THE IMPORTANCE OF MOLD ACTION IN THE SOIL¹

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INTRODUCTION

When a group of microorganisms is studied in relation to soil fertility, the first question that presents itself is: What is the nitrogen metabolism of these organisms? What part do they play in the nitrogen cycle of the soil? The largest number of investigations dealing with soil microorganisms, whether in pure or in mixed cultures, whether studied in solution or in the soil, in the laboratory or in the field, are concerned with the possible nitrogen changes in the soil produced as a result of the activities of these organisms. Since the nitrogen changes in the soil are among the most important ones in the study of soil fertility, the wildest speculations have often been made in an attempt to interpret the nitrogen changes produced in the laboratory upon artificial culture media from a few organic or inorganic substances by microorganisms and account thus for the different soil processes which are important from the point of view of soil fertility problems. Fewer investigations were devoted to the study of the carbon, phosphorus, iron, sulfur, and potash changes in the soil as a result of microbial activity.

The very methods employed in soil bacteriological investigation can be subjected to a great deal of criticism. Without going at present into a detailed discussion of the different methods used in the study of nitrogen metabolism of the soil flora as a whole, an attempt will be made in this paper to take up the study of the metabolism of molds which commonly occur in the soil. This may help to throw some light upon the part played by these organisms in different soil biological processes.

From the early period of soil microbial investigations up to the last four or five years, nearly all the attention of the soil bacteriologists was centered upon the study of bacteria, entirely neglecting the other groups of microorganisms, although these must have come to their attention here and there. The fact could not be overlooked that the soil has an abundance of molds, actinomycetes, protozoa, rotifers and, under certain conditions, algae, which exist in the soil in an active state. Whether the cause for neglect was the use of media more favorable to bacteria than to the other microorganisms for their isolation from the soil; whether it was due to the fact that the bacteria form the

¹ The species of fungi isolated from the soil belong to widely scattered groups; the common term "mold" is applied here collectively to these organisms, although no sharp limitation can be placed on the use of the term.

more numerous group of soil organisms, and, as always happens in such cases, the attention is centered upon the more frequently-occurring types; whether the important advance along bacteriological lines from the point of view of pathogenicity to men and animals has centered upon it so much of the attention even of the soil bacteriologist, that the other organisms (non-bacterial) were neglected—whichever was the cause, it was only within the last half-dozen years that the great abundance of other microorganisms in the soil besides bacteria, has been demonstrated and an attempt has been made to explain their probable part in soil fertility.

The work of Russell and his associates (48) upon the possible part played by protozoa in soil fertility has called forth a series of investigations on the occurrence of soil protozoa and their activities. The algae of the soil were studied by several investigators. The occurrence of actinomycetes and their possible rôle in the soil have been recently summarized in several papers. It will be the object of this paper to point out several metabolic processes of the molds occurring in the soil and thus attempt a suggestion as to their part in soil fertility. An extensive bibliography on this subject can be found elsewhere (56). The several soil biological processes will be taken up and, by comparing the activities of the molds with those of bacteria, the important transformations which the organic matter and the mineral matter undergo in the soil and which may be due to the action of the molds, will be discussed.

The observations and conclusions are based on the work of different investigators, but chiefly on that of the writer.

OCCURRENCE OF MOLDS IN THE SOIL

It has been definitely established (56, 58) that the molds are common inhabitants of the soil and form a large and important group of the soil flora.

Hundreds of species of molds have been isolated repeatedly from the soil; it has been found that many molds occur in different soils, under different topographic, climatic and crop conditions. The same species have been isolated by a number of investigators in different European countries and from numerous soils in this country. A number of new species never encountered before were isolated from the soil, which would serve as additional proof that some of these are typical soil organisms. Although as many as 1,000,000 fungus colonies developed from 1 gm. of soil in some cases, this cannot be taken as proof that so many pieces of mycelium were present in that quantity of soil, but merely that a mass of spores were present and each of these spores developed into a colony when the soil was shaken with water and plated out. The method of determining the number of molds in the soil is very inefficient, and since usually only two or three plates are made from each dilution, the probable error is much greater than anything that should be allowed for exact work. This was the reason why the writer, in one of the earlier publications (58), attached little importance to the plate count of molds in the

soil. And before more exact work has been done along this line and a large enough number of samples and soils have been studied, we shall not be able to state definitely as to how many molds (or rather mold spores and pieces of mycelium) can be expected in each gram of soil; even then, the uncertainty will exist because of the fact that at one spot an organism might have sporulated, which would give a tremendously large number of spores, not indicating, however, any predominant rôle for this organism over others which may occur in much smaller numbers. For example, such organisms as the *Aspergilli* or *Penicillia*, which produce a very large number of spores separating with relative ease, are often found in predominant numbers, while at a different period of the year these organisms may be entirely absent.

This may account for the statement of Hagem (20) that certain *Aspergilli* occur in larger numbers in the soil than all the *Mucors* taken together. This statement was made for Norway soils, and as the writer pointed out elsewhere (58), the *Aspergilli* are more abundant in a warmer climate, while the colder climates show a greater proportion of *Mucorales* in the soil.

A detailed discussion as to the occurrence of the different genera and species of fungi in different soils, as well as the methods of isolation, both in vegetative mycelial and spore forms can be found elsewhere (56, 58). In reviewing the work of others and his own, the writer concluded that there is a fungus flora of the soil and that many molds occurring in one soil will also be found in other soils, under different conditions of cultivation topography, origin and climate. The fact was pointed out that the Northern soils have a greater abundance of *Mucorales* and *Penicillia*, while the Southern soils are richer in *Aspergilli*; *Trichodermae* are found in large numbers in acid soils; *Fusaria*, *Cladosporia*, *Chaetomia*, *Alternaria*, etc. will be always found in most soils, if a large enough number of samples are studied and care taken in isolation. The list will no doubt be extended a great deal with a more intimate knowledge of the different more or less obscure forms of fungi present in the soil which require the careful study of the specialist in the different groups of these organisms.

Ramann (44) found that molds develop readily in acid soils and are more active in the forest and in the compact poor soils, while bacteria predominate in loose soils, rich in nutrients, cultivated and fertilized. Hagem (20) stated that in the well cultivated lands containing little humus the bacteria play an important part and occur in predominant numbers and the molds are of minor importance; while the upper layers of pine forests, rich in humus, contain a large number of molds. The rainy seasons of the year, fall especially, favor the surface growth of these organisms, otherwise they live and sporulate below the surface, among the plant residues and living roots. Since the last publication of the writer (58) there appeared a paper by Pratt, who isolated from Idaho soils a number of organisms, particularly *Mucors*, *Fusaria* and *Penicillia*, many of which were isolated by the writer previously from different soils of North America.

The fact that molds are found in the soil would not warrant as yet any extensive study of these organisms, before it has actually been demonstrated that they are not only present in the soil, but actually live there and produce mycelium, which would necessitate their taking an active part in the different biological transformations taking place in the soil. The writer (57) suggested a method by which it could be demonstrated that molds actually live in the soil, and, although not all the organisms found in the soil could be demonstrated by this method to have produced mycelium (curiously enough, the organisms found in the largest numbers, such as *Aspergilli* and *Penicillia*, could be demonstrated in the soil by this method only in very few cases), a number of groups were found to be present in the soil in an active state.

Conn (9), using a direct microscopic method for examining soils, found that mold mycelium is present in the soil only to a very limited extent. If plate counts of molds present in the soil do not have the same significance as plate counts of bacteria, as pointed out by Conn (9) who was preceded in this by the writer (58, p. 571), the microscopic method of Conn (9) is probably of not much greater value in this respect. The relative numbers of spores and hyphae of typical soil molds is such that, as Conn himself states, 3000 microscopic fields would have to be examined before a piece of mycelium could be found, while enough spores were produced by the same organisms to give a plate count of 300,000 per gram of the soil in which it would be grown.

Brown (4) repeated the work of both the writer and Conn and demonstrated that mycelium was present in all soils examined. Conn, using very small quantities of soils, namely 10 mgm., could easily have overlooked the mycelium that might have been present, although Brown stated that by using the writer's method he could demonstrate mold mycelium not only in 10 mgm. but also in smaller quantities of soil. Thom and Church (53) observed that different *Aspergilli* and *Penicillia* planted upon the surface of sterilized soil are capable of growing into the soil to considerable depths and even of producing spores.

The fact should not be overlooked that most of the investigations upon soil molds were made by botanists, who were either interested more in the types of the organisms as such, or as to their possible pathogenicity to plants, but not from the point of view of soil fertility. To be able to interpret the proper part played by these organisms in the soil we must study them not only as vegetative or other forms of plants, but as living organisms which exist in the soil, and by their metabolic processes help in the various transformations through which both organic and inorganic soil constituents undergo and thus affect the fertility of the soil.

The universal presence of molds in the air, on decaying fruit and different forms of organic matter, has brought them early to the attention of the biochemist, who used some of these organisms in investigations on metabolism. But since the fact has been established that these organisms exist in the soil, their biochemical activities could then be interpreted so as to understand their possible part in the fertility of the soil.

NITROGEN-FIXATION

The question of nitrogen-fixation by molds commonly isolated from the soil was under dispute, some investigators demonstrating that it was positive, while others could not find any fixation at all. The very recent work of Goddard (19), Waksman (56), Duggar and Davis (17) and Chambers (7), using more exact chemical methods and taking all precautions to eliminate any possible error that may creep into such experiments, has shown that the fungi commonly occurring in the soil, such as the different species of *Aspergilli*, *Penicillia*, *Macrosporia*, *Alternaria*, *Mucors* and others do not fix any atmospheric nitrogen. The amount of nitrogen-fixation by molds claimed to be positive by certain recent workers is so slight (below 5 mgm. per 50 cc. of solution) as to be of practically no importance in soil fertility when compared with the nitrogen-fixing bacteria. The slight quantities of nitrogen-fixation obtained by several workers may possibly be explained by the fact that no precautions were taken to eliminate any nitrogen compounds from the air of the laboratory, to have the chemicals carefully analyzed and to make a large enough series of determinations so as to eliminate any possible error. A detailed discussion of the literature on the subject can be found in the papers of the writers mentioned above. The mycorrhiza fungi, as shown recently by Peklo (39) and certain other fungi, not commonly occurring in the soil, such as cultures of *Phoma Betae*, studied by Duggar and Davis (17), may show a definite nitrogen-fixation.

It can therefore be definitely concluded that, with the exception of certain organisms, which are not very common in the soil, the typical soil molds do not play any direct part in the economy of the nitrogen enrichment of the soils.

NITRIFICATION

Neither nitrite nor nitrate formation has ever been demonstrated for any of the molds, so that this important process in soil fertility will have to be eliminated, as well as the nitrogen-fixation, from the field of mold activities in the soil. As will be demonstrated later, the nitrogen activity of the molds tends rather to a process of reduction than to oxidation. The breaking down of the complex proteins to proteoses and peptones, then to polypeptides and amino acids, and finally to ammonia, is accomplished by means of molds just as rapidly and thoroughly as by means of protein-decomposing bacteria. Whether this is accomplished as a result of the feeding of the organism, or by means of enzymes, and also whether it is due to the action of the organism upon the nitrogen part of the protein molecule or its carbon part, will be taken up later. All that can be pointed out here is that the organism decomposes the proteins of the soil with the liberation of ammonia, which is either left in the soil or absorbed by the organism in the process of the building up of microbial proteins. There is no need for the mold to oxidize the ammonia

into nitrates, since the ammonia as such is just as good a form of nitrogen for molds as nitrates are, when other conditions are equal.

Schloesing and Müntz (51) showed in 1878 that *Aspergillus niger* and other molds do not nitrify. The molds may exert an indirect effect upon nitrification, due to the fact that in acid soils the normal bacterial activities may be repressed and the growth of molds encouraged, as pointed out by Hall and his associates (21).

AMMONIFICATION

When we approach the subject of the disintegration of organic matter in the soil, particularly the first stages of decay, the molds are found to play a very important part: both the disintegration of nitrogenous organic compounds, which will be considered under the above heading and the decomposition of celluloses, hemicelluloses and starches in the soil taken up later.

The subject of ammonification has been often misunderstood and such importance attached to the information obtained from the determination of ammonia in the soil that could not possibly be warranted at all by the data. It has not been even definitely settled as yet as to what part ammonia plays in the metabolism of microorganisms. Czapek (12), in an exhaustive study of the utilization of different substances as sources of nitrogen by molds, found that amino acids are used much more economically than other nitrogen compounds. He assumed that molds, in building up their proteins from simple nitrogen compounds such as ammonia salts, have to produce first amino acids, and these are used as building-stones for the production of complex proteins; if amino acids are offered as sources of nitrogen, the organism will be spared the waste of energy which would be necessary if it had to build up its proteins from simpler nitrogenous substances. Hagem (20) claimed that the utilization of amino acids by molds takes place in the following manner. By the addition of water to the amino acid, ammonia and the corresponding oxy-acid are produced; and the protein synthesis takes place from the ammonia thus formed.

Whatever may be the process of formation of complex proteins by molds one thing is certain—that ammonia is left in the medium as a waste product of the protein metabolism of the organism. But even assuming that, we cannot state definitely that ammonia produced by a certain organism or a mixture of organisms will point to a definite condition and interpretation, because the ammonia accumulated as a result of the action of one organism from one nitrogen compound will depend not only on the source of nitrogen, but also on the amount and availability of carbon compounds present in the medium. When we therefore claim that a certain form of life or a complex mixture of different forms, such as a soil flora, will produce so much ammonia from peptone, dried blood, cottonseed meal or other organic nitrogen compounds, and try to interpret from it the activities of the given flora, we may be entirely wrong even if the ammonia produced could be taken as a factor

of the metabolism of the organisms, because the amount of ammonia produced may depend more on the character of the carbon compounds present than on the source of nitrogen. Hagem (20) has shown that although certain amino acids can be used both as sources of energy and nitrogen by molds, the ammonia produced will depend on the presence of carbohydrates in the medium. Kendall and his associates (26) have studied in detail the protective action exerted by available carbohydrates upon the decomposition of proteins by microorganisms. The writer (59, 60), in taking up the same work and applying it to the study of molds isolated from the soil, has tried to develop the theory further and apply it to the changes of the organic matter in the soil due to the action of microorganisms. The bacteria and molds will attack the protein molecule in the soil to derive from it the nitrogen needed for structural purposes, if available carbohydrates are present to supply the energy required; only small quantities of ammonia will be liberated under these conditions. But, in the absence of available carbohydrates, the organisms will attack the protein molecule not only as a source of nitrogen, but also as a source of energy; since the energy requirement of the organism is greater than the nitrogen need, only a small part of the nitrogen of the protein molecule will be used for the building up of the proteins of the microorganism, and the greater part of the nitrogen will be left in the medium as a waste product in the form of ammonia. We should therefore take up the study of ammonification by molds with this idea in mind: the ammonia merely indicates the activities of the organism in the presence of a certain source of carbon and nitrogen. It will indicate, as Hagem (20) stated, the mineralization of the organic matter.

The early workers on ammonification studies used both molds and bacteria. Müntz and Coudon (36) and Marchal (32) demonstrated in 1893 the fact that soil molds are as active ammonifying organisms as bacteria. Marchal (32) even attempted to ascribe the ammonia production in soils (particularly acid soils) chiefly to the action of molds. But following the work of these investigators, the greatest attention has been paid to soil bacteria. Only here and there a paper appeared calling attention to the occurrence of molds in the soils or to the chemical changes produced by them when grown on artificial culture media. Butkewitch (6) observed that in the decomposition of proteins by molds, ammonia and amino acids were formed, among the latter tyrosin and leucin being identified. He has been able to show that, by changing the cultural conditions of the same organism, either a rapid transformation of the peptone into ammonia with the production of only a small quantity of amino acids, or a slow accumulation of ammonia with a large quantity of amino acids takes place. The accumulation of ammonia runs parallel with that of oxalic acid; when the latter is neutralized by means of CaCO_3 , the ammonia accumulation is suppressed and other nitrogen compounds such as tyrosin and leucin, appear in the substratum. He agrees with Czapek, that amino acids are used directly by the organisms, while ammonia is a by-product

in the decomposition of the complex nitrogen compounds. The least energy was spent by the organism in utilizing amino acids, more energy was used when ammonium salts and nitrates were offered as a source of nitrogen, and still more when peptone and egg-albumen were the nitrogen sources.

Iwanow (23) has shown that, in the decomposition of the seeds of yellow lupins by *A. niger*, small quantities of tyrosin, larger amounts of leucin and still more ammonia, in the form of ammonium oxalate, were produced. Kosyachenko (31) found among the products of decomposition of the proteins of peas by the same organism tyrosin; leucin; the hexone bases histidine, arginine, and lysin; and ammonia. Hagem (20) has shown that, in the utilization of amino acids and peptone by *Mucors*, ammonia is always produced. Kappen (24) found that several molds decompose cyanamide with the production of ammonia. Other references to the work on the decomposition of organic matter by molds and liberation of ammonia will be found elsewhere (56). McLean and Wilson (34) found that soil molds produce in pure culture a much greater accumulation of ammonia than bacteria. Waksman and Cook (62) confirmed these observations and called attention to the fact that the accumulation of ammonia as a result of mold activities seems to take place in definite cycles and expressed the idea that there may be a relationship between the growth period of the organism and ammonia accumulation. Further information on the subject of decomposition of organic matter by fungi isolated from the soil can be found in the work of the writer (56), who has shown that the growth of molds on artificial culture media affects the ability to decompose organic matter by some molds and not by others. Kopeloff (28) found that an increase in the number of mold spores inoculated into the soil is responsible for a proportionate increase in ammonia production up to a certain point; additional information on the environmental factors influencing the decomposition of organic matter added to sterilized soil by soil fungi are supplied by Coleman (8). The decomposition of urea, uric and bipuric acids and glycocoll by molds with the subsequent liberation of ammonia was studied by Kossovicz (30).

Although, as shown above, the production of ammonia from organic matter, particularly when this has been added to sterilized soil, making the conditions distinctly different from normal, cannot be taken as a true indication of the rôle of these organisms in soil fertility, we are still able to obtain some information from these studies. In nearly all cases reported, some molds, such as *Trichoderma Koningi*, *Monilia sitophila*, and others are able to decompose the organic matter much more rapidly than the strongest ammonifying bacteria known, such as *Bacillus mycoides* or *Bacillus subtilis*. Even if the ammonia is looked upon only as an indication of the amount of organic matter decomposed by the organism, some of the molds, commonly occurring in the soil, are found to possess distinctly greater powers of decomposing the organic matter than do the bacteria. The writer (60) has shown that *A. niger* grown in culture media containing peptone or asparagine as a source

of nitrogen will bring about a rapid decomposition of the material, although the total ammonia production will depend entirely on the amount of available carbohydrate present. It was also pointed out that the ammonia production by *A. niger* is an autocatalytic phenomenon. A word should be said here that, although it has been definitely established by Miyaki (35) for bacteria and by the writer (60) for molds that the ammonia production obeys the law of autocatalysis, as shown by a mathematical interpretation of the results, it has not been demonstrated as yet that a catalyst actually exists, or that the action and counteraction combining to give the curve of autocatalysis may not be merely a result of the growth of the organism (action) and the injurious action of some of the by-products in the culture medium (reaction).

The action of the molds upon the nitrogenous organic matter of the soil consists, therefore, in the mineralization of these materials, with the production of ammonia and the building up of fungus proteins. The ammonia is either used by the higher plants as such or oxidized by the nitrifying bacteria into nitrates and used by the plants in this form, or absorbed again by the microorganisms of the soil either in the form of ammonia or even as nitrates and used for the production of the complex microbial proteins, as will be shown later.

DECOMPOSITION OF CARBON COMPOUNDS IN THE SOIL

The molds play an active part in the decomposition of celluloses and other carbon compounds in the soil; the importance of this process is well known to the student of soils, since the addition of green and animal manures, plant roots and other residues, necessitates this process before the minerals and nitrogen compounds can be brought to a form, in which they could be either taken up by the higher plants directly or after they have undergone another transformation due to the action of other groups of molds or bacteria. Celluloses, pectins, vegetable gums and other similar substances are rather inert and cannot be attacked by all microorganisms harbored in the soil; among those that are able to do it, the molds occupy a prominent place.

The work of Van Iterson (55), Koning (27), Dascewska (13), McBeth and Scales (33), and others has established the fact that the rôle of molds in the soil in the destruction of cellulose has been greatly underestimated; a number of molds were isolated from soil which decompose cellulose very rapidly. The different molds differ greatly in this respect: while some, such as different *Penicillia*, *Aspergilli*, *Trichodermae*, and others dissolve the cellulose very rapidly, others, such as the *Mucorales* (20) cannot attack it at all. Schellenberg (50) demonstrated that the ability of the molds to dissolve different celluloses does not depend on the solubility of these in acids, but on the chemical composition of the substance. Hagem (20) found that the *Mucorales* can use, out of all the carbon compounds found in the soil, only the pectin bodies and the mono-saccharides, and partly the disaccharides, while the cellu-

loses and hemicelluloses are left intact. He concluded that the Mucorales must take only a limited part in the decomposition of carbon compounds in the soil, when compared with the other molds and bacteria. Out of nearly a hundred organisms tested by the writer for the ability to decompose cellulose according to the method of McBeth and Scales (33), only the Mucorales and a few other organisms (several *Fusaria* and *Sporotricha*) produced little or no decomposition of the cellulose, while the *Trichodermae*, *Cephalosporia*, *Aspergilli*, *Penicillia*, *Verticillia*, and others produced a strong or very strong decomposition. A detailed discussion of the action of different compounds would be here out of place; it may only be stated that nearly all the simple and complex organic carbon compounds in the soil can be attacked by one or another group of soil molds, and these, through this action, play an important part in the fertility of the soil.

The question of the humin substances of the soil may be brought up here. Ramann (44) states that Nageli, Hoppe-Seyler, Kostytschew, Müller and others concluded that the molds are the proper humus builders in the soil. The fallen leaves, at the end of the vegetative period in the fall, are found to be penetrated with mold mycelium, which decomposes the leaves readily, with the production of humic substances. Hoppe-Seyler (22) claimed in 1889 that no plant or animal is able to use humin substances as food, and no bacterium can bring forth their decomposition; they afford an habitat and substratum to many bacteria, molds, algae and animals. Reinitzer (45) and Nikitinsky (38) have shown that humin substances cannot serve for molds and bacteria both as a source of carbon and nitrogen, but in the presence of available sources of carbon, they can be used as a source of nitrogen. The value of the study of humin substances in the soil and the action of microorganisms upon them has to be proven as yet, since they are artificial in nature, and the fact has not been even established as yet that they exist as such in the soil, and are not merely decomposition products due to the action of chemicals upon the soil.

As to the utilization of starch and production of amylases, the molds seem to be very active. *Aspergillus Oryzae*, *Aspergillus niger* and other *Aspergilli* and *Penicillia* have been found to produce very active starch-splitting enzymes.

The production of carbon dioxide can be taken as an index of the decomposition of soil organic matter. It is a much more accurate index of the biological transformations going on in the soil, since once it is liberated, it is not utilized again by the soil microorganisms, while ammonia and nitrates which are used more commonly by the soil bacteriologists, as indicating soil biological processes, can be utilized again by other organisms in the soil. Neller (38) made recently some interesting observations on the correlation between the production of carbon dioxide and accumulation of ammonia by soil organisms. The molds tested oxidized more of the carbon and produced less ammonia than the bacteria did; mixed soil infusions resembled the molds in the low accumulation of ammonia, but produced larger quantities of carbon

dioxide. Neller, therefore, suggested that the soil molds were the more active components of the natural soil flora, and a low accumulation of ammonia with alfalfa as the source of carbon, may not necessarily indicate a low activity, contrary to the usual conclusions of soil bacteriologists, who took only ammonification, nitrification, or bacterial numbers, as an indication of soil biological processes. A very active production of carbon dioxide from organic matter by molds isolated from the soil was made recently also by Potter and Snyder (40), who have demonstrated that several fungi isolated from the soil liberated nearly as much carbon dioxide from sterilized soil as did a mixed soil flora (soil infusion), both in the presence and absence of dextrose.

These two observations together with the work of the writer (59, 60) on the action of available carbohydrates upon the ammonia production by molds will help to throw a great deal of light upon the activities of these organisms in the soil. The molds attack the carbohydrates very readily, perhaps even more readily, concluding from these few observations, than the bacteria do. This rapid decomposition of the carbohydrates, both complex and simple, indicates a strong activity and rapid need and utilization of energy; the index of the rapid respiration is the carbon dioxide production. The nitrogenous organic compounds may be decomposed only to a smaller extent than they would be in the absence of these available carbohydrates; very little ammonia is therefore produced. But even when the nitrogenous compounds are decomposed rapidly and when a large amount of ammonia would be expected as a waste product of protein metabolism of the organisms, the ammonia will not be accumulated in the medium, but will be used, in its turn, by the organism for the further building up of mold protein, as long as carbohydrates are available to supply the energy. The ammonia will therefore not accumulate in the soil from the nitrogenous compounds, in the presence of available carbohydrates, for two reasons: first, less of the nitrogenous substances will be decomposed and, therefore, less ammonia will be left as a waste product, as shown by the writer (59, 60); second, the ammonia will be further utilized by the organism in the building up of the fungus protein, because of its rapid growth, as shown by the carbon-dioxide production; this can be readily seen from the data obtained by Neller (37). Of course, more information is necessary before we may be able to construct an exact theory as to chemical changes taking place in the soil and underlying soil fertility. One thing is certain that *we will have to construct our theories on soil fertility, particularly on the nitrogen part of it, not only from the point of view of nitrogenous manures and fertilizers and nitrogen content of the soil, but also by taking into consideration the nature and amount of carbon compounds added to the soil.* A study of the action of pure and mixed cultures of molds, as well as of bacteria, will help us to throw light on this subject. These observations clearly indicate *of how little value is the study of ammonia production by different pure or mixed cultures of soil organisms, when other factors are not taken into consideration.*

UTILIZATION OF NITROGEN COMPOUNDS

A knowledge of the utilization of nitrogen compounds by molds in the soil is important for a thorough understanding of soil biological processes, particularly from the point of view of soil fertility problems. Besides a knowledge of the production of ammonia, nitrates, and other simple nitrogenous compounds in the soil, or the addition of these in the form of an artificial fertilizer, we have to keep in mind that the lower organisms present in the soil will always compete with the higher plants in utilizing these compounds and converting them into complex proteins. The utilization of the different forms of nitrogen compounds has attracted the attention of many chemists, and some very interesting work has been done along these lines. Two molds chiefly have been studied: *Aspergillus niger*, whose identity can be recognized without much difficulty, although it has been fairly well established that different strains of this organism exist, which differ somewhat in their biochemical activities (54); and *Penicillium glaucum*, which is a rather vague term, since there exist many species of green *Penicillia* which could be termed *P. glaucum*. Czapek (12) made an extensive study of the nitrogen compounds that can be utilized by *A. niger* for the building up of the fungus protein; albumoses and peptones are utilized; peptones are produced out of amino acids, and these are in turn condensed to proteins. Since amino acids are necessary for the synthesis of fungus proteins, therefore, he argued, amino acids are the best sources of nitrogen for these organisms, since they will be spared the expense of energy which would be necessary to produce the amino acids out of simpler nitrogenous compounds. The nutritive value of the other nitrogen compounds depends on how easily they can be transformed into amino acids. Puriewitsch (42) started out with the idea that the more easily a substance is utilized by an organism, the fewer will be the stages through which the substance will have to undergo and the less will be the expenditure of energy. As a measure of this expenditure of energy he used the carbon dioxide production per unit of dried body weight of the organism. He confirmed the observation of Czapek (12): the utilization of energy was least for amino acids; ammonium derivatives followed, then nitrates, and peptone, and finally egg-albumen requiring the largest expenditure of energy. Raciborski (43), Hagem (20), Abderhalden (1) were of the opinion that the amino acids (also nitrates and nitrites) are first reduced to ammonium salts and in this form utilized for the production of proteins. Raciborski (43), Puriewitsch (41) and Brenner (3) stated that nitrites are poisonous for *A. niger* in an acid solution, while in an alkaline medium assimilation is positive and in some cases even just as good as nitrates. Ritter (46) found that the ability of an organism to assimilate nitrogen from inorganic ammonium salts is in direct relation to the ability of the organism to withstand the mineral acid liberated.

Without going into a detailed discussion on the assimilation of ammonium salts, nitrites, nitrates, and other inorganic and organic nitrogenous compounds by molds [such a discussion can be found in the paper of Brenner (3),] we may merely note here that this is of great importance from the point of view of soil fertility problems, since these organisms will use up the nitrogen compounds in the soil available for higher plants and will exert thus a very unfavorable action. This was pointed out by several investigators, particularly by Rothe (47), who stated that the molds exceed the bacteria and actinomycetes in acid as well as in neutral media in the assimilation of the available nitrogen and storing it away in an organic microbial form; under favorable circumstances, namely in the presence of calcium carbonate, large quantities of nitrogen added to the soil in the form of ammonium salts are transformed by these organisms into very insoluble nitrogen compounds. Hall and associates (21) stated that, under certain conditions, molds must compete with higher plants for the nitrogen added to the soil.

Sullivan (52) suggested that the complex organic substances found in the soil may be a result of the growth of the mold mycelium. The nitrogen which would have been otherwise available for higher plants may be transformed into an insoluble, concentrated, and mostly undecomposable form. This question is discussed in detail by Ehrenberg (18). He stated that fungus protein is much less available for further decomposition than bacterial protein; the spores contain a large quantity of nitrogen stored away in an unavailable form. The disappearance of the available nitrogen added to the soil in the form of ammonium salts or nitrates is to be looked for more in the mold metabolism rather than bacterial. Denitrification due to the action of molds is discussed in detail by Ehrenberg (18). When ammonium salts are added to the soil together with manure, the large quantities of energy material introduced allow a rapid growth of the soil molds, which assimilate a large quantity of ammonium sulfate nitrogen, thus preventing it from becoming available to higher plants.

We can thus see that the molds of the soil may produce a very unfavorable effect upon soil fertility in competing with the higher plants for available nitrogen compounds, particularly in the presence of large quantities of available carbohydrates. Although this injurious action, under certain conditions, cannot be denied, even if the extent of it has not yet been definitely established, two other factors should be considered here which may counterbalance the possible injury to higher plants. First, we know very well that an excess of ammonium salts and nitrates in the soil will lead to large losses due to natural or artificial irrigation and drainage, particularly in humid climates; the utilization of some of these nitrogen salts by the soil molds, bacteria and other organisms may serve for the conservation of a great deal of this nitrogen in the soil. Second, the autolysis of mold mycelium, resulting in the splitting off of the fungus protein with the liberation of ammonia, as shown by Dox and Maynard (16), Brenner (3) and the writer (60) will tend toward the

giving back to the soil of the nitrogen assimilated before by the molds in an available form. The molds and the other organisms may act in the soil, from this point of view, as a storing agent for the soluble nitrogen compounds added to the soil, and the injury caused by them at times, in competing with the higher plants for the available nitrogen, may be more than balanced by their ability to store the nitrogen and make it afterwards slowly available for the plants. This subject requires further studies, particularly the question of symbiotic and antagonistic relationship between the higher plants and soil microorganisms.

ENZYME PRODUCTION BY MOLDS

A review of the literature on the enzyme production by molds can be found in the paper of Dox (14). Dox (14) found that *P. camemberti* contains the following enzymes: erepsin, nuclease, amidase, lipase, emulsin, amylase, inulase, raffinase, sucrase, maltase, and lactase; the protease digested casein, gelatin, and proteoses, yielding a large percentage of amino acids; the amidase liberated ammonia from urea, asparagin, benzamid, and alanin; another enzyme could split hippuric acid into benzoic acid and glycocoll. Kellerman (25) has demonstrated the production of a cellulose-splitting ferment by *Penicillium pinophilum*. Scales (49) demonstrated that *Aspergillus terricola* produced inulase, diastase, invertase, maltase, alcoholoxydase, emulsin, lipase, protease, and amidase. The production of proteolytic enzymes by molds was studied by the writer (61). The production of the enzymes enumerated by molds will bring forth the proposition that enzymes formed by soil molds as well as bacteria may be concerned in the decomposition of the various organic substances in the soil. Many of the products of this decomposition form good sources of nitrogen and carbon for bacteria, while the ammonia and some of the amino acids may be directly assimilated by higher plants.

THE POSSIBLE MODIFICATION OF THE SOIL REACTION BY THE ACTION OF MOLDS

It is a common belief among soil bacteriologists that the mold flora is more active in acid than in neutral or alkaline soils. The scant exact information that we have on this subject confirms this belief, although it does not preclude the growth of the molds just as well in neutral and perhaps alkaline media. The work of Thom and Currie (54) and Currie (10, 11) clearly demonstrate the fact that a number of *Penicillia* and *Aspergilli* produce a great deal of acid (citric and oxalic) due to the fermentation of cane sugar. Currie (10) found that different strains of *A. niger* and a certain *Penicillium* will grow at as high acidity as pH = 1.8–1.4, this point of acidity being produced by the use of hydrochloric, oxalic or citric acids. Very few of the soil molds have been studied for their acid production, but those used by the previously-named investigators were isolated by the writer from the soil. If not all

molds, then some of them at least are able to produce large quantities of acid from available carbohydrates. Currie found that a certain strain of *A. niger* can produce 10 to 12 per cent of citric and some oxalic acid in a 15 per cent cane sugar solution. If an assumption should be made that some of the soil molds are also as active in the production of acids from available carbohydrates, we might be able to account for at least some of the increasing acidity in soils and the necessity of lime to neutralize the acidity. It may be very possible that for a great deal of the soil acidity we should look not only to the production of mineral acids, due to the oxidation of minerals in the soil, or added fertilizers, but also to the organic acids, such as citric and oxalic, produced by soil molds as a result of the fermentation of the available carbohydrates. Hagem (20) found that several *Absidia* isolated from the soil produced a large quantity of oxalic acid, which, he thought, was probably due to the incomplete oxidation of glucose; the *Mucors* produced no oxalic acid, but another unidentified acid. These acids produced in the soil may have still another function: acting upon the insoluble phosphates and other minerals in the soil. They may thus bring about their transformation into a soluble form available for higher plants.

Kopeloff (29) found that a maximum ammonification by soil fungi was obtained between the neutral point and an acidity equivalent to 2,000 pounds of CaO per acre (Veitch method). These results point to a possibility that where the soil reaction may be unfavorable for the activities of the bacteria concerned in the decomposition of the organic matter, the molds might prove an important compensating factor in maintaining fertility. Hall and associates (21) stated that molds are the active agents in producing the acidity of the soil manured with ammonium salts. When ammonium chloride and sulfate are added to the soil, the basic ions are used up by molds, while the acid ions are left in the soil and are subsequently converted into hydrochloric or sulfuric acids.

THE EFFECT OF MOLDS UPON THE MINERAL TRANSFORMATIONS IN THE SOIL

The recent investigations on the mineral nutrition of fungi have been reviewed by Dox (15). The mineral nutrition of lower fungi and the other action of these organisms, direct and indirect, on the minerals of the soil, are still awaiting investigation. It is sufficient to mention that we know very little about the production of available phosphorus, oxidation of sulfur and iron in the soil and the production of available potassium from insoluble silicates, and particularly on the part played by molds in this respect. Results secured in this laboratory on the oxidation of sulfur by certain species of *Fusaria*, and the results of Brown and Corson (5) that *A. niger* is very active in the oxidation of iron in the soil, point to the fact that the molds probably are an important factor in these transformations.

RELATION OF SOIL FUNGI TO PLANT DISEASES

It is known to plant pathologists that a soil may become sick with respect to a particular crop, due to the fact that yearly continuation of one crop on the same soil has introduced the organisms pathogenic to the particular crop into the soil, which therefore became a carrier for these disease-producing organisms. But parasitic fungi have been isolated also from virgin soils, or from soils on which the particular crop has never been grown before. Pratt (41) recently isolated fungi known to be parasitic on the Irish potato, from Idaho soils never cropped with potatoes and from virgin desert lands. We can also cite the work of Bolley (2) on the fungi parasitic to wheat isolated from the soil. More work is also needed on this subject, before we could definitely conclude how far the soil should be considered as a possible medium for facultative parasitic fungi.

SUMMARY

1. Molds have been isolated in large numbers from different cultivated and uncultivated soils, and the identity of many genera and species isolated from widely different localities has been established. The cultivated soils contain by far a smaller number of molds than they do bacteria and actinomycetes.

2. Molds live and produce mycelium in the soil, and therefore take an active part in the transformation of some of the organic and inorganic substances, which are important factors in the fertility of the soil. The plate count of molds in the soil cannot be taken as an indication of the actual numbers of molds living in the soil.

3. The molds present in the soil, at least most of them, do not fix any atmospheric nitrogen, and even where fixation was shown to be positive, the quantities are so small as to be negligible in the study of soil fertility problems.

4. Molds do not seem to play any part in the process of nitrification.

5. The molds play an important rôle in the decomposition of organic matter with the subsequent liberation of ammonia. The amount of ammonia produced depends not only on the source of nitrogen, but also on the carbohydrates available.

6. The molds take an active part in the decomposition of the simple and complex carbohydrates in the soil, with the production of carbon dioxide; this brings about a mineralization of the organic matter which is thus made available for higher plants.

7. The molds utilize very readily the nitrogen compounds usually added to the soil in the form of different fertilizers and convert them into complex body proteins, thus competing with the green plants and exerting an injurious effect upon soil fertility. This may be somewhat counterbalanced by the fact that some of the soluble nitrogen compounds are thus saved from loss by drainage from the soil and that the fungus body undergoes autolysis thus liberating in a soluble form most of the nitrogen that it has assimilated.

8. The molds isolated from the soil produce a number of enzymes which may help to bring about decomposition processes which are important to the upkeep of the fertility of the soil.

9. The production of acids by some molds in the soil may account for some of the soil acidity and may help to dissolve the insoluble phosphates and other minerals necessary for the growth of the green plants.

10. A number of organisms parasitic to green plants have been isolated from soils, upon which these plants have often never been grown before.

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