Lectures on

Soil Organic Matter

by

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Foreword

To my friends and the friends of the subject.

In this manuscript are some unpublished results and therefore only for friends and not to use for publication.

I would enjoy having any comments on this material.

W. Flaig

Acknowledgments

This manuscript came about as a result of the kind invitation of Prof. Dr. W. H. Pierre, Head, Department of Agronomy, to give lectures about soil biochemistry. I am very thankful for this opportunity.

I would like to express my best thanks also to my colleague, Prof. Dr. Lloyd Frederick, who stood by me helpfully at all times during the writing of these lectures in the English language and I appreciate his suggestions during our many discussions.

Without the help of his co-workers, Messrs. McIntosh Sims, Horton, Brown, and of the secretaries, Mrs. McLaughlin, Misses Sansgaard and Zart, it would not have been possible to mimeograph the lectures. Also to these, many thanks.

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W. Flaig

SOIL ORGANIC MATTER

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There are some points of view which cause us to put the enzymes at the mid-point of the biological processes in the soil. (Compare: Hoffman, Ed. u. G. Hoffman: Uber Herkunfe, Bestimmung und Bedeutung der Enzyme im Boden. Z. Pflanzenernahr., Dung., Bodenkunde 70, 9-16. (1955)), Hofmann, Ed. Die Enzyme im Boden und ihre Bedeutung fur seine Biologie und Fruchtbarkeit. Z. Acker-u. Pflanzenbau 100, 31-55, (1951)), Hofmann, Ed. u. W. Huunius: Die Auswirkung verschiedener physiologischer Dungung auf einige Faktoren der Bodenfruchtbarkeit. Z. Pflanzenernahr., Dung., Bodenkunde 70, 104-114 (1955)), and others). In a soil sample there are enzymes of different specivity. Their activity depends upon the pH. The enzymes generally come from bacteria and fungi. In the first case the optimum is more in the neutral, in the second case, more in the acid range. If a soil sample is taken from an area covered by plants during the time of vegetative growth, the enzymes of the roots are also determined, after mixing with toluene.

According to known experience, the living roots do not excrete many, if any, enzymes in the soil. If large amounts of plants have been added to the soil and have decomposed in it, then the enzymes which are in the plants are determined. The amount of roots which are distributed in the soil are decomposed by micro-organisms after harvesting of the plant. The microorganisms multiply very rapidly in this case. After the experience of Hofmann, the enzymes of plants and roots are destroyed during decomposition. In most cases the microorganisms are the most important producers of enzymes. After a short life, the micro-organisms are autolyzed. During this process other microorganisms and processes of decomposition occur. The enzymes then enter the soil. The amount of plant residues in the soil are only indirectly responsible for the increasing of enzymes because the number of microorganisms increases and with those also the amount of enzymes.

In so far as it would be possible that large amounts of enzymes of plants would come into the soil, they would also be one of its enzymes systems; but mostly they are decomposed by the emzymes of the bacteria.

After these considerations all possible enzymes can be in the soil. According to the investigations of the last years some seem to be more important. These are the hydrolases, for hydrolyzing the proteins; the amylase for the decomposition of starch; the saccarase, the B-glucosidase, the urease and in some cases also the phosphatases.

The activity of the enzymes is determined by splitting off products of known substrates. Therefore, it is necessary to run preliminary tests before the substrate is decomposed so that the pH value is adapted to the process which will be investigated. In most cases it is necessary to make a test experiment. It must be proved that the soil is free of such substances which are to be determined during hydrolysis.

The soil samples are mixed with toluene to destroy the living microorganisms. By this process, their enzymes are liberated.

The content of enzymes depends upon the different properties and components of the soil. According to known investigations and comparisons of the different cultivated soils, the content of enzymes is influenced by the following factors:

1. The soil type (the content of enzymes is lowest in sandy soils and increases with the content of clay minerals; but decreases again in the case of loamy soils.

2. The content of humus.

3. The content of calcium, respectively pH value.

4. The vegetation, effects by cultivation.

5. The amount of inorganic nutrients, such as fertilizer.

The presence of organic substances and lime increases the content of enzymes. It decreases in the case of physiological acid fertilizing and acid reaction. In the case of cultivated land, it is about half as high as in the case of grassland under the same conditions. Some kinds of cultivated plants increase the soil content of enzymes; others cause a decrease. It is suggested that there are also some other influences, especially that of climate.

In numerous papers there is described that the microflora and the microfauna of the soil depends on the same factors and in the same sense as has been found true for the amount of enzymes. This is understandable because the organisms are producing the enzymes.

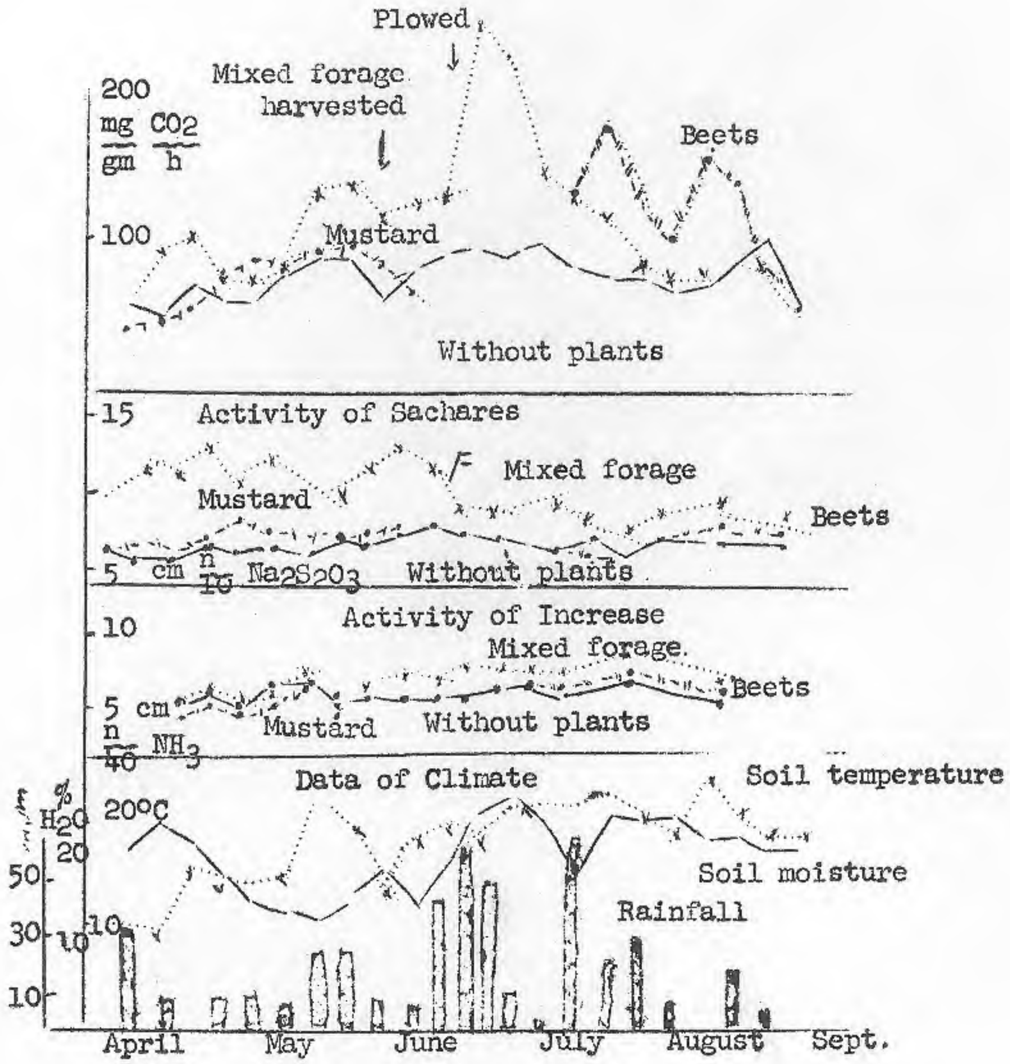
The mentioned factors determine in equal manner soil productivity. Also this parallelity is self evident. According to known experiences the microorganisms have the same demands on the soil as the cultivated plants. All modes of action which increase the soil productivity favors also the development of microorganisms and therefore the production and content of enzymes.

Therefore the determination of the content of enzymes can be under certain circumstances a measure of the soil productivity. It is sure that there are different facts to be considered as it must be done in the case of all other kinds of soil testing. A value which can be too high in the case of the sandy soil and would be too low for a loamy soil, etc.

Most of the enzymes which I mentioned are important for the decomposition of plant material; mostly in the case of proteins and polysaccarides. Their importance therefore is only indirect in connection with the formation of humic acids. In this case dehydrogenation reactions are more important. Therefore, some investigations about the activity of dehydrogenases of the soil as a measure of the activity of microorganisms in the soil shall be mentioned. (Lenhard, G. Die Dehydrogenaseaktivitat.

As an example for the different factors which influence the activity of enzymes, compare diagrams with the content of saccharase and urease. The determination of the biological activity by enzymes seems to Koepf to be more adapted for experiments in the laboratory than in the fields. In this case three different plants have been measured against plots without plants. To get relations with other factors which determine the biological activity of the soil, the respiration curves and the data of the climate such as soil temperature, soil moisture, and rainfall have been given in comparison to the measurements of the activity of saccharase and urease. (Koepf, H. Untersuchungen uber die biologische Aktivitat des Bodens. Teil I: Atmungskurven des Bodens und Fermentaktivitat unter dem Kinfluss von Dungung und Pflanzenwachstum. Z. Acker-u.Pflanzenb. 98, 289-312 (1954)). Summarizing it can be said:

1. Respiration curves: The respiration curves of plots with plants increase in the spring much more than on the plotswithout plants. After harvesting, the increasing of the evolved CO_2 depends on the amount of roots.


Plowed
Mixed forage harvested
200
mg CO2 / gm h
Beets
100
Mustard
Without plants
15
Activity of Sachares
Mixed forage
Mustard
Beets
5 cm n/10 Na2S2O3
Without plants
10
Activity of Increase
Mixed forage
Beets
5 cm n/40 NH3
Mustard
Without plants
Data of Climate
Soil temperature
% H2O
20°C
20
50
Soil moisture
Rainfall
30
10
10
10
April
May
June
July
August
Sept.

2. Content of saccharase: Only in the case of mixed plants, is the change in content of saccharase with time significant. The increase of saccharase is in accordance with the increase of the sprouts and roots. The fluctuation may be caused by the different formation of roots with time. After harvesting the values decrease. The beets do not influence the content of saccharase measurably.

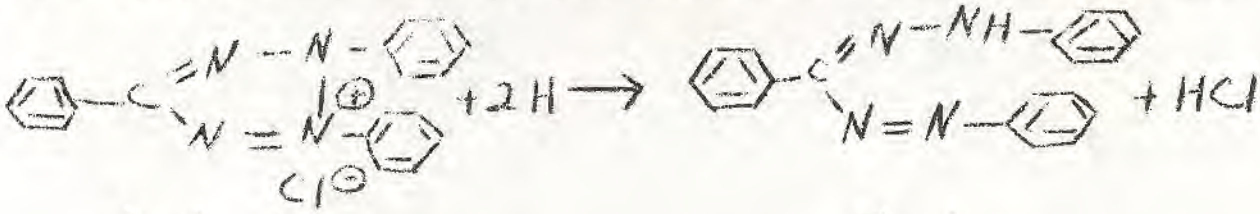
Dried soil samples of the different plots show that it is possible to determine the times in which the roots in the soil had been formed, according to the content of saccharase.

3. Content of urease: During most of the time of vegetative development of the plants the activity of urease is not significantly influenced in the part of the soil, in which the roots grow. In the second half of the vegetation time there is to be noted an increase in the content of urease in the part of the soil in which there has been the most roots. It may be that the intensive decomposition of roots during summer time led to a significant accumulation of urease.

Summarizing it can be said, that the respiration curves are more an expression of the degree of intensity of the biological activity influenced by the effect of plant roots. It has not been possible to determine the intensity of the biological activity. Des Bodens als Massfur die Mikroorganismentatigkeit im Boden. Z. Pflanzenernahr., Dung., Bodenkunde 73, 1-11 (1956)).

In the metabolism of organisms there are many reactions which transfer hydrogen from compounds which contain hydrogen to other compounds. These processes are catalyzed by dehydrogenases. Kuhn und Jerschel (Ber. dtsch. chem. Ges. 77, 591 (1944)) have shown this first with the transfer of hydrogen on to the nitrogen of tetrazoliumchloride.

Triphenyltetrazoliumchloride is colorless and by addition of two hydrogen atoms, triphenylformazane is formed, which possesses a characteristic red color.



Colorless Red

Matzen and co-workers have shown for instance the respiration of different plants; Wallhauser und Rippel-Baldes the activity of different microorganisms, and according to Hayer, the organs of high metabolism intensity produce more formazane than the others. The reduction of tetrazolium-salts is considered as a measure of the intensity of respiration (Mattson, S.: Science 106, 294 (1947). Hayek, H. V.: Naturwissenschaften 37, 263 (1950), Black, M., und Zweifach, B.: Tetrazoliumsalze, ein neues Hilfsmittel der allgemeinen experimentellen Pathologie. Am. J. Clin. Pathol. 23, 332 (1953), Udsin, E.: Tetrazoliumautobiographie. Applied Microbiol. 2, 29-33 (1954) USA. Wallhauser, K. H., und Rippel-Baldes, A.: Naturwissenschaften 37, 450 (1950). Stolp, H.: Arch. Microbiol. 17 209 (1952). Lambou, M. G.: 2,3,5 Triphenyl-tetrazoliumchlrid als Schnellindikator der Lebensfahigkeit von Baumwollsamen. Ref. Chem. Zentralbl. 125, 3724 (1954), Lakon, G.: Ber. dtsch. bot. Ges. 60, 299 und 434 (1942), Seyfarth, W.: Naturwissenschaften 39, 91, 162 (1952), Hofmann, E.: Z. Pflanzenern., Dung., Bodenkunde 56, 68-72 (1952), Kremkus, Fr.: Z. Pflanzenern., Dung., Bodenkunde 62, 108-128 (1953)).

Lenhard measured the intensity of microbial transformations in the soil with the formation of formazane out of tetrazolium salts. With other words, the amount of dehydrogenases is a measure of the microbial activity. As is well known, the intensity of microbial transformations in the soil depends on the number of microorganisms present and the conditions for their growth. The conditions for their growth are determined by the available carbon compounds, by the inorganic nutrients such as N, P, K, Ca, etc., by temperature, aeration and moisture. It could be shown that in soils with a high microorganism population and therefore a high biological activity, much formazane is formed. While in sand soils with a low content of humus and therefore poor conditions for the microorganisms, only a small amount of formazane has been found.

Summarizing, the experiments have been made in the following way:

10 gm. of soil are mixed with 100 mgr. calcium-carbonate and water added to an amount of 90% of water capacity. In the water are 0.35 ml. of a solution which contains 2% of glucose and 1% of peptone. The flasks are incubated at 29 C for 24 hours. After this time, 1 ml. of a solution of 2,3,5-triphenyl-tetrazolium-chloride are added and mixed with the soil.

After further incubation at 29° C for 24 hours, the soils are extracted with methanol and the formazane formed is measured at a wave length of 546 mμ. The extinction of a liquid layer of 10 mm in thickness is the measure for the dehydrogenase activity.

The different factors which influence the formation of formazane have been investigated.

Soils sterilized with methanol showed no dehydrogenase activity.

Dehydrogenase Activity of Sterilized and Non-Sterilized Soils

	Non-sterile	Sterile
Compost earth	0,64	0,01
Low-land moor	0,18	0,001
Sand	0,02	0,00

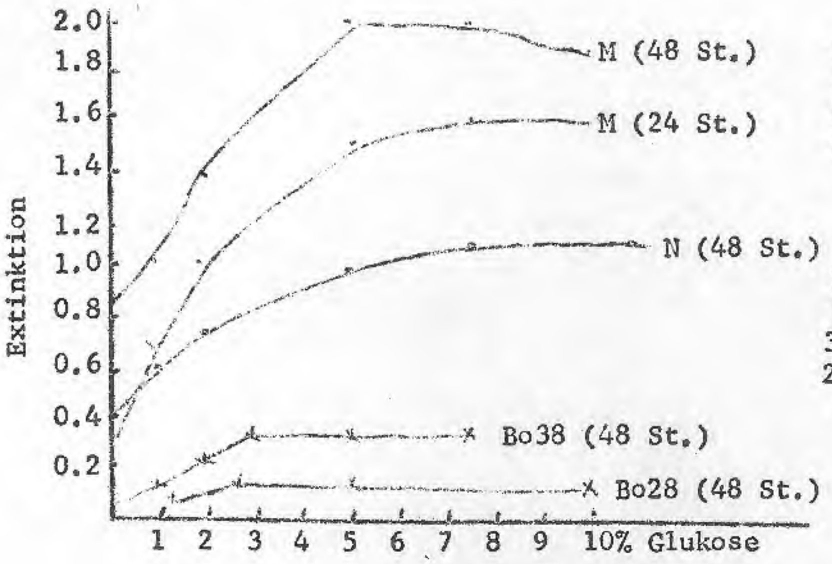
In further experiments, the influence of the concentration of glucose on the formed amount of formazane has been investigated.

S 1 228 (see next page)

Different soils have been mixed with different amounts of glucose and incubated 48 and 24 hours. With increasing amount of glucose in humic soils, the formation of formazane increases and a maximum value results. The amount of formazane depends on the soil type.

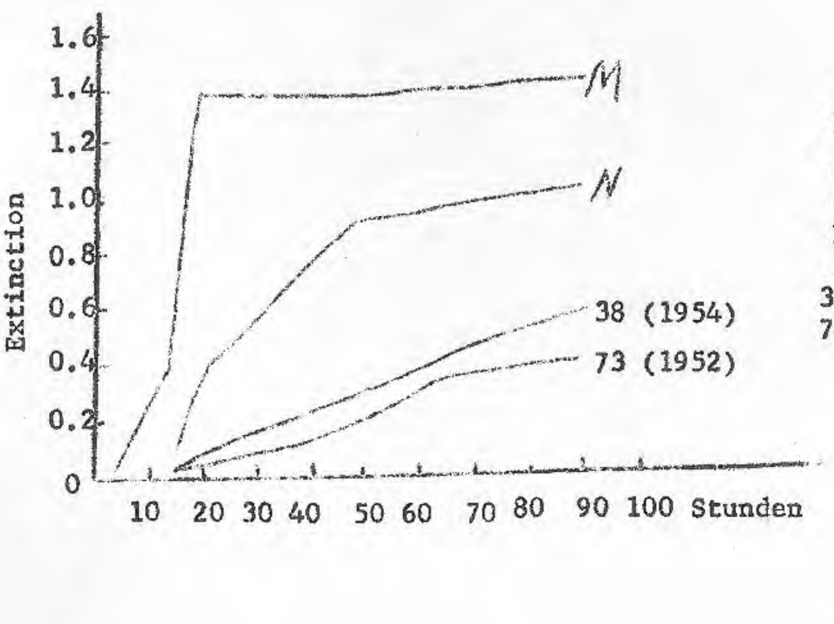
S 2 229 (see next page)

S 1 228



Einfluss der Glucosemenge in der Bodenlosung auf die Dehydrogenaseaktivitat verschiedener Boden
(Je Versuch 0,5 ccm Glucoselosung verschiedener Konzentration.)
M = Mistbeeterde
N = Niederungsmoor
38 = Versuchsfeld CI Ca I
28 = Versuchsfeld CI Ca I

S 2 229



Einfluss der Einwirkungszeit von Glucose und Tetrazoliumchlorid auf die Dehydrogenaseaktivitat der Boden
M = Mistbeeterde
N = Niederungsmoor
38 = Versuchsfeld CI Ca I 1954
73 = Versuchsfeld CI Ca II 1952

Furthermore, the influence of time of incubation has been investigated. In soils with high biological activity, the maximum value results in a shorter time than in inactive soils.

S 3 230

Einfluss von N (P und K) auf die Dehydrogenaseaktivitat verschiedener Boden

Zugabe von mg N	Boden M	N	38	0
0	1,53	1,05	0,28	0,13
0,1	1,54	----	0,31	0,24
0,2	1,54	----	0,35	0,26
0,3	1,56	1,06	0,36	0,24
0,4	----	----	0,33	0,23
0,5	1,57	----		0,20
0,6	----	1,04		
0,7	1,55	----	0,26	0,16

Einfluss von P und K auf die Dehydrogenaseaktivitat verschiedener Boden

Zugabe von	Boden M	38	0
0	1,53	0,28	0,13
0,1	----	0,30	0,18
0,2	----	0,30	0,19
0,3	1,50	0,30	0,19
0,4	----	0,30	0,19
0,5	1,51	----	----
0,7	1,50	0,23	0,14

In further experiments, the influence of nitrogen on the one hand and that of P- and K-salts has been investigated. The addition of nitrogen to compost and to the low-land moor soil has no effect on the activity of dehydrogenase. The light soil 38 and the soil 0, which have not been fertilized for a long time, showed an effect by addition of nitrogen and also more or less by the addition of P and K.

In further experiments, the dependency of the activity of the dehydrogenase has also been investigated in connection with physiologically acid, neutral and basic fertilizers and also in connection with organic fertilizing.

The results are summarized in the following table:

Dehydrogenase Activity of Soils Fertilized with Fertilizers of Different Physiological Reaction

Kind of fertilizing	$CaCO_3$	Extinction	pH (KCl)	Total humus
acid	without	0.186	3.97	0.93
neutral	"	0.333	4.60	0.82
basic	"	0.321	5.75	0.78
acid	small amount	0.276	4.53	0.82
neutral	"	0.286	5.23	0.88
basic	"	0.331	6.69	0.77
acid	large amount	0.286	5.62	0.91
neutral	"	0.330	6.30	(0.9)
basic	"	0.396	7.30	0.85
acid	peat	0.693	3.40	2.46
neutral	"	0.605	3.86	2.09
basic	"	0.708	4.63	2.20

G. Lenhard, Z.Pflanzenernahr., Dung., Bodenkunde 73, 1-11, (1956).

In the summary, especially the dehydrogenase activity of these soils fertilized with peat is remarkably higher than the others. The amount of humus is also higher. In the other cases, there are only tendencies which are not significant. The dehydrogenase activity of the physiologically acid fertilized plots is smaller than those of physiologically neutral and basic fertilized soils. Therefore, it seems to be that the content of organic matter in soils is the determining factor for the dehydrogenase activity.

In my opinion, the experiments with peat showed that not only are the dehydrogenase which are responsible for carbohydrate metabolism, respiration respectively, important for increasing dehydrogenase activity. But it may also be that the dehydrogenases which take part in the transformation of the aromatic compounds in the peat, affect the measurement of the activity. But this must be proved by further experiments. It is also interesting to remark that the activity of dehydrogenases increases in the case of fertilizing straw with nitrogen.

Dehydrogenase Activity of Soils Fertilized with Fertilizers of Different Physiological Reaction.

Kind of Fertilizing	$CaCO_3$	Extinction	pH (KCl)	Total Humus
Acid	Without	0.186	3.97	0.93
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Basic	" "	0.331	6.69	0.77
Acid	Large amount	0.286	5.62	0.91
Neutral	" "	0.330	6.30	(0.9)
Basic	" "	0.396	7.30	0.85
Acid	Peat	0.693	3.40	2.46
Neutral	"	0.605	3.86	2.09
Basic	"	0.708	4.63	2.20

G. Lenhard, Z. Pflanzenernähr., Düng., Bodenkunde 73, 1-11 (1956).