

## MICROBIOLOGIC PROCESSES IN LIMELESS ALKALINE SOILS

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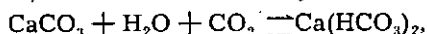
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A thorough knowledge of soils is the preliminary condition of solving the problems arisen from the growing of rice in Hungary. For this reason, in addition to the usual chemical and physical investigations, also the continuous microbiological study of the soil and flooding-water has become necessary. In the course of these investigations, light was thrown also on the processes contributing to the formation of the limeless alkaline soils (solonetz).

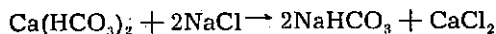
According to FEKETE, (4) in South-Eastern Europe alkaline soils develop on the border line of chernosem from soils of younger alluvium, meadow clay, and degraded chernosem. Opposite is the case in the Soviet Union, where alkaline soils are hardly to be found within the chernosem-belt, and they occur mainly on the brown and grey desert soils to the south of the chernosem-belt. Thus the alkaline soils of this country are not quite the same as those of Russia. Their formation is different and, consequently, so is the way of their reclamation. The location of alkaline soils is determined by plenty of factors even within a small region. A good example for this is given in the region Tiszazug, thoroughly investigated by M. A. NAGY. (10) From the fact that utterly different circumstances as well as processes may lead to alkalization it follows that in these regions also the formation of sodium carbonate may take place on the base of different processes. Thereafter, calcium will be replaced by sodium in the absorption complex.

There are several theories concerning the processes of alkalisation as well as the formation of sodium carbonate. In the literature HILGARD (3) and GEDROIC's (7) theories are predominant.

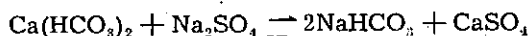
According to HILGARD, sodium carbonate is formed by interaction between calcium carbonate and neutral sodium salts ( $\text{NaCl}$ ,  $\text{Na}_2\text{SO}_4$ ) in the soil. He believes that calcium carbonate, under the influence of water as well as carbon dioxide dissolved, turns into calcium hydrocarbonate:



then this latter reacts with sodium chloride or sodium sulphate to give  $\text{NaHCO}_3$ :



or



respectively.

According to VENDL, (21) in areas flooded with water, the possibility of formation of sodium carbonate exists when in the soil a quantity of calcite of fine distribution occurs and the sodium compounds have not been leached. In Hungary and also in other countries sodium carbonate is to be mainly found in alkaline soils containing calcite in large amounts; this would mean that  $\text{CaCO}_3$  plays an important role in the formation of sodium carbonate. The alkaline soils in the areas to the east of the Tisza however contain, apart from sodium carbonate,  $\text{Na}_2\text{SO}_4$ , and may be  $\text{NaCl}$ , and in lower horizons also gypsum ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ).

HILGARD's theory was disputed by GEDROIC who ascribed the formation of sodium carbonate to the hydrolytic decomposition of the Na-complex.

According to GEDROIC the formation of sodium carbonate starts but then when, after adsorption of the Na-ion, surplus sodium salts had been leached out. In his opinion  $\text{Na}_2\text{CO}_3$  is formed from some part of the Na adsorbed, under the influence of carbon dioxide and water. VENDL believes that in the region between the Danube and Tisza GEDROIC's theory comes closer to truth.

The alkaline soils to the east of the Tisza, however, contain plenty of  $\text{Na}_2\text{SO}_4$ , and those between the Danube and Tisza contain, in addition to their high sodium carbonate content, also some  $\text{NaCl}$ . These facts are at variance with Gedroic's theory.

I think that the processes outlined in HILGARD's as well as GEDROIC's theory give an insufficient explanation of the accumulation of sodium carbonate in either the surface soil or the sub-soil, and, the formation of alkaline soils to the east of the Tisza can by no means be interpreted purely in terms of either of the two theories.

On the basis of my investigations it seems clear that, in addition to geographic, climatologic, and pedologic factors, some microbiologic processes are also responsible for the alkalization, or rather, the former factors facilitate the development of the microbiologic processes playing part in the alkalization. The role of climatologic and geographic factors has been considerably cleared up by our investigations. The knowledge of microbiologic processes summed up below may contribute to the solution of the problems arisen.

The elucidation of the etiology and therapy of the browning disease of rice in Hungary, as well as the study of the influence of rice growing on soils, and also the reclamation of alkaline soils, required a thoroughly chemical and microbiological investigation of soils of some rice plots situated in alkaline areas with for the most part limeless surface horizons.

PRETTENHOFFER's thorough investigations (11, 12) have cleared up the role of lime and organic matter in the appearance of the bruzone.

The results of my own investigations have revealed other possibilities of explaining the formation of sodium carbonate and the alkalization process, too. This theory can be easier applied to conditions in Hungary than those of HILGARD and GEDROIC.

The Hungarian investigators of the alkalization problem are all of the opinion that alkaline soils, both calciferous and limeless, have developed in places of marshes of old. The first Hungarian author who put this down was K. MURAKÖZY. (9). He wrote in 1902: »My observations of several years have led to the conclusion that the formation of alkaline soils should be related to the life activity of marshes, bogs and swamps.« In support of this statement.

MURAKÖZY mentioned several examples. — According to 'SIGMOND the formation of alkaline soils in Hungary is due to the joint effect of three factors: 1. arid climate, 2. an impermeable layer in the subsoil which prevents the penetration of water, and 3. a periodical excess of watercovering.

Since rice-flooding periodically restores the original primitive, as it were wild, conditions which had caused the solonization of the soil, the rice plots, separated from one another by dikes, presented themselves as excellent experimental plots for a detailed study of solodization processes.

These studies have thrown light on the fact that, in soils periodically subjected to natural or artificial flooding, and where the infiltration of water is hindered by an impervious layer, the microbiological processes determined by local conditions may lead to both ammonification and subsequent oxidation, i. e. to formation of sodium carbonate, or rather, to solonization.

*The Role of Sulphur in the Formation of Limeless  
Alkaline Soils to the East of the Tisza*

The rice growing in Hungary mainly proceeds on the limeless alkaline soils of the areas to the east of the Tisza. The chief characteristic of these soils is the absence of lime in the surface layers. The symptoms of the browning disease could chiefly be explained in terms of the effect of hydrogen sulphide, therefore it became necessary to examine the soils from the point of view of the circulation, reduction, and oxidation, of sulphur.

It is well-known that some rice plots are liable to, and others exempt from, the browning disease. The exchangeable basic conditions of soils of liable and immune plots were thoroughly cleared up by SIK (16, 17) and other investigators. On the base of their investigations the ratios calcium: magnesium as well as calcium: sodium have been estimated. The average ratios are as follows:

Basic ratios	On liable plots	On immune plots
Calcium: magnesium	1,4	3,4
Calcium: sodium	1,3	4,4

Since the alkalization process means at the same time also the progressive replacement of  $\text{Ca}^{++}$  by  $\text{Na}^+$ , from the fact that alkaline soils containing magnesium are to a greater extent liable to the disease, it follows that the disease indicates processes, taking place in the soils, rather than a mere static condition!

For the formation of hydrogen sulphide and for the effectiveness of its toxicity, a favourable coincidence of various factors is indispensable. The very process may be concisely summed up as follows:

In stagnant waters covering the limeless alkaline soils, as, in the flooding-water of rice-fields, the sulphate-reducing bacteria (*Desulfovibrio desulfuricans*), vigorously multiplying under anaerobic conditions chiefly established by the increased decomposition of organic matters, may reduce the sulphate

contents of these soils as well as of the flooding-water. The hydrogen indispensable for the reduction is secured by decomposition of organic material (rice-stubble and rot-remains). The sulphate-reducing bacteria meet their requirements of energy through combustion of hydrogen to what they get the oxygen by reduction of sulphates present. As a result of this reduction hydrogen sulphide will be liberated by the carbon dioxide, and also by other acids, formed from decomposition of organic matters.

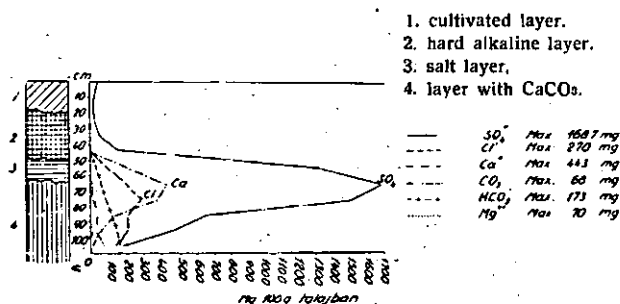
Thus the conditions for the hydrogen sulphide development are:

- a) *plenty of organic matter* (decomposing cellulose and other organic substances), as a source of hydrogen;
- b) *high sulphate content* of soil and flooding-water, as an oxygen source;
- c) *anaerobic conditions*, the development of which is facilitated by decomposition the organic nitrogen content;
- d) *acidic medium*,  $\text{CO}_2$  resulting from the decomposition of organic matter, as well as of  $\text{H}_2\text{SO}_4$  produced by sulphur bacteria so that the hydrogen sulphide may be released from the sulphides.

The sulphate ion content of the Tisza-water has been examined by J. MEZŐSI and É. DONÁTH. (8). They pointed out that the average sulphate ion content of the Tisza-water amounts to 30 mg per liter. According to SOMORJAI, (18), for flooding a cadastral acre of field (= 1,42 acres), 8700 m<sup>3</sup> of water is used up in a year, that is, some 261 kg  $\text{SO}_4$ -ion, or rather, 89 kg of sulphur. Whether remained after irrigation or flooding, or produced by a high water-table, the periodical water-covering continuously increases its sulphate content by evaporation and transpiration of the vegetation.

Table 1. shows the average salt content of limeless alkaline soils, according to TREITZ. (20).

Table 1.



The distribution of salts in limeless alkali soils.

### *The Sulphate-reducing Bacteria and their Method of Investigation*

The presence of the sulphate-reducing bacteria (*Desulfovibrio desulfuricans*) was demonstrated by the Starkey culture-medium. The sterilized media were packed into sterile 100 ml common jars suitable for this purpose, to the brim, and then inoculated with known dilutions (of 1000 and 10.000) of the soils to be examined. The jars were incubated in thermostat at 25 C°. Depending on the presence and number of sulphate-reducing bacteria, the reduction of  $\text{FeSO}_4$ , used for indicator led to the blackening of the media. By continuous

microscopic examination of the slides made from the culture-media was established also that, depending on the oxygen content, at the start, a mixed culture precedes the dominance of the sulphate-reducing bacteria.

Hydrogen sulphide in soil dissociates, and consequently, apart from the undissociated molecules, hydrosulphide and sulphide ions occur, too. The degree of dissociation is determined by the reaction of the medium. Table 2. demonstrates, according to RUBENCIK, (13), the percentage of the molecular hydrogen sulphide, hydrosulphide, and sulphide ions, observed as a result of the pH-value variation.

Table 2.

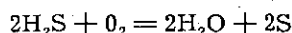
Molecular and dissociated $H_2S$	pH — value						
	5	6	7	8	9	10	11
	percent						
$H_2S$	99.41	94.61	63.66	14.93	1.72	0.18	0.02
$HS^-$	0.59	5.39	36.31	85.07	98.28	99.82	99.97
$S^{--}$	—	—	—	—	—	—	0.01

According to Table 2, the molecular  $H_2S$  would be, due to the acidic reaction of the medium, of the most toxic character, that is, the amount of the undissociated form increases.

In the course of a later investigation it has been also established that the bacteria, similarly to the lactic or the alcoholic fermentation, perish through their own metabolic products. Namely, after a definite concentration of the gas developed, multiplication of the sulphate-reducing bacteria comes to a standstill. During investigation of the cultures grown old it could be observed that, under the influence of hydrogen sulphide developed, dark spots appeared in the bodies of bacteria, and, thereafter, every bacterium went into as many pieces as dark spots were in its body previously. Since the same quantity (100 ml) of the media has been always inoculated with the same 1 ml soil suspension of a dilution of 1:1000, it was also possible to draw a conclusion from the time required for blackening, or rather, from the colour shade of the medium, for the number of the sulphate-reducing bacteria. With the soils examined, the blackening set in every case; as regards the quantity, however, they differed considerably.

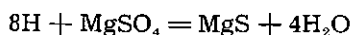
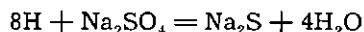
On the base of the investigations the course of reduction conditions may be summarized as follows:

In the mud of marshy tracts flooding the sulphate-containing limeless alkaline soils, or in that of rice plantations, the microorganisms of oxidative action gradually consume the oxygen absorbed by water. The establishment of anaerobic conditions is facilitated also by hydrogen sulphide developed during the decomposition of proteins. Hydrogen sulphide, namely, uses up the oxygen with precipitation of sulphur:

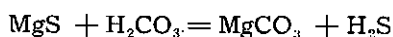
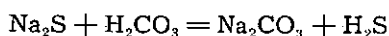


At first, the deficiency of oxygen of the mud more and more promotes the multiplication of anaerobic bacteria. The absence of oxygen, therefore,

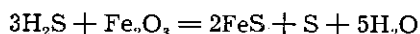
makes possible the multiplication of those bacteria which obtain, through reduction, the oxygen indispensable for their life activities. In consequence, the absence of oxygen as well as of light, and an adequate temperature are quite necessary for reducing bacteria to come into dominance. These optimum conditions are secured by the mud. This leads to the multiplication of the sulphate-reducing bacteria present. Hydrogen, obtained through decomposition of organic material, chiefly of cellulose, and oxygen, developed through sulphate reduction, are utilized by the sulphate-reducing bacteria as follows:



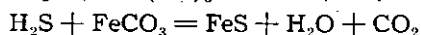
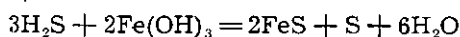
From the sodium sulphide produced, the hydrogen sulphide will be released by carbon dioxide developed through decomposition of organic matter:



The hydrogen sulphide formed by organic matter decomposition or sulphate reduction, with soluble ferri-compounds of the soil, gives rise to ferrosulphide:

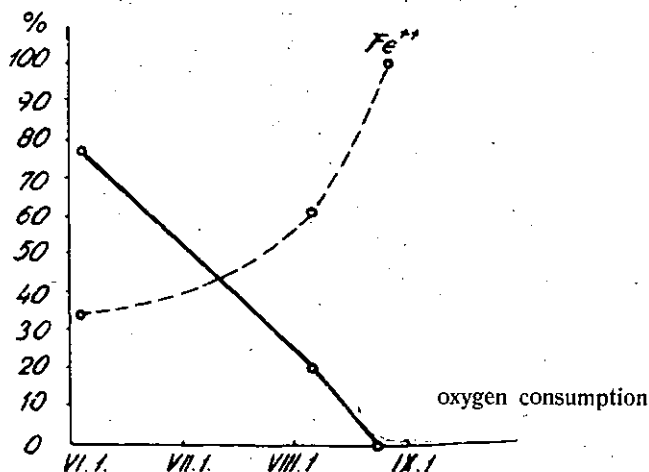


The formation of FeS may be illustrated also through other equations:



The accumulation of ferro-compounds as well as the decrease of oxygen consumption are shown, according to SIK's investigations, in Table 3.

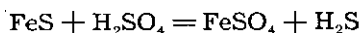
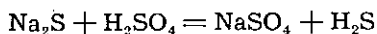
Table 3



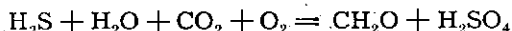
The ferrosulphide produced forms a deposition on portions, roots, of the plants, covered by water, and colours the mud black, too.

The liberation of hydrogen sulphide, effected by the influence of carbon dioxide, soon would lead to equilibrium, hindering the further hydrogen sulphide precipitation through dissociation of sodium carbonate produced. This,

however, will not take place, for sulphur bacteria (mainly the *Thiobacillus thiooxidans*) form sulphuric acid from the sulphides. The flooding-water becomes acidic, and hydrogen sulphide will be liberated from the sulphides.



The  $\text{H}_2\text{S}$  thus formed will be further oxidised by sulphur bacteria, in metabolism, according to the equation:



Apart from carbon dioxide and sulphuric acid, of course, also other acids may share in the liberation of hydrogen sulphide. In the course of butyrous, and another types of, fermentation may arise organic acids, too, which, being stronger than hydrogen sulphide, promote the release of hydrogen sulphide as well.

The hydrogen sulphide, not immediately oxidizing in the soil covered with water, and accumulating, may exert a pernicious effect upon the vegetation of the flooded area. The toxic influence, as well as the accumulation of hydrogen sulphide, hindering the metabolism and transpiration, are, after all, the cause of the brusone of rice. The decrease of temperature in August results in an increase of gas absorptivity of flooding-water, and, consequently, in an accumulation of hydrogen sulphide. The disease, therefore, is due to a process taking place in the soil independently from the plant; to a process of activities of the limeless alkaline soils, proceeding in middle of summer, when, in addition to the oxidation processes of surface water layer, reduction processes in the airless lower layer are also intense. Thus, in my opinion, as regards time, the disease will appear when, owing to sulphate reduction and decomposition of organic matters, as much  $\text{H}_2\text{S}$  had developed already, that the sulphuric acid, produced by oxidation of sulphides arisen through the reaction, together with the other acids, acidify the flooding-water and intensify the separation of hydrogen sulphide. This, as a rule, occurs in the middle of August or later, according to the weather. The sooner the reduction processes ensue, the thicker the reduction layer will be. According to measurements, this latter may also be in the neighbourhood of 14—17 cm. In such a case the  $\text{H}_2\text{S}$  may affect the whole surface soil, with a damage of 100%. In alkaline circumstances, the sulphides taken up by the plant cause the illness of the leaves, nodi and heads of corns.

Also the green algae producing oxygen will be driven away by hydrogen sulphide accumulated in the flooding-water. Owing to the toxic effect of hydrogen sulphide, the sulphate reducing bacteria conquer more and more territory, act on the vegetation of rice-field. The plant as a sensitive indicator shows the spreading of intense reduction processes as if the appearance of disease were connected with the spreading of some pathogenic microorganism.

#### Detection of Products of Reduction

In flooded soils the formation of hydrogen sulphide was shown in a simple way, by putting silver objects into the mud, which there became black because of the hydrogen sulphide formed.

The reduction process usually taking place in flooded soils was reproduced artificially, under laboratory circumstances, as follows:

The limeless alkaline soil samples, taken from lower lying, and saturated with water, spots, were packed into hermetic boxes made of aluminium. At room temperature, after a few days had passed, the samples became black, except for the surface. Along the vegetable parts, that is, where, on account of the anaerobic decomposition, more hydrogen was developing, the ferrosulphide formation was particularly vigorous.

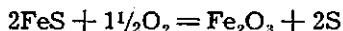
The connection between anaerobic decomposition of cellulose and the sulphate reduction was proved by the following laboratory experiment:

Hermetic aluminium boxes, provided by inserts, were packed with surface-layer sample of original limeless alkali soil to the brim. The material-disk thus obtained was cut into two halves horizontally, then sterile cotton-wool was inserted between the two half-disks which were, thereafter, crammed back into the box. After irrigation with water, the boxes were hermetically closed. After 14 days' standing in a thermostat of 14 C° the boxes were opened. The cotton-wool disappeared, in its stead a black colloidal Fe — precipitation was noticed. This experiment also evidences that the hydrogen formed from decomposing cellulose is utilized for reduction of sulphates by the sulphate-reducing bacteria.

Decomposing cellulose (organic material) as a source of hydrogen facilitates the multiplication and vigorous life-activity of sulphate-reducing bacteria, which will be pointed out by the increasing Fe<sup>++</sup> as indicator. In the course of field experiments, the changes in Fe<sup>++</sup> taking place owing to the covering of straw of rice and wheat are illustrated by Table 4., according to PRETTENHOFFER's investigations. (Table 4.)

In order to collect further observations, the mud of a plot perished by brusone was examined straight after drainage when water was oozing from the plot. The surface soil being in contact with air had, in a thickness of a few mm, a brown colour, whereas the layer thereunder was of black colour as if it were mixed with soot. The black colour was due to ferrosulphide.

After the soil had dried up, through its crevices air enters into the deeper soil horizons, too, the black ferrooxide adhered to the vegetable roots will be oxidised and by then it takes on a red-rusty brown colour because of the ferrooxide. That is, the following process would proceed:



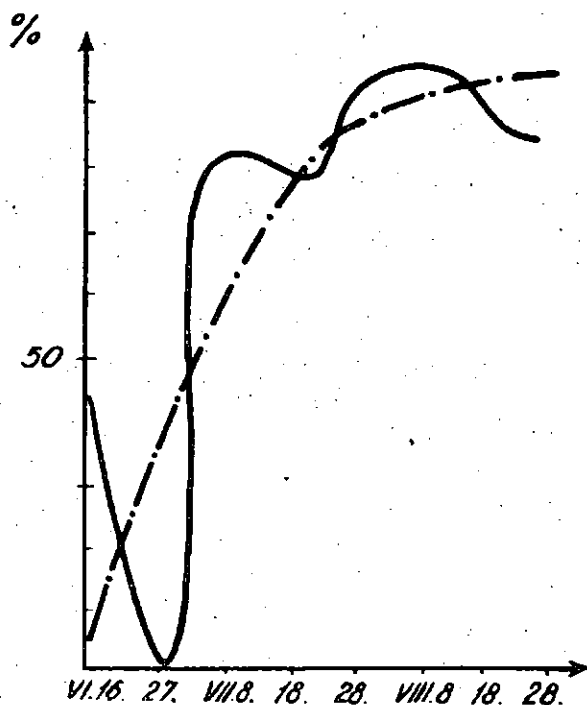
Hydrogen sulphide might have had a share in the decalcification of the surface soil and the leaching, as well. Namely, hydrogen sulphide, with Caions of the absorbing complex, joins into calcium hydrosulphide. This compound owing to its great solubility, percolates into deeper soil horizons where later, when airy circumstances ensue, that is, when soils had become fissured from dryness, it may turn into calcium sulphate.

In the alkaline soil profile of acidic character, sodium carbonate can be detected only in calcareous layers. Treitz, too, found that in alkaline soils with acid topsoil, the carbonates of sodium and calcium occur usually together, in a depth of about 55 cm. He also established that the amount of carbonate of sodium as well as of calcium increases with depth so that in the depth of 1 m

the latter amounts to about 16%, and the former — to about 2,5 g. The accumulation of other salts is considerably influenced by local conditions.

The next question is: what happens to the sulphur, when these processes had taken place and what is the role the sulphur bacteria play when oxidizing S to sulphuric acid.

Table 4.



The influence of straw-dung on the  $\text{Fe}^{++}$ -variation.

The formation of sulphuric acid explains the heavy texture of the unsaturated, leached out top-soil, and the presence of sulphates in subsoil, and throws light upon the biological factors of limeless alkaline soil formation.

#### *Relations between nitrogen circulation and sulphate reduction*

It is well known that anaerobic conditions, starting the reduction processes responsible for the outbreak of the bruzone, usually appear at first on lower-lying places with high water-covering. These low-lying spots, as reported above, always have larger total nitrogen contents than those in higher sites, which are sooner relieved from water when drained off. The higher nitrogen contents are due to the accumulation of humus-colloids and to the enormous masses of microorganisms populating the flooding-water that draws back here, when the water level sinks. Their carcasses represent a considerable amount of protein nitrogen. Also the carcasses of higher-class animals increase the protein-nitrogen supply of the soil. For instance, immense number of young fish remain and perish there after drainage.

According to my investigations, sulphate reduction in flooded soils is always preceded and introduced by a considerable degree of ammonification. The gaseous products: ammonia and carbon dioxide, namely appear as nutrients of sulphate reducing bacteria, and the  $H_2S$ , through the binding of the oxygen, creates suitable conditions for their multiplication.

The ammonia serves also for binding the carbonic acid.

### *Marks Indicating the Solonization Process*

The sulphur circulation process reported above may give an explanation of the leaching of the surface soil and, of the formation of a calcium sulphate layer in a depth of 60—65 cm. Results of the tests carried out hitherto clear up the role of the sulphur circulation process and, of the action of sulphur bacteria, in the formation of limeless alkaline soils.

Experimental chemical data that would prove the acid-solonetz-forming action of the sulphur compounds, cannot exist for lack of knowledge of the conditions of soil before solonization. The process, however, may be rendered perceptible. Here the rice plant succours the investigation as being a sensitive bioindicator. It indicates the reduction oxidation processes mentioned above by the symptoms of its disease or by its death. As the degree of the disease depends upon the intensity of these processes, the outbreak of disease conspicuously indicates the spots where they are intensive. On the basis of detailed soil tests the sound and affected plots may be compared. Of course, a single comparison of soils of one, or a few, sound plots with plots definitely liable to disease, does not give any, even generally acceptable, result. For the application of the usual statistical methods, many test data are required in order to get reliable average values suitable for comparison. The use of this method was rendered possible by the courtesy of PRETTENHOFFER who placed FRANK's (6) comparative analyses at my disposal. These data as well as my own results allowed the estimation of the average values. The average and limiting values just as the averages of the limit values are included in the following table:

Test	averages		limit values		averages of limit values	
	sound	ill	sound	ill	sound	ill
1. pH-value in $H_2O$	7,22	6,54	6,3-8,1	6,2-7,7	7,2	6,85
2. pH-value in $KCl$	5,89	5,37	5,2-6,9	4,9-6,9	6,05	5,9
3. Stickiness number	55,7	46,3	40 - 68	35-61	50,40	48,0
4. Nitrogen mg/100g available	1,1	2,9	0,46-2,1	1,4-6,1	1,28	3,9
5. Total nitrogen %	0,139	0,20	0,137-0,157	0,165-0,253	0,147	0,209
6. Phosphor mg/10 g available	0,55	3,1	0,45-9,55	0,4-9,1	5,0	4,8
7. Total Fe mg/100g	102	147	42-182	63-210	112	131
8. Hydrolytic acid.	7,8	12,7	2,9-10,1	3,9-25,5	6,5	14,7
9. Humus %	2,88	3,41	2,7-2,96	3,1-3,85	2,83	3,46
10. Total salt, dissolved in water	0,09	0,086	0,06-0,165	0,042-0,23	1,14	1,36

From the data of the above table the following conclusions may be drawn:

**Latent acidity:** Liable soils possess a large latent acidity.

**Nitrogen:** The amounts of available as well as total nitrogen of the diseased plots are the multiples of those in a sound plot. As to the total nitrogen content, there is a distinct separation line between the sound and the diseased plots: the total nitrogen content above 0,16% may lead to disease.

The rate of development of the anaerobic conditions is more rapid on these spots than on places poorer in nitrogen.

The available **phosphor** content is twice as high on diseased plots as on sound ones. It seems obvious that the dissolving effect in these places must be more intensive. The limiting values, however, show that the appearance, or absence, of disease is not influenced by the amount of available phosphor. The larger amount of water-soluble phosphoric acid, in my opinion, is the consequence of the dissolving effect of sulphuric acid produced by sulphur bacteria. Thus, the dissolving processes in the soil are the same as those applied in technology.

The total **iron** content of the affected plots is near 30% higher than that of the sound ones.

**Hydrolytic acidity:** A relation exists between the process reported above and the appearance of the disease or the solonizing processes. On soils liable to disease, the hydrolytic acidity exceeds the value 10.

**Humus:** The same applies also to the quantity of humus. The limiting value of the humus producing disease may be drawn at 3,0%.

**Total salts:** The averages of the total water-soluble salts showed no differences, but considering the limiting values, the higher salt contents are significant.

By the agrobiological processes reported above light is thrown upon the hitherto unknown cause and control of the brusone. In 1954, this disease caused damage to a half milliard forints in Hungary. From the results of my investigations it may be expected that these enormous damages could be considerably reduced or even avoided.

### *Summary*

To raise rice production it has become necessary to investigate the flooded soils chemically and microbiologically. As a result of these researches it turned out that the physiological disease of rice depends on the microbiologic processes of the soil flooded periodically. According to these investigations, the reduction and the oxidation processes running off after flooding and draining are connected with solonization. Rice, as a sensitive indicator, marks by its disease those spots where the above processes go on intensely.

## Literature.

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