney, Paris, Braunschweig, (1966).
- KRATZL, K. and P. CLAUS: Zur quantitativen Bestimmung der monomeren Athenolysenprodukte aus dem Lignin monocotylar und dicotylar Angiospermen, *Monatsh. Chem.* 93, 219-229 (1962).

- KRATZL, K., and E. RISNYOVSKY: Zum Mechanismus der alkalischen Hydrolyse der Ligninsulfosäure. *Chimie et Biochimie de la Lignine de la Cellulose et des Hemicelluloses. Actes du Symposium International de Grenoble, Juillet (1964).*
- KRATZL, K., G. BILLIK, E. KLEIN, K. EUTSCHER: Über das Verhalten von markiertem Coniferin in der verholzenden Pflanze. *Monatsh. f. Chemie* 88, 721-734 (1957).
- KRATZL, K., E. RISNYOVSKY, P. CLAUS and E. WITTMANN: Über den Mechanismus der alkalischen Hydrolyse von Guajacylglycerin- β -guajacylather- α -sulfosäure. I. Mitteilung, Modellversuche zur Fragmentierung der Ligninsulfosäure in alkalischem Medium. *Holzforschung* 20, 21-27 (1966).
- KRATZL, K., W. SCHAFER, P. CLAUS J. GRATZL and P. SCHILLING, Zur Oxydation von ^{14}C -markierten Phenolen (Ligninmodellen) *Mh. Chemie* 98, 891-904 (1967).
- KUBISTA, K.: The influence of bentonite and of aeration on the dynamics of the development of microorganisms during the decomposition of lucerne in a sand culture. *Rostlinna Vyroba (Prague)* 15, 221-228 (1969).
- KUMADA, K.: Studies on the colour of humic acids. Part. I. On the concepts of humic substances and humification. *Soil Sci. and Plant Nutr.* 11, 11-16, (1965).
- KUMADA, K. and A. AIZAWA: The infrared spectra of humic acids. *Soil and Plant Food* 3, 1 2-159 (1958).
- KUMADA, K. and K. AIZAWA: The infra-red absorption spectra of soil components. *Soil and Plant Food* 4, 101-188 (1959).
- KÜSTER, E.: Umwandlung von Mikroorganismen-Farbstoffen in Huminstoffe. *Z. Pflanzenernähr., Düng., Bodenkunde* 57, 51-57 (1952).
- KÜSTER, E.: Humusbildung und Phenoloxidasen bei Streptomyceten. *Z. Pflanzenernähr., Düng., Bodenk.* 69, 137-142 (1955).
- KÜSTER, E.: Beiträge zur Physiologie der Streptomyceten. VI. *Congres International de la Sci. du Sol. Paris* 3, 67-72 (1956).
- KYUMA, K.: A fractional precipitation technique applied to soil humic substances. *Soil Sci. Plant Nutr.* 10, 33-35 (1964).
- LANTZ, R. and H. MICHEL. Action de l'ammoniaque en des amines primaires sur le benzene substitué en 1,3 et par des groupes amino ou hydroxyles, non substitués ou substitués. *Bull. Soc. Chim. France* 12, 2402-2408 (1961).
- LAATSCH, W., L. HOOPS and O. BIENECK: Über Huminsäuren des Pilzes *Spicaria elegans*. *Z. Pflanzenernähr., Düng., Bodenkunde* 58, 258-268 (1952).

- LEVESQUE, M. and M. SCHNITZER: Organo-metallic interactions in soils: 6. Preparation and properties of fulvic acid-metal phosphates. *Soil Sci.* 103, 183-190 (1967 b).
- LIM, S.: Beiträge zur Aufklärung der Zusammenhänge zwischen dem mikrobiellen Abbau des Lignins und der Bildung von Humusstoffen. Dissertation der Rheinischen Friedrich Wilhelms-Universität Bonn (1965).
- LYINCH, D.L., L.M. WRIGHT and L.J. COTNOIR: The adsorption of carbohydrates and related compounds on clay minerals. *Proc. Soil Sci. Soc. Amer.* 20, 6-9 (1956).
- LYNCH, D.L., L.M. WRIGHT and L.J. COTNOIR: Some factors affecting the adsorption of cellulose compounds, pectins and hemicellulose compounds, on clay minerals. *Soil Sci.* 84, 113-126 (1957).
- MAEDER, H. : Chemische und pflanzenphysiologische Untersuchungen mit Rottestroh. Diss. Justus Liebig Universität Giessen (1960).
- MARSHALL, K.C.: Methods of study and ecological significance of Rhizobium-clay interactions. In methods of study in Soil Ecology. (J. Phillipson, Ed) Proceedings of UNESCO and IBP, Paris 1970. pp. 107-110. UNESCO, Paris (1967).
- MARTIN, A.E. and R. REEVE: The extraction of organic matter from podzolic B-horizons with organic reagents. *Chem. and Ind.* 1955, 356.
- MARTIN, A.E. and R. REEVE: Chemical studies on podzolic illuvial horizons. I. The extraction of organic matter by organic chelating agents. *J. Soil Sci.* 8, 268-270 (1957 a).
- MARTIN, A.E. and R. REEVE: Chemical studies on podzolic illuvial horizons. II. The use of acetylacetone as extractant of translocated organic matter. *J. Soil Sci.* 8, 279-286 (1957 b).
- MARTIN, A.E., P. DUBACH, N.C. MEHTA and H. DEJEL: Bestimmung der funktionellen Gruppen von Huminstoffen.-Z. Pflanzenernähr., Düng., Bodenkunde 103, 29-39 (1963).
- MARTIN, J.P. and K. HAIDER: Phenolic polymers of *Stachybotrys atra*, *Stachybotrys chartarum* und *Epicoccum nigrum* in relation to humic acid-formation. *Soil Sci.* 107, 260-270 (1969).
- MARTIN, J.P. and K. HAIDER: Microbial activity in relation to soil humus formation. *Soil Science* 111, No. 1, 54-63 (1971).
- MARTIN, J.P., S.J. RICHARDS and K. HAIDER: Properties and decomposition and binding action in soil of humic acid synthesized by *Epicoccum nigrum*. *Soil Sci. Soc. Amer. Proc.* 31, 657-662 (1967).

- MASON, H.S: Chemistry of melanin. III. Mechanism of oxidation of 3¹⁴-dihydroxyphenylalanine by tyrosinase. *J. Bio. Chem.* 172, 83-99 (1948).
- MAYAUDON, J. and P. SIMONART: Etude de la decomposition de la matière organique dans le sol au moyen de carbone radioactif. II. Decomposition du glucose radioactif dans le sol. A. Répartition de la radioactivité dans fractions humiques du sol. *Plant and Soil* IX, 376-380 (1958).
- MAYAUDON, J. and P. SIMONART: Etude de la decomposition de la matière organique dans le sol au moyen de carbone radioactif. III. Decomposition des substances solubles dialysables des protéines et des hemicelluloses. *Plant and Soil* XI, 170(1959 a).
- MAYAUDON, J. and P. SIMONART: Etude de la décomposition de la matière organique dans le sol au moyen de carbone radioactif. V. Decomposition de cellulose et de lignine, *Plant and Soil* XI, 181-192 (1959 b).
- MAYAUDON, J. and P. SIMONART: Stabilité de l'humus du sol étudiée à l'aide d'humus marqué au ¹⁴C-*Arch. Internat. de Phys. et de Biochim.* 68, 512 (1960).
- MAYAUDON, J. and P. SIMONART: Decomposition of cellulose C¹⁴ and lignin C¹⁴ in the soil. *Ecology of Soil Fungi* (1961).
- MAZUMDAR, B.K., S.K. CHAKRABARTTY and A. LAHIRI: Aromaticity and oxidation of coal. *Sci. Ind. Res. (India)* 16, B. 275 (1957).
- McCALLA, T.M.: The adsorbed ions of colloidal clay as a factor in nitrogen fixation by *Azotobacter*. *Soil Sci.* 48, 281-286(1939).
- McCALLA, T.M.: Physico-chemical behavior of soil bacteria in relation to the soil colloid. *J. Bact.* 40, 33-43 (1940).
- McLAREN, A.D. and G.H. PETERSON: Introduction to the biochemistry of terrestrial soils. In *Soil Biochemistry* (A.D. McLaren and G.H. Peterson, Eds.) pp. 1-15, MarcelDekker, New York(1967).
- MEYER, B.: Exkursionsführer zur Jahrestagung 1969 in Hannover. *Mitt. Deutsche Bodenkundl. Ges.* 9, 104 u. 105 (1969).
- MIGITA, N. and M. KAWAMURA: Studies on lignin. I. A comparison between the insoluble and soluble portions of lignin sulfate isolated from various trees.- *J. Agr. Chem. Soc. Japan* 20, 348-352 (1944 a).
- MITCHELL, L.P.: Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. *Bio. Rev. Cambridge philos. Soc.* 41, 445-502 (1966).
- MITSCHERLICH, E.A: An der Grenze der Ertragssteigerung. *Z. Pflanzenernähr., Düng., Bodenkunde* 40, 193-200 (1948).

- MOHTADI, S.: Beitrag zur Isolierung von pflanzenphysiologisch wirksamen Stoffen während der Rotte von Stroh. Dissertation Giessen (1962).
- MORRISON, R.I.: Products of the alkaline nitrobenzene oxidation of soil organic matter. *J. Soil Sci.* 14, 201-216 (1963).
- MOSCHO PEDIS, S.E.: *Fuel*, 31, 425 (1962).
- MUSSO, H.: Über Phenol-Oxydationen. - *Angew. Chem.* 75, 965-977(1963).
- MUSSO, H., U.V. GIZYCKI, H. KRAMER and H. DOPP: Über Orceinfarbstoffe. XXIV. Über den Autoxydationsmechanismus bei Resorcinderivaten. *Chem. Ber.* 98, 3952-3963 (1965).
- NAUMOVA, A.N. and E.P. GROMYKO: The influence of gumbrin on the micro-organism of gray-soil. *Mikrobiologiya*. 22, 1 (1953).
- NEHRING, K. and R. SCHIEMANN: Untersuchungen zum Humusproblem. I. Mitt. Beiträge zur Kenntnis der Vorgänge bei der Rotte von Stallmist und Komposten sowie zur Kenntnis der Huminsäuren. *Z. Pflanzenernähr., Düng., Bodenkunde* 57, 97-113 (1952 a).
- NEHRING, K. and R. SCHIEMANN: Untersuchungen zum Humusproblem. I. Mitt. Beiträge zur Kenntnis der Vorgänge bei der Rotte von Stallmist und Komposten sowie zur Kenntnis der Huminsäuren. 2. Teil Beiträge zur Kenntnis der Huminsäuren. *Z. Pflanzenernähr., Düng., Bodenkunde* 57, 193-215 (1952 b).
- NICOLAUS, R.A.: Biogenese der Melanine. *Conf. VII Corso chim. Acad. naz. Lincei, Milano* (1962).
- NOVAK, B.: Contribution to the Theory of microbial formation of humus. *For. Social Agri. Sci. (Prague)* 12, 401-418 (1963).
- NOVAKOVA J.: The effect of different amounts of bentonite and kaolinite on the decomposition of glucose. In *Studies about Humus. Trans. Intern. Symp. "Humus et planta IV"*, Prague:185-188 (1967).
- NOVAKOVA J.: The influence of different quantities of bentonite and kaolinite on glucose mineralization. *Rostlinna Vyroba(Prague)* 15, 215-220 (1969).
- OBERLANDER, H.E. and K. ROTH: Transformation of ^{14}C -labelled plant material in soils under field conditions. In: *Isotopes and radiation in soil organic matter studies. International Atomic Energy Agency, Wien 1968*, 251-264.
- ORLOV, D.S. and N.M. GRINDEL: Spectrophotometric determination of the humus content in soil. *Pochvovedenie Nr. 1*, 112-122(1967) (russ.)

- ORLOV, D.S., O.H. ROZANOVA and S.G. MATYUKHINA: Infrared absorption spectra of humic acids. Pochvovedenie Nr. 1, 17-25 (1962) (russ.)
- OTTEY, L., E.L. TATUM: Protocatechuic acid oxidase of Neurospora. J. Biol. Chem. 223, 307-311 (1956).
- PEOVER, M.E. and J.D. DAVIES: Einfluß der Ionenassoziation auf die Polarographie der Chinone in Dimethylformamid.-J. electroanalyt. Chem. 6, 46-53 (1963).
- PHILLIPS, M: The chemistry of lignin. Chem. Rev. 14, 103-170 (1934).
- PLOETZ, Th.: Beiträge zur Ligninbestimmung mit starker Schwefelsäure. Cellulosechemie 18, 49-57 (1940).
- PLOETZ, Th.: Polymere Chinone als Huminsäuremodelle.-Z. Pflanzenernähr., Düng., Bodenkunde 69, 50-58 (1955).
- PLOTHO, O.v.: Die Humusbildung der Mikroorganismen. Z. Pflanzenernähr., Düng., Bodenkunde 51, 212 (1950).
- PLOTHO, O.v.: Weitere Untersuchungen zur Humusbildung der Mikroorganismen. Z. Pflanzenernähr., Düng., Bodenkunde 55, 151-169 (1951).
- POSNER, A.M.: The humic acid extracted by various reagents from a soil. Part. I. Yield, inorganic components, and titration curves. J. Soil Sci. 17, 65-78 (1966).
- POSNER, A.M., B.K.G. THENG and J.R.H. WAKE: The extraction of soil organic matter in relation to humification. 9th Internat. Congr. Soil Sci., Adelaide, Australia, Transaction III, 153-162 (1968).
- RAPER, H.S.: Die Einwirkung von Tyrosinase auf Tyrosin. Fermentforschung 9, 206 (1927).
- RAPER, H.S.: The aerobic oxidases. - Physiol. Rev. 8, S.245-248 (1928).
- REINHARDT, G.: Untersuchungen über die Einwirkung von Lignin und dessen Abbauprodukten auf das Wachstum und den Stoffwechsel von Pflanzen. Dissertation Universität Giessen 1961.
- RIEMER, H.: Polarographische Untersuchungen über Beziehungen zwischen Struktur und Reaktivität bei Benzochinonen. Dissertation TU Braunschweig 1970.
- RIFFALDI, R. and M. SCHNITZER: Electron spin resonance spectrometry of humic substances. Proc. Soil Sci. Soc. Am. 36, 301-305 (1972).

- RITTER, G.J., R.M. SEBORG and R.L. MITCHELL: Factors affecting quantitative determination of lignin by 72 percent sulfuric acid method. *Ind. Eng. Chem. Analyt. Edit.* 4, 202 (1932).
- RUHEMANN, H.: Untersuchungen zur Auftrennung physiologisch aktiver Stoffe aus Lignin unter Anwendung von Radiokohlenstoff. Dissertation Th Braunschweig 1964.
- RUIZAMIL, M. and W. FLAIG: The effect of Thymohydroquinone on the organic-acid metabolism and the respiration of plants. *An. Edafol. Agrobiol.* 19, 1-9 (1964).
- RUIZAMIL, M. and W. FLAIG: Influence de la timohidroquinona sobre el metabolismo de carbohidratos en algunas dicotiledoneas. *Annles de Edafologia y Agrobiologia XIX*, 1, 11-22 (1960 b).
- SAALBACH, E.: Zur Kenntnis der Huminsäuren, XIV. Mitt. Einfluß von Modell-substanzen von Humusstoffen auf den Stoffwechsel von Getreide, *Landw. Forsch.* 9. Sonderheft 95-100 (1957).
- SAGAVE, B.: Grundzüge der Abschätzungslehre. P. Parey, Berlin 125-131 (1934).
- SALFELD, J. Chr.: Zum Reaktionsmechanismus der Purpurogallinbildung. *Angew. Chem* 69, 723-724 (1957).
- SALFELD, J. Chr.: Fraktionierung eines Huminstoffpräparates mit wasserhaltigen Lösungsmitteln. *Landbauforsch. Völknerode* 14, 131-136 (1964).
- SALFELD, J. Chr.: Die Charakterisierung von Huminstoffen durch Differenzen-Spektrogramme. *Mitt. Dtsch. Bodenkundl. Ges.* 4, 289-290 (1965).
- SALFELD, J. Chr.: Untersuchungen zur Klassifizierung der organischen Bodensubstanz. *Mitt. Dtsch. Bodenkundl. Ges.* 8, 133-135 (1968)
- SALFELD, J. Chr.: Optical measurements on humic systems. *Symposium Humus at Planta V, Prag* (1971), 257-266.
- SALFELD, J. Chr. and E. BAUME: Über die Oxydation von Pyrogallol und Pyrogallolderivaten. IV. Die Konstitution der Purpurogallin-carbonsäure -(9). *Chem. Ber.* 97, 307-311 (1964)
- SALFELD, J. Chr. and H. SÖCHTIG: Ergänzung zum Exkursionsführer der Jahrestagung der Dtsch. Bodenkundl. Ges. in Hannover (1969).
- SAUERBECK, D.: Stability of recently formed humus compounds in soil. In: *Isotopes and radiation in soil organic matter studies. International Atomic Energy Agency, Vienna 1968 b*, 57-66.
- SAUERBECK, D.: Die Umsetzung markierter organischer Substanzen im Boden in Abhängigkeit von Art, Menge und Rottegrad. *Landwirtsch. Forsch.* 21, 91-102 (1968 a).

- SAUERBECK, D.: Comparison of plant material and animal manure in relation to their decomposition in soil. In: Isotopes and radiation in soil organic matter studies. International Atomic Energy Agency, Vienna 1968 c, 219-225.
- SAUERBECK, D. and F. FÜHR: Alkali extraction and fractionation of labelled plant material before and after decomposition - a contribution to the technical problems in humification studies. In: Isotopes and radiation in soil organic matter studies. International Atomic Energy Agency, Vienna 1968, 3-11.
- SAUERBECK, D. and F. FÜHR: Die Umsetzung von markiertem Pflanzenmaterial im Boden unter Feldbedingungen. Mitt. Deutsche Bodenkundl. Gesellsch. 10, 174-177 (1970).
- SCHARPENSEEL, H.W.: Herstellung und Reinigung von tritiummarkierten Graublau Braun/Huminsäurepräparaten sowie von Tritium-Purpurgallin. -Z. Pflanzenernähr., Düng. und Bodenkunde 91, 131-146 (1960 a).
- SCHARPENSEEL, H.W.: Untersuchungen mit radioaktiv markierten Huminsäuren und Vorstufen. 2. Teil.-Z. Pflanzenernähr., Düng. und Bodenkunde 91, 193-202 (1960 b).
- SCHARPENSEEL, H.W.: Labelling of soil organic matter. In: The use of isotopes in soil organic matter studies. Report of the FAO/IAEA Technical Meeting, Pergamon Press. Ltd. Oxford 1966a, 351-364.
- SCHARPENSEEL, H.W.: Aufbau und Bindungsform der Ton-Huminsäurekomplexe. Teil IV. Z. Pflanzenernähr. Düng. V. Bodenk. 55, 151-169 (1970).
- SCHARPENSEEL, H.W. and W. ALBERSMEYER: Infrarotspektroskopische Untersuchungen an Huminsäuren, Huminsäureaufschlüssen und phenolisch-chinoiden Vergleichssubstanzen. Z. Pflanzenernähr. Düng., Bodenkunde 88, 3, 203-211 (1960).
- SCHARPENSEEL, H.W. and H. BECKMANN: Untersuchung zur Kohlendioxid-Entbindung des Bodens. II. Teil. Spezielle Studien unter Verwendung radioactiver Tracer.-Z. Pflanzenernähr., Düng. Bodenkunde 104, 110-119 (1964).
- SCHARPENSEEL, H.W. and R. KRAUSSE: Aminosäureuntersuchungen an verschiedenen organischen Sedimenten, besonders Grau- und Braunhuminsäurefraktionen verschiedener Bodentypen (einschließlich C^{14} -markierter Huminsäuren). Z. Pflanzenernähr., Düng. Bodenkunde 26, 11-34 (1962).

- SCHARPENSEEEL, H.W., E. KÖNIG and E. MENTHE: Infrarot- und Differentialthermo-Analyse an Huminsäureproben aus verschiedenen Bodentypen, aus Wurmkot und Streptomyces, Z. Pflanzenernähr., Düng., Bodenkunde 106, 134-150 (1964).
- SCHNEFFER, F. and B. ULRICH: Lehrbuch der Agrikulturchemie und Bodenkunde. III. Teil. Humus und Humusdüngung 1, Enke Stuttgart, pp. 266 (1960).
- SCHNEFFER, F., O.v. PLOTHO and E. WELTE: Untersuchungen über die Bildung von Humusstoffen durch Actinomyceten. - Landwirtschaftl. Forsch. 1, 81-92 (1950).
- SCHMID, G. and W. FLAIG: Pflanzenstoffwechsel und Wirkstoffe. Landbauforsch. 12, 51-56 (1962).
- SCHNITZER, M.: The application of infrared spectroscopy to investigations on soil humic compounds. Canad. Spectroscopy 10, No. 5, 121-127 (1965).
- SCHNITZER, M. and J.G. DESJARDINS: Molecular and equivalent weights of the organic matter of a podzol. Soil Sci. Soc. Amer., Proc. 26, 362-365 (1962).
- SCHNITZER, M. and J.G. DESJARDINS: Carboxyl and phenolic hydroxyl groups in some organic soils and their relation to the degree of humification. Can. J. Soil Sci. 45, 257-264 (1965).
- SCHNITZER, M. and I. HOFFMAN: Thermogravimetry of soil humic compounds. Geochim. Cosmochim. Acta 29, 359-370 (1965).
- SCHNITZER, M. and SU. KHAN: "Humic substances in the Environment". Marcel Dekker Inc. New York. (1972).
- SCHNITZER, M. and S.I.M. SKINNER: Organo-metallic interactions in soils: 3. Properties of iron- and aluminium-organic matter complexes, prepared in the laboratory and extracted from a soil. Soil Sci. 98, 197-203 (1964).
- SCHNITZER, M. and S.I.M. SKINNER: Organo-metallic interactions in soils: 4. Carboxyl and hydroxyl groups in organic matter and metal retention. Soil Sci. 99, 278-284 (1965 a).
- SCHNITZER, M. and S.I.M. SKINNER: The carboxyl group in a soil organic matter preparation. Soil Sci. Soc. Amer., Proc. 29, 406-405 (1965 b).
- SCHNITZER, M., D.A. SHEARER and J.R. WRIGHT: A study in the infrared of high molecular weight organic matter extracted by various reagents from a podzolic horizon. Soil Sci. 87, 252-257 (1959).

- SCHNITZER, M. and U.C. GUPTA: Some chemical characteristics of the organic matter extracted from the C and B2 horizons of a gray wooded soil. Proc. Soil Sci. Soc. Am. 28, 374-377 (1964).
- SCHNITZER, M. J.R. WRIGHT and J.G. DESJARDINS: A comparison of the effectiveness of various extractants for organic matter from two horizons of a podzol profile. Can. J. Soil Sci. 38, 49-53 (1958).
- SCHOBINGER, U. : Chemische Untersuchungen über die Umwandlung von Weizenstrohlignin im Laufe der Verrottung.- Dissertation, ETH Zurich (1958).
- SCHREINER, O. and E. SHOREY: Chemical nature of soil organic matter. U.S. Dept. Agric. Bureau of Soils Bull. 74, 5-48 (1910).
- SIMONART, P. and J. MAYAUDON: Etude de la décomposition de la matière organique dans le sol au moyen de carbone radioactif. I. Cinétique de l'oxydation en CO₂ de divers substrats radioactifs. Plant and Soil 9, 367-375 (1958 a).
- SIMONART, P. and J. MAYAUDON: Etude de la décomposition de la matière organique dans le sol au moyen de carbone radioactif. II. Décomposition du glucose radioactif dans le sol. B. Répartition de la radioactivité dans l' a-humus. - Plant and Soil 9, 381-384 (1958 b).
- SIMONART, P. and J. MAYAUDON: Etude des transformations de la matière organique du sol au moyen du carbone-14. In: The use of isotopes in soil organic matter studies. Report of the FAO/IAEA Technical Meeting. Pergamon Press. Ltd. Oxford 1966, 245-258.
- SIMONART, P., J. MAYAUDON and L. BATISTIC: Etude de la décomposition de la matière organique dans le sol au moyen de carbone radioactif. IV. Décomposition des pigments foliaires. Plant and Soil 11, 176-180 (1959).
- SKUJINS, J.J.: Enzymes in soil. In soil Biochemistry (A.D. Melaren and G.H. Peterson, Eds.) pp. 371-414, Marcel Dekker, New York. (1967).
- SLYKE, D.D. van, D.A. MacFAYDEN and P. HAMILTON: Determination of free amino acids by titration of the carbon dioxide formed in the reaction with ninhydrin. J. Biol. Chem. 141, 671-680 (1941).
- SMITH, J.H.: Some inter-relationships between decomposition of various plant residues and loss of soil organic matter as measured with carbon-14 labelling. In: The use of isotopes in soil organic matter studies. Report of the FAO/IAEA Technical Meeting. Pergamon Press. Ltd. Oxford 1966, 223-233.

- SMITH, F.B., W.H. STEVENSON and P.E. BROWN: The production of artificial manures. Agr. Exp. Sta. Res. Bul. 126, (1930).
- SNEATH, P.H.A.: The application of computers to taxonomy. J. Gen. Microbiol. 17, 201-226 (1957).
- SÖCHTIG, H.: Beeinflussung des Stoffwechsels der Pflanzen durch Humus und seine Bestandteile und die Auswirkung auf Wachstum und Ertrag. - Landbauforschung Völkenrode 14, 9-16 (1964)
- SÖCHTIG, H.: Ein Pflanzentest zur Feststellung der Wirkung stoffwechselaktiver Substanzen, Methodik und Erfahrungen. Deutsch-Polnisches Torf-Kolloquium DDR-Polen in Rostock 1967.
- SÖCHTIG, H.: Über den einfluß von N-Lignin auf die Nitrifizierung im Boden sowie den Ertrag und den Nitratgehalt der Pflanzen - Qual. Plant. Mater. Veg. XX, 1-2, 137-150 (1970).
- SÖCHTIG, H.: (1972) In press.
- SÖCHTIG, H. and H. HARMS: Über den Einfluß von Torf auf Keimung und Anfangs-wachstum von Pflanzen.- Landw. Forsch. 26/II (1971)
- SÖCHTIG, H. and F. MACIAK: Bindung des Stickstoffs und Vorkommen phenol-ischer Verbindungen im Torf. Telma, Band 1, 49-61 (1971).
- SÖCHTIG, H. and J. Chr. SALFELD: Characterization of humic systems in a black earth-Griserde-morphosequence in the area of Hildesheim. Symposium Humus et Planta V, Prag 1971, 247-256.
- SØRENSEN, H.: Studies on the decomposition of ¹⁴C-labelled barley straw in soil. Soil Sci. 95, 45-51 (1963).
- SØRENSEN, H.: Formation of soil organic matter during decomposition of plant components. In: The use of isotopes in soil organic matter studies. Report of the FAO/IAEA Technical Meeting. Pergamon Press. Ltd. Oxford 1966, 271-274.
- SOWDEN, F.J. and H. DEJEL: Fractionation of fulvic acids from the B-horizon of podzol. Soil Sci. 91, 44-47 (1961).
- SPRINGER, U.: Stoffabbau und Humusaufbau untersucht an einem Strohmis und Strohlignin ist (Laboratoriumsversuch). Prakt. Blätter f. Pflanzenbau u. Pflanzenschutz 21/22, 1-57(1944/45).
- SPRINGER, U.: Über Komposthuminsäuren aus verschiedenen pflanzlichen Ausgangsstoffen.-Z. Pflanzenernähr., Düng., Bodenkunde 69, 66-71 (1955).

- SPRINGER, U., and A. LEHNER: Stoffabbau und Humusaufbau bei der aeroben und anaeroben Zersetzung landwirtschaftlich und forstwirtschaftlich wichtiger organischer Stoffe. I. Z. Pflanzenernähr., Düng., Bodenkunde 58, 193-231 (1952 a).
- SPRINGER, U. and A. LEHNER: Stoffabbau und Humusaufbau bei der aeroben und anaeroben Zersetzung landwirtschaftlich und forstwirtschaftlich wichtiger organischer Stoffe. II. Z. Pflanzenernähr., Düng., Bodenkunde 59, 1-27 (1952 b).
- SPRINGER, U. and F. SELSCHAB: Zur Kenntnis der bei der Kompostbereitung auftretenden stofflichen Veränderungen. Mit Ergebnissen eines dreijährigen Gefäßversuches. Bayr. Landw. Jahrbuch 38, 250-300 (1961).
- STAHLER, R.Y. and J.L. INGRAHAM: Protocatechuic oxidase.- J. Biol. Chem. 210, 799-808 (1954).
- STEELINK, C.: Free radical studies of lignin, lignin degradation products and soil humic acids. Geochim. Cosmochim. Acta 28, 1615-1622 (1964).
- STEELINK, C. and G. TOLLIN: Stable free radicals in soil humic acid. Biochim. Biophys. Acta 59, 25-34 (1962).
- STEELINK, C., J.W. BERRY, A.HO and H.E. NORDBY: Alkaline degradation products of soil humic acid. Sci. Proc. Roy. Dublin Soc. Ser. A 1, 59-67 (1960).
- STEINMETZ, A.: Modellversuche zur Beteiligung von Polyphenoloxidasen bei der Bildung natürlicher Huminsäuren. Dissertation TH Braunschweig (1956).
- STOCKLI, A.: Über den Abbau von Lignin, Cellulose und Hemicellulose durch Pilze. Versuche mit Streumaterialien, Holz und Ligninsulfonsäure. Promotion ETH Zurich (1952).
- STOTZKY, G.: Influence of clay minerals on microorganisms. II. Effect of various clay species, homoionic clays, and other particles on bacteria. Can. J. Microbiol. 12, 831-843 (1966).
- STOTZKY, G.: Clay minerals and microbial ecology. Trans. N.Y. Acad. Sci. Ser II, 30, 11-21 (1967).
- STOTZKY, G. and L.T. REM: Influence of clay minerals on microorganisms. I. Montmorillonite and Kaolinite on bacteria. Can. J. Microbiol. 13, 1535-1550 (1967).
- SUNDMAN, W., K. HARO: On the mechanism by which cyclolignolytic agro-bacteria might cause humification. Finiska Kemists Med. 75, 11-118 (1966).

- SWABY, R.: Soil organic matter. -8th, 9th, 10th C.S.I.R.O. Annual Reports. Government Printers, Sydney. (1956-1958).
- THENG, B.K.G. and A.M. POSNER: Nature of the carbonyl groups in soil humic acids. *Soil Sci.* 101, 199-201 (1967).
- TINSLEY, J. and A. SALAM: Extraction of soil organic matter with aqueous solvents. *Soils and Fertilizers* 24, 81-84 (1961).
- TOKUDOME, S. and I. KANNO: Nature of the humus of some Japanese soils. 9th Internat. Congr. Soil Sci., Adelaide, Australia, Transactions III, 163-173 (1968).
- TRAYNARD, Ph. and A. EYMERY: Delignification par les solutions hydrotropiques. II. Etude des lignines hydrotropiques. *Holzfor-schung* 10, 6-11 (1956).
- TRIPPETT, S., S. DAGLEY and D.A. STOPHER: Bacterial oxidation of protocatechuic acid. *Biochem. J.* 76, 9p (1960).
- TSCHAPEK, W. and A.J. GARBOSKY. The principles of adsorption of the Azotobacter, Transactions of the Fourth International Congress of soil science, Proceedings, Amsterdam 3, 102-104 (1950).
- WAKSMAN, S.A. and J.W. SMITH: Transformation of methoxyl group in lignin in the process of decomposition of organic residues by microorganisms. *J. Amer. Chem. Soc.* 56, 1225 (1934).
- WAKSMAN, S.A. and F.G. TENNEY: The composition of natural organic materials and their decomposition in the soil. I. Methods of quantitative analysis of plant materials. *Soil Sci.* 24, 275-283 (1927 a).
- WAKSMAN, S.A. and F.G. TENNEY: The composition of natural organic materials and their decomposition in the soil. II. Influence of age of plant upon the rapidity and nature of its decomposition - rye plants. *Soil Sci.* 24, 317-333 (1927 b).
- WAKSMAN, S.A., F.C. TENNEY and R.A. DIEHM: The chemical and microbiological principles underlying the transformation of organic matter in the preparation of artificial manures. *J.A.S.A.* 21, 533-545 (1929).
- WHITEHEAD, D.C. and J. TINSLEY: Extraction of soil organic matter with dimethylformamide. *Soil Sci.* 27, 34-42 (1964).
- WIESEMÜLLER, W.: Untersuchung über die Fraktionierung der organischen Bodensubstanz. *Albrecht Thaer Archiv* 9, 419-436 (1965).
- WINTER, A.G.: Untersuchungen über die Aufnahme von Penicillin und Streptomycin durch die Wurzeln von *Lepidum sativum* und ihre Beständigkeit in natürlichen Boden. *Z. Bot.* 40, 153 (1952)

- WINTER, A.G., H. PREUSS and F. SCHÖNBECK: Untersuchungen über die Aufnahme organischer Substanzen durch die Wurzeln höherer Pflanzen. I. Phenolische Verbindungen. *Naturwissensch.* 46, 536-537 (1959).
- WRIGHT, J.R. and M. SCHNITZER: Oxygen containing functional groups in the organic matter of the Ao and Bh-horizon of a podzol. 7th Internat. Congr. Soil Sci. Madison, Wisc. USA 2, 120-121 (1960).
- WRIGHT, J.R. and M. SCHNITZER: An estimate of the aromaticity of the organic matter of a podzol soil. *Nature* 190, 4777, 703-704 (1961).
- ZENK, M.H.: Einbau von p-Hydroxybenzoesäure in die Hydrochinonkomponente des Arbutins in *Bergenia crassifolia*. - *Z. Naturforsch.* 19 b, 856-857 (1964).
- ZIECHMANN, W.: Spectroscopic investigations of lignin, humic substance and peat. *Geochim. Cosmochim. Acta* 88, 1555-1566 (1964).
- ZIECHMANN, W.: Infrared spectra of humic acids. *Brennstoff-Chem.* 39, 353 (1958).
- ZIECHMANN, W.: Die Darstellung von Huminsäuren im heterogenen System mit neutraler Reaktion. *Z. Pflanzenernähr., Düng., Bodenkunde* 84, 155-159 (1959).
- ZIECHMANN, W. and H. SCHOLZ: Spektroskopische Untersuchungen an Huminsäuren. *Naturwissenschaften* 47, 193-196 (1960).
- ZVJAGINCEV, D.G.: Some regularities of adsorption of microorganisms on ion exchange resins. *Mikrobiologiya.* 31, 399 (1962).
- ZVJAGINCEV, D.G. and L.L. VELIKANOV: Effect of adsorbents on the activity of bacteria growing on media containing amino acids. *Mikrobiologiya* 37, 1017-1023 (1968) (russ.).