

Biochemistry of Soil Organic Matter  
in Relation to Crop Production

Bundesforschungsanstalt  
für Landwirtschaft  
Braunschweig-Völkenrode (FAL)  
**ZENTRALBÜCHEREI**

91/300

W. Flaig

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THE IMPORTANCE OF MODERN TRENDS IN HUMUS RESEARCH FOR

AGRICULTURAL PRODUCTION

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Introductory Lecture for FAO Consultant for Project No. 472

19. May - 23. Juli 1971

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For about 100 years one knows the importance of mineral fertilizers for plant production. Only a little precise knowledge exists about the effect of soil organic matter on production capacity of soils, because the problem is very complex. But the farmers know that the addition of organic matter in the form of farmyard manure or green manuring has an additional effect on yield and quality of plants, which cannot be explained by a sufficient mineral nutrition alone. The farmers speak about "humus effect" or "old strength of the soil" or some other explanations. If such a "humus effect" exists, then it must be possible to illustrate it with physical, chemical and biological effects.

The report concerns itself with investigations for elucidation of a part of the factors of "humus effect", which may be important for plant production and important from an economical point of view.

The processes during humification of dead plant material are briefly summarised.



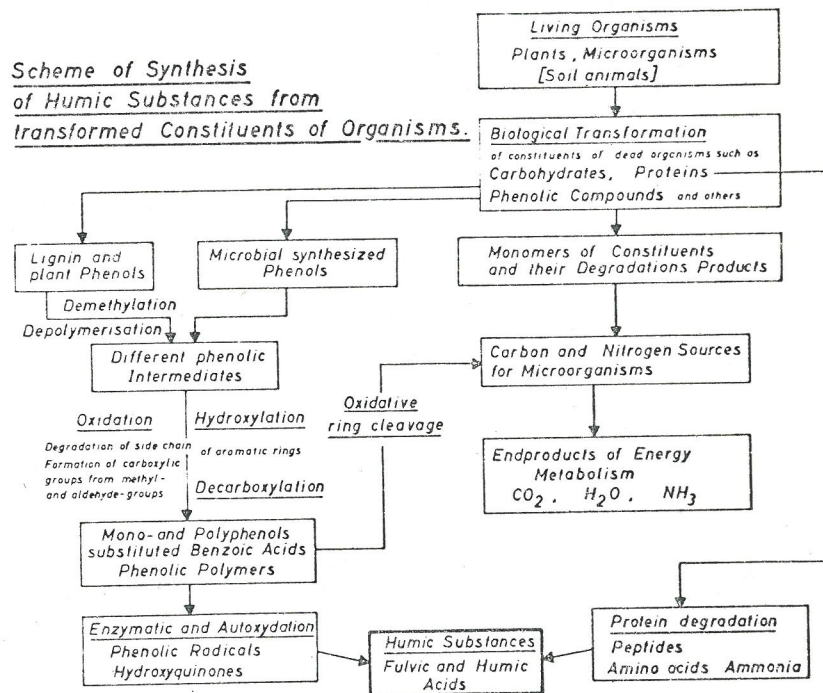


Fig. 1: Scheme of synthesis of humic substances from transformed constituents of organisms.

The most important organisms for humification processes are plants and microorganisms.

The most important constituents of the organisms are carbohydrates, protein, phenolic compounds which are transformed to the monomers and their degradation products used as carbon and nitrogen sources for microorganisms or formation of humic substances.

The end products of the energy metabolisms are CO<sub>2</sub>, H<sub>2</sub>O and NH<sub>3</sub>. Phenolic compounds can be derived from lignin and other plant phenols or are synthesised by microorganisms. Lignin is demethylated and depolymerised. Different phenolic intermediates are formed.

The main reactions are:

- oxidation
- hydroxylation
- decarboxylation

In this way mono- and polyphenols, substituted benzoic acids and phenolic polymers are formed.

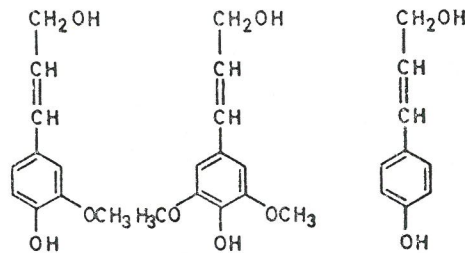
By oxidative ring cleavage aliphatic compounds are formed, which can be used by the microorganisms as carbon source and disappear in this way from the mixture of compounds which form humic substances.



By enzymatic and autoxidation phenolic radicals and hydroxyquinones are formed.

By condensation reactions between the mentioned phenolic compounds and protein degradation products such as peptides, amino acids and ammonia humic substances are formed, which are mainly divided up into fulvic, humic acids and humins by different separation operations.

Monomers of different Lignins.



- Coniferous trees +
- Deciduous trees +
- Graminees +

Structure Scheme of Spruce Lignin

(FREUDENBERG et al. 1964, 1968)

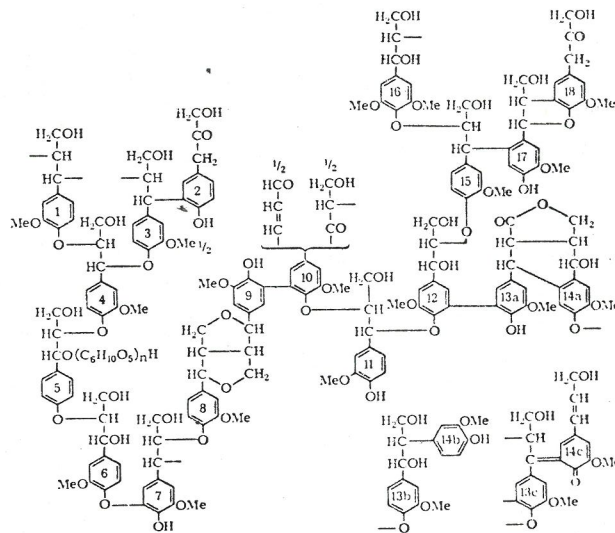


Fig. 2: Structure scheme of lignin

For remembrance I will briefly report about the structure scheme of lignin according to Freudenberg (1968). Coniferous lignin is formed by the condensation of coniferyl alcohol. The lignin of deciduous trees is a polymerisation product of two monomers, graminaceous lignin consists of the three monomers: coniferyl, sinapyl and p-coumaryl alcohol.

We labelled different carbon atoms of the monomers, synthesized the different types of lignin and studied the microbial decomposition. In this way we have been able to elucidate single steps of lignin degradation during humification (summarised in: MARTIN and HAIDER, 1971). The most important reactions are the following:

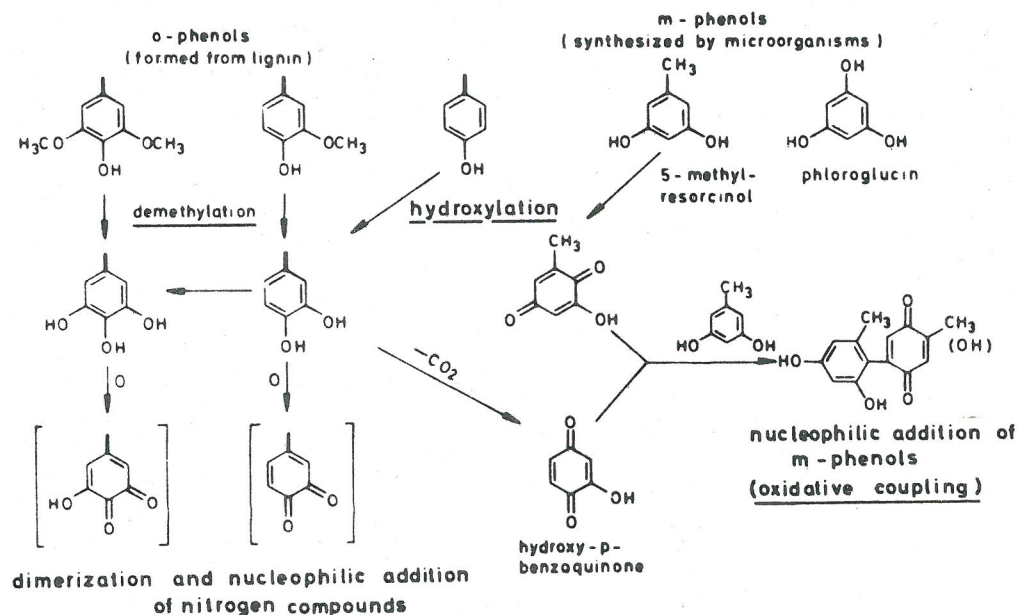


Fig. 3: Scheme of transformation of phenols during humification.

In the case of lignin and its degradation products the following reactions have been studied:

1. Demethylation, cleavage of phenolmethylether
2. Hydroxylation, formation of o-diphenols
3. Intermediary formation of o-benzoquinones
4. Oxidative decarboxylation, formation of p-diphenols or respectively p-benzoquinones

In the case of microbial synthesis, also m-diphenols are formed, such as 5-methylresorcinol and phloroglucin as two representatives of several. m-Diphenols can only be oxidised to quinones after hydroxylation. In the two ways formed p-benzoquinones, add nucleophilic m-diphenols to the corresponding diphenyl compound. These are the principles of chemistry which are needed to understand the following considerations.



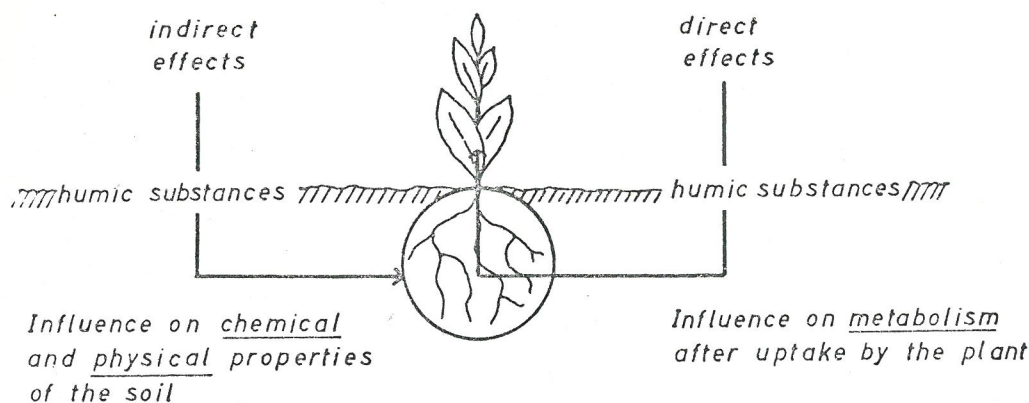


Fig. 4: Scheme of the possibilities of the effect of humic substance on growth and yield of plants.

According to the existing environmental conditions humic substances can have a more or less large effect on growth and yield of plants. The uptake of inorganic ions as nutrients can be influenced by humus in different manners. One differentiates between indirect and direct effects. (Summarised in FLAIG and SÖCHTIG. 1962).

The indirect effect is characterised by the fact that the organic substances are not taken up by the plant. Those processes influenced by soil organic material occur outside of the plant and are concerned mainly with alterations of physical and mechanical properties of the soil.

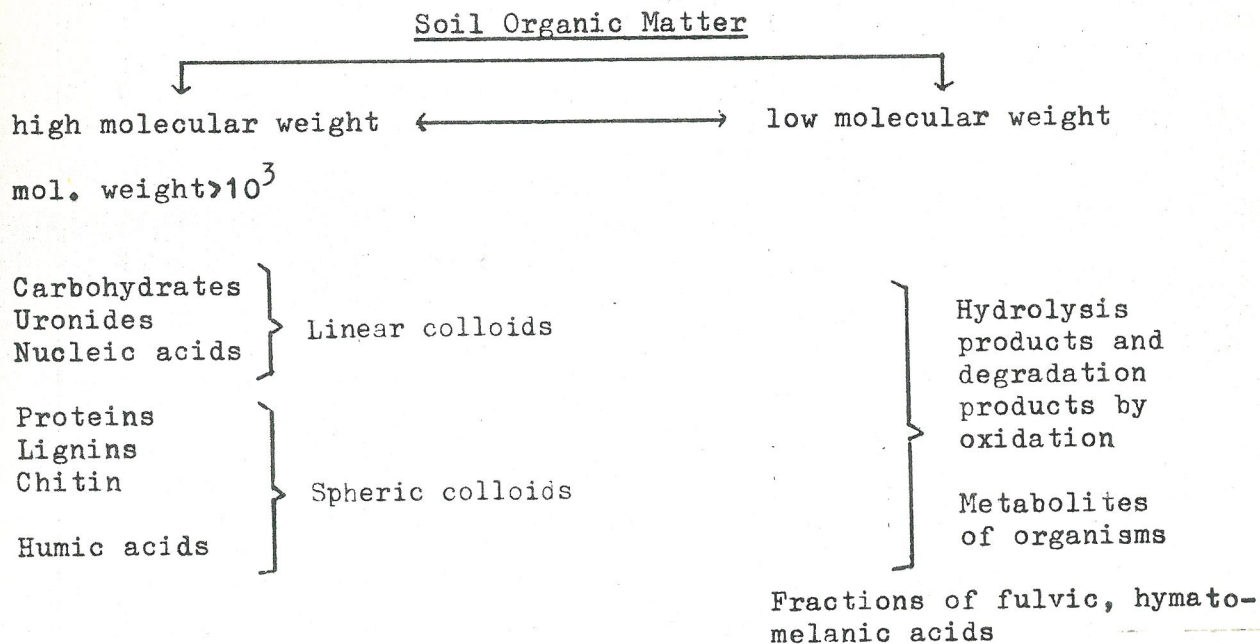
We define a direct effect, when the organic substances are taken up by the plant through the roots and enter in the metabolism. Before the particulars of the different manners of effects of soil organic components are mentioned, I should like to show a scheme in which the effects of organic soil constituents are summarised from a chemical and physical point of view (tab. 1).

The organic substances in the soil can be divided up according to their molecular weights into high molecular weight and low molecular weight substances.

Such a proposal to divide up the organic substances in the soil results from the fact that mainly only low molecular weight substances up to a molecular weight of about 1000 are taken up by the plant, while high molecular weight substances almost do not penetrate into the plants. As the most important high molecular weight substances are considered the carbohydrates, uronides, nucleic acids, which are mainly linear colloids, and proteins, lignins, chitin, and also humic acids which are spheric colloids.



Tab. 1: Division of organic substances in soil according to their effect on the plant



Indirect effects

Alteration of physical properties of soils

Participation in chemical reactions in soil

Direct effects

None

Influence of metabolism of organisms

The low molecular weight compounds are formed by hydrolysis or oxidative degradation of the high molecular substances. Besides this, low molecular weight compounds from metabolisms of the living organisms are in the soil. The fractions of fulvic and humato-melanic acids contain on average mainly low molecular compounds.

On the whole it can be said that the high molecular weight compounds effect alterations of physical properties of soils, while the low molecular weight substances participate in chemical reactions in the soil and alterations of the metabolism of organisms after sorption by roots.

In this presentation no more will be said about the indirect effects. There are many publications about the influence of soil structure, exchange capacity, chemical weathering, redox processes, etc. The most important direct effects are the following:

Tab. 2: The direct effect of organic substances on the uptake of inorganic ions by the plant

- 1) Easier uptake and better distribution of heavy metals in form of complexes (Chelates).
- 2) Influence on ion adsorption
- 3) Direct use of uptaken organic substances for formation of plant constituents.
- 4) Catalytic influence on metabolic pathways

By formation of complexes with heavy metals, the uptake and distribution of heavy metals in the plant is easier.

The effect number 2, influence on ion adsorption, may occur when the ratio of the quantity of high molecular weight plant constituents of the root is changed by the uptake of physiologically active substances, then the possibility exists that the Donnan-equilibrium is shifted, which influences ion adsorption.

The influence of a different composition of adsorbing polyanions on sorption and on Donnan-equilibrium is shown by investigations of KELLER and DEUEL (1958) with roots of mono- and dicotyledons. How far soil organic matter has an influence on the composition of plant polyanions is not well enough known.

As an example of direct utilisation of take-up organic constituents for production of plant substances are the investigations with amino acids of VIRTANEN (1947) and VIRTANEN et al. (1933, 1946), with peas or with citrate and sucrose by RATJE (1960, a, b, c, d) and WENT and CARTER (1942). These processes do not seem to be very important for plant production.

The fourth effect on organic components of the soil on plant growth, the catalytic effect of metabolism of organisms, may be the most interesting. Because its contribution for the formation of plant mass is much more unknown than in the case of the indirect effect.

From many papers (BLANCHET, CHAMINADE, CHRISTENA, GUMINSKI, PRAT and co-workers, MIKLEWSKI, TYPACEK, SLADKY, SAALBACH, SÖCHTIG, TICHY summarisssd in FLAIG 1962) it is known that fractions of humic substances can have a favourable effect on growth and yield in nutrient solutions, sand sultures and in some cases also in soils as substrates for culture. But in literature, results are also published which do not confirm the favourable effect of humus or its fractions (summarised in SAX, 1963). We tried to elucidate these contradictions (summarised in SÖCHTIG, 1964).

Before speaking about details, some common remarks are made on relations between physiological activity on the one hand and chemical constitution



concentration and plant species on the other.

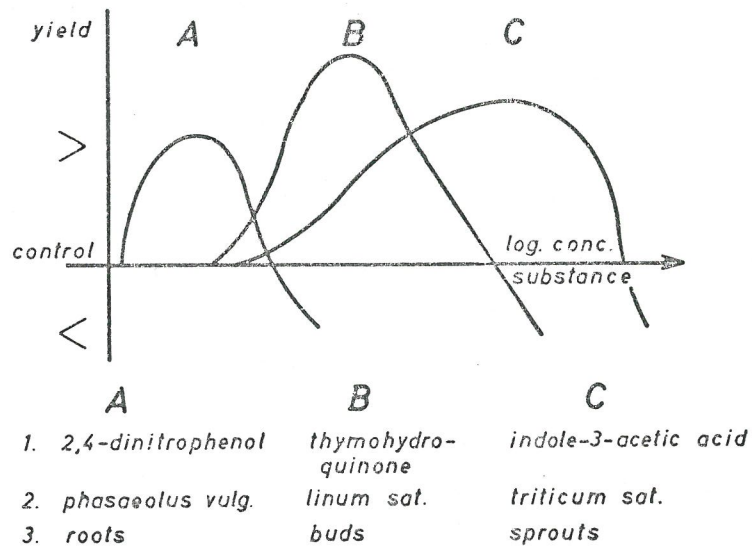


Fig. 5: Scheme about the influence of different factors on the effect of physiologically active substances.

The effect dependent upon concentration is depicted in a scheme, in which the concentration in logarithmic scale is put against the positive or negative deviation of the yield of the untreated control. The position and the form of the curves are only schematic.

Case No. 1:

3 substances A, B, C, different in their chemical constitution, have a maximum of their effects for increase of dry weight in the case of one plant at three different concentrations. Neither the amount of stimulation nor the amount of inhibition must be the same in all cases.

Case No. 2:

Otherwise one substance can have a different effect in the case of the different plants A, B, and C.

I am only going to remind you of the different effects of herbicides on mono- and dicotyledones. Furthermore I mention the observation of CHRISTEVA (1947, 1948, 1949), that carbohydrate-producing plants are more affected by humic substances than protein or at least oil-producing plants.

Case No. 3:

Finally different organs A, B and C of one plant are differently influenced by one physiologically active substance. The growth of roots is inhibited by indole-3-acetic acid at lower concentrations than is the growth of buds or sprouts (THIMANN, 1937).



It must be especially mentioned that all those observations have been made by the addition of lignin degradation products such as phenol carboxylic acids, their oxidation products such as the corresponding quinones, and also by the addition of low molecular weight fractions of humic substances to plant culture in nutrient solution or in sand cultures and even by the addition to soils.

Furthermore, it must be mentioned that the extent of influence of metabolically active substances depends also on the environmental conditions such as temperature, light and humidity, as well as on nutrient supply. Some examples may explain this.

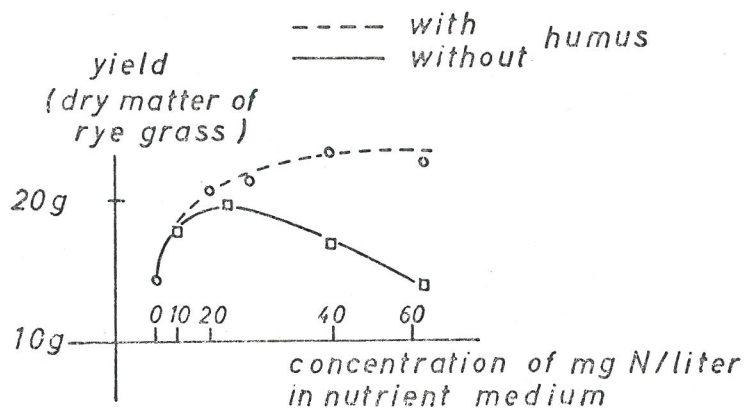


Fig. 6: Influence of humic substances on yield formation in the case of overdoses of inorganic nitrogenous salts according to CHAMINADE (1965).

So, for instance, CHAMINADE (1965) found that overdoses of inorganic nitrogenous salts remain effective for yield production in the presence of humus, whilst without humus a decrease of yield occurred. The investigations were made in pots with sand cultures and rye grass. The same was observed also with oxidatively ammonified ligninsulfonates from the paper industry as nitrogen fertilizer (unpublished). More will be reported later on this subject.

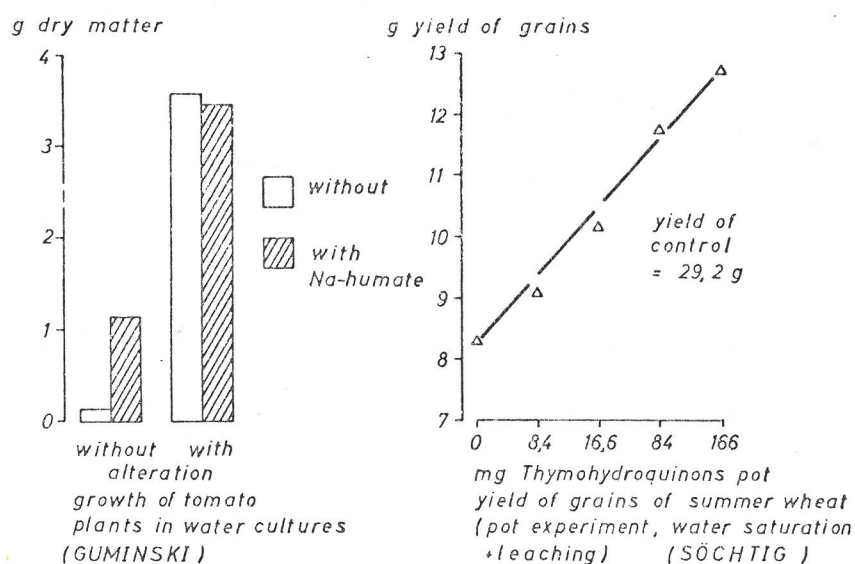


Fig. 7: Scheme of investigation of oxygen deficiency in nutrient cultures (GUMINSKI) and on the leaching of the water in the presence of thymohydroquinone (SÖCHTIG)

GUMINSKI and GUMINSKA (1953) investigated the influence of fractions from humus on the production of plant mass of tomatoes at low oxygen tension in water cultures.

The yield of unaerated nutrient solution was higher by the addition of humic substances than without them. But the yield was only the same in aerated culture with and without the addition of humic substances.

In pot experiments SÖCHTIG (1964) investigated the influence of a model substance of oxidised lignin degradation product; the substance was thymohydroquinone. The soil was always water saturated and the water flow was about 100 ml. The leaching of nutrients was not influenced by the addition of thymohydroquinone. In spite of these unfavourable conditions such as leaching of nutrients, water saturation of soil, small oxygen tension, the hydroquinones increased the yield (up to 13 g.) remarkably, but not up to the amount of control (29.2 grams) without leaching of nutrients and water saturation of the soil. Further observation of the favourable effects have been made in the case of deficiency of light and water; in the latter case the wilting resistance is increased.



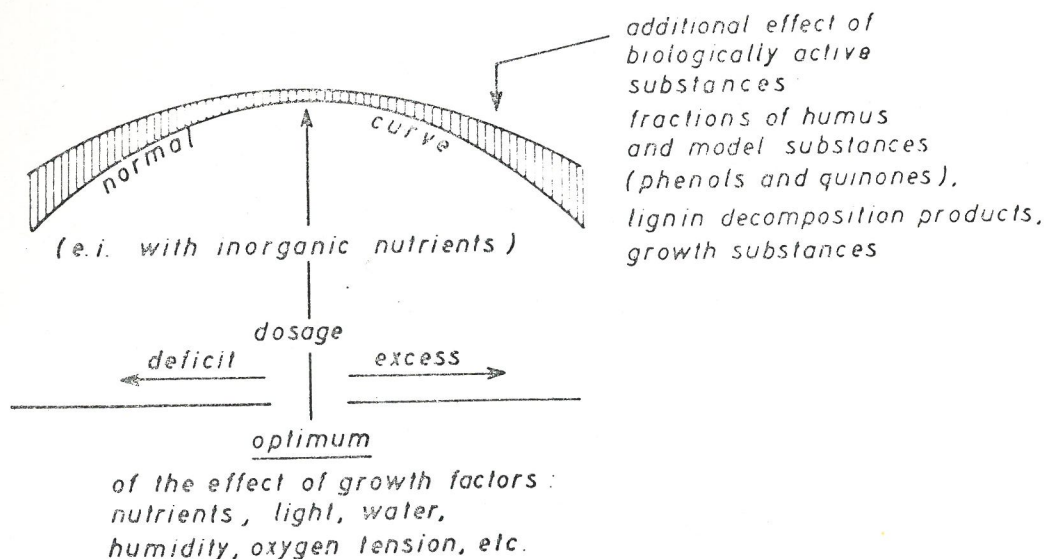


Fig. 8: Influence of physiologically active substances on yield formation in dependence of environmental factors.

The experiments mentioned demonstrate that the effect of a metabolic active substance on plants depends on the environmental conditions. We explained the favourable effect of humus on plant growth and yield in the following way (summarised in SÖCHTIG, 1964):

When growth factors, such as temperature, humidity, light, oxygen tension of the culture medium, water saturation of the soil, as well as the supply of nutrients are in the "optimum", the yield on plant mass is the highest.

The effect of physiologically active substance improved the formation of yield if one or several growth factors are in deficit or in excess. With this explanation of the effect of substances from humus, of lignin degradation products or all other physiologically active substances, all the contradictory results from literature can be explained.

Economically, the results mentioned mean a partial diminution of the risk of yield formation. Some of the environmental conditions cannot be predicted, such as climate factors, heavy rainfall, dry seasons, etc.

The observations that humic substances have an effect on plant growth and yield, are not sufficient to find a causal connection. It must not only be proved that the organic substances are taken up and have an influence on metabolism, but it must also be elucidated in which way they are transported or transformed in the plant itself.



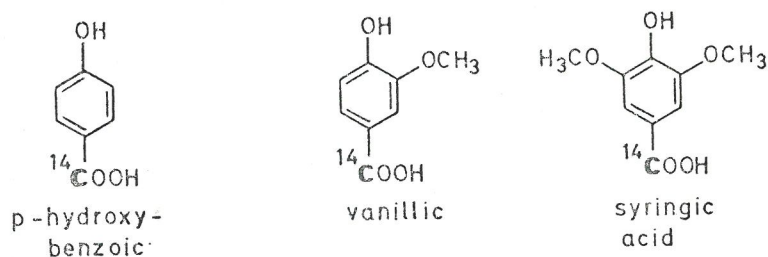


Fig. 9:            <sup>14</sup>C-carboxyl-labelled phenolcarboxylic acids as lignin degradation products

In this connection some of the results of our investigations with <sup>14</sup>C-carboxyl-labelled phenolcarboxylic acids such as o-hydroxybenzoic-, vanillic- and syringic acid will be briefly mentioned. Special investigations have to be done in a closed system and under absolutely sterile conditions for this purpose (HARMS et al. 1969, a, b, some results in press). Seedlings of wheat have been cultivated for seven days in the nutrients and then phenolcarboxylic acids were added so that the concentration was about 10<sup>-3</sup> molar. After three or six days the seedlings were harvested.

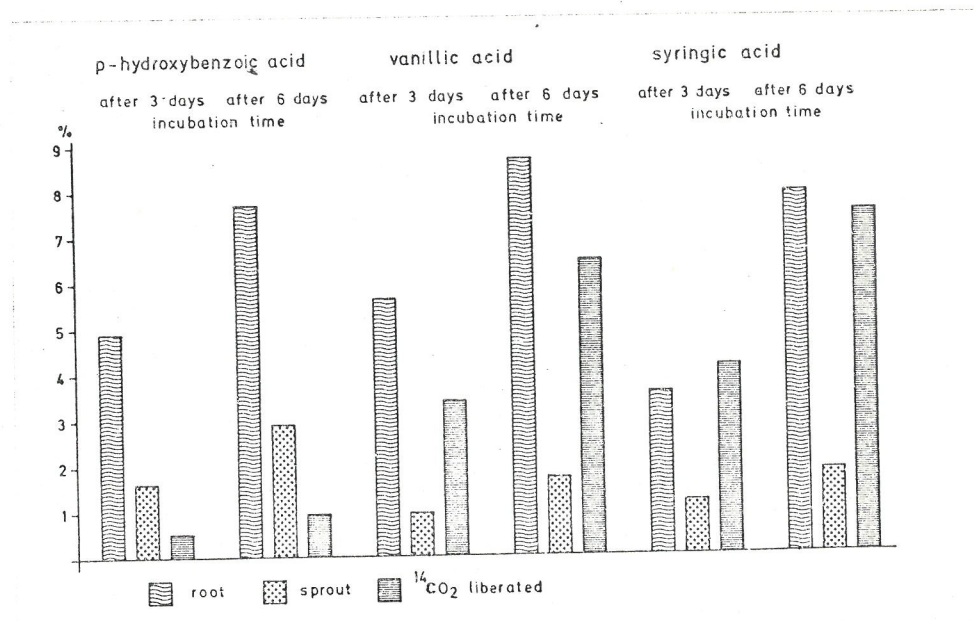


Fig. 10:            Uptake of labelled phenolcarboxylic acids in % of added quantity (HARMS 1967)

The diagram summarises the results:

1. With incubation time the activity in roots, sprouts and released carbondioxide increases. The active carbondioxide is caused by oxidative decarboxylation of the phenol carboxylic acids. Methoxy-hydroquinone respectively methoxy-p-benzoquinone from vanillic acid or 2,6-dimethoxy-hydroquinone and also the corresponding quinone from syringic acid were identified.
2. The highest amount of activity is in the roots. Presumably a part of the activity is only sorbed on the surface of the root, because it cannot be displaced by dilute sodium hydroxide solution.
3. Sorption and decarboxylation depend on the substitution of the phenolic acids with methoxyl groups.

The determination of the activity in the plant organs does not answer the fixation of the activity in different compounds. Therefore the plants were extracted with different solvents successively.

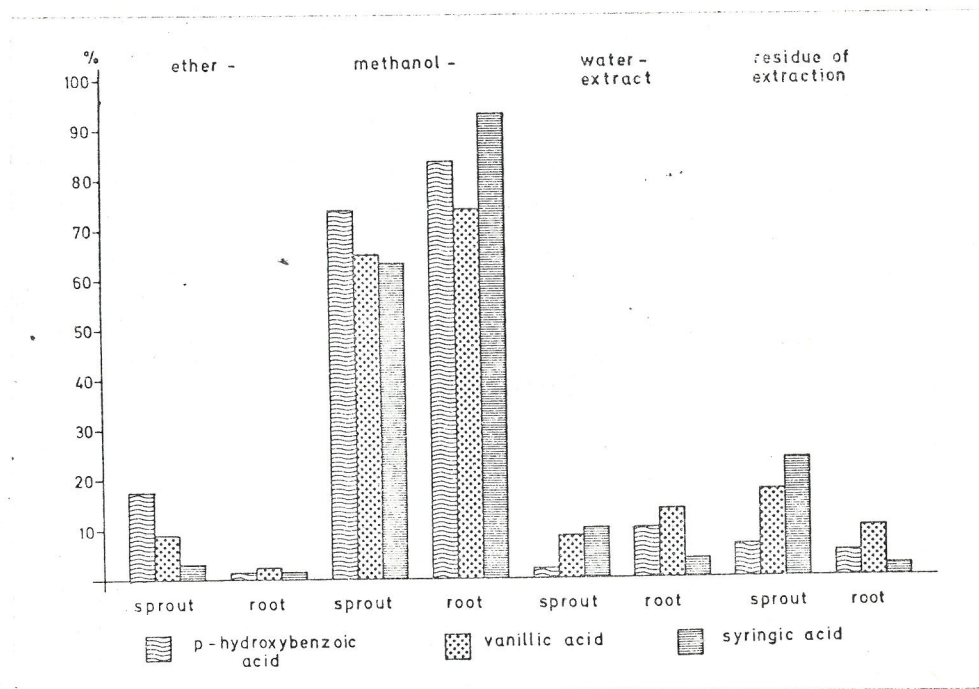


Fig. 11: Relative distribution of the activity in the different extracts (sprout or root resp. = 100%) (HARMS 1967)

The low activity in the other extract shows that only small amounts of free carboxylic acids are in the plant organs.



The high amount of activity in the methanol extracts belongs mainly to the presence of glycosides of the phenol carboxylic acids or their glucose esters. The importance of these depots of glycosides must be elucidated. It should be mentioned that lignin is only formed from coniferin, the glucoside of coniferylalcohol, when the glucoside is split off enzymatically (FREUDENBERG, 1956).

The relatively low activity in the water extract and in the residues of extraction is caused by fixation of  $^{14}\text{C}$ -carbondioxide and formation of different metabolites of carbohydrate and protein cycle. This means also that the released  $^{14}\text{C}$ -carbondioxide is fixed in plant metabolism to a low extent.

The reported scientific results led us to try to find a technical solution and the knowledge for use in practice. Lignin sulfonates from cellulose industry have been oxydatively ammonified. The resulting product was a slow-acting nitrogen fertilizer with physiologically active components from the degradation of the lignin derivatives. We call this product "N-lignin". At the moment a pilot plant is running with 500 to. per year.

The composition of N-lignin is:

Total N = 18 - 20%  
from this (Total N content = 100%)  
30% - 40%  $\text{NH}_4^+$ -N  
ca. 10% "Amide-N"  
50% bound in other organic form

"Amide-N" is nitrogen which can be hydrolized with 30% sodium hydroxide solution after the distillation of the ammonium ions with magnesium oxide.

The largest part of organic bound nitrogen becomes available for the plant during the first vegetation period by the activity of microorganisms. The total for plant growth required amount of nitrogen can be given in one dose. No plasmolysis occurs because the concentration of ions is not increased too much in soil by the addition of N-lignin, or even in mixtures with inorganic nitrogen fertilizers.

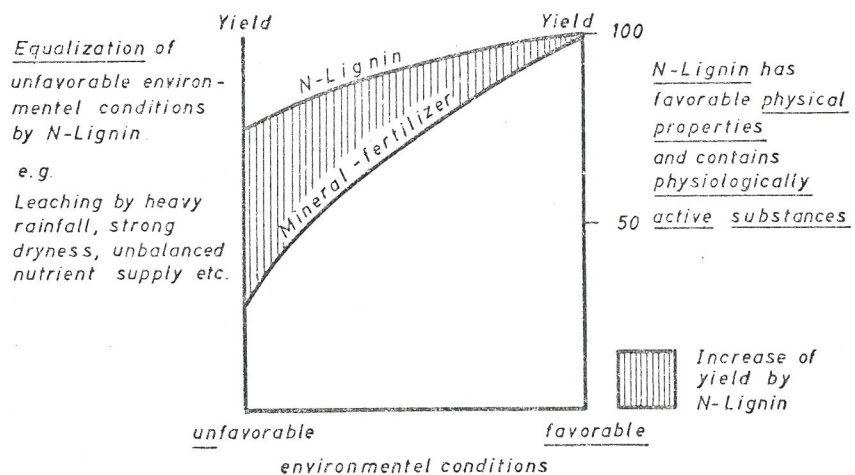


Fig. 12: Favourable properties of N-lignin. Diminution of risk of yield formation by N-lignin.

With N-lignin also the effect of the diminution of the risk of the yield formation was observed. In pot experiments under artificial - but not under natural - unfavourable conditions the yield is sometimes increased up to double. Last year we had a dry season and therefore unfavourable climatic conditions. We observed in a field experiment with barley an increase of the yield of about more than 20%.

But this is exceptional.

The nitrogen of N-lignin cannot be leached so much as the nitrogen of inorganic fertilizers, because about 50% of N-lignin are high molecular weight substances which contain 40% - 45% of the total nitrogen content. Furthermore, some constituents are in N-lignin, which inhibit nitrification in soil. By fertilization with N-lignin different legumes, such as spinach, can be produced with a low nitrate content for baby food, (SÖCHTIG 1970),

In many cases an increase of phosphate content of plants was observed. Some other properties will be mentioned in a special lecture.

To fulfill the demands of mankind in agriculture, science has to go new ways. One of these is the use of bioregulators to increase yield production per hectare, and to increase the biological value of plant production for the nutrition of man and animal.

ARMY and GREER (1967) made interesting predictions.



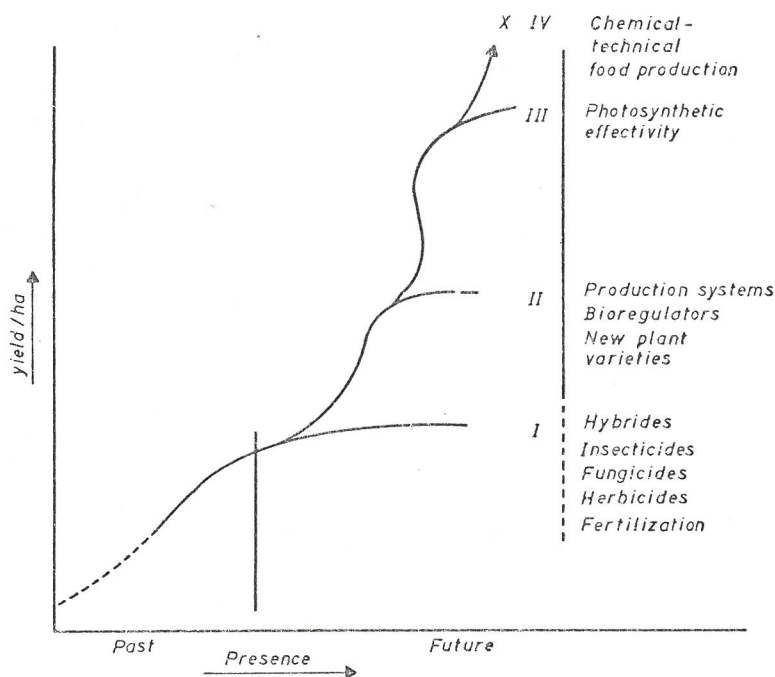


Fig. 13: The level of yield in present and future production of plants (ARMY and GREER, 1967)

In the past up to the present the increase of plant production per hectare was effected by plant breeding (hybrids), use of pesticides and especially by the use of mineral fertilizers.

The second step of yield increase could be caused by new plant types and plant systems, as well as by the use of bioregulators.

The next two steps are the increase of the effectivity of photosynthesis - partly also a problem of bioregulators - and the production of food without organisms, e.g. the reduction of carbondioxide by special catalyts.

Since the soil will be the main substrate for plant production also for the next decades, the research on bioregulators should be promoted.

Some bioregulators are already used in practice, for instance CCC (trichloro-cholin-chloride), to decrease the length of stem in wheat, or for instance slow-acting nitrogen fertilizers. But there may exist other possibilities, which are not yet known. In this connection the importance of soil organic matter is also to be mentioned in relation to soil pollution.

Properties of soils.

No.	pH	CEC meq/100g	Clay %	Organic carbon %
1	8.1	6.3	8	0.7
2	8.1	10.4	18	0.9
3	6.8	10.2	6	1.1
4	6.8	10.6	6	1.3
5	7.5	13.0	26	1.4
6	8.0	21.9	36	1.7
7	8.1	10.6	10	1.7
8	7.2	14.0	21	1.8
9	6.5	10.7	7	1.8
10	7.8	19.8	18	2.7
11	6.3	18.2	2	2.8
12	7.5	44.8	11	7.6
13	7.7	48.2	16	8.8
14	7.6	74.0	36	11.0
15	7.2	66.4	25	12.0
16	5.1	83.4	62	15.0
17	6.9	118.8	peat soil	31.0

Relationship between soil organic carbon and adsorption.

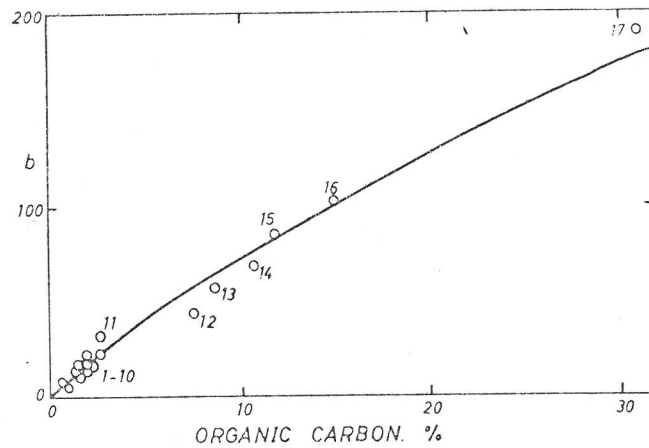


Fig. 14: Relationship between soil organic carbon and adsorption of pesticides (GRAHAM - BRUCE, 1967)

The higher the carbon content of the soil, the stronger is the adsorption of all types of pesticides and not only for "Disulfoton" as in this graph. Cation exchange capacity and clay content do not correlate so significantly as does organic carbon content.

This summarising report of new trends in humus research demonstrates clearly that new knowledge about humus exists to be transmitted in practice, but also that many problems in this direction are not yet solved. Their elaboration should be urgently promoted, also from economical points of view. Plant production means not only tons per hectare, but also biological value of the harvested products and preservation of the substrate of production - the soil.



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