A PRELIMINARY SURVEY OF THE PROTOZOAN FAUNA OF MANITOBA SOILS, WITH A HISTORICAL REVIEW OF THE SUBJECT.

by

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ABSTRACT

The results are given of an examination of 128 different soils from 30 different localities of cultivated and virgin land of Manitoba, made during the months of October, 1933 to June, 1934.

The results are based upon the microscopic observations of the 128 soils and of cultures of them, during a period of two months each; also upon chemical tests of these soils.

An attempt has been made, firstly to determine which species constitute the protozoan fauna of Manitoba soils, and the extent to which variations in soil type and soil texture cause changes in this fauna; secondly, to detect the influence of such factors as hydrogen ion concentration, soil depth and variations in amount of precipitation upon the kind and abundance of protozoa; thirdly to determine the effects of various natural and artificial fertilizers applied to soils on Manitoba Agricultural College farm plots.

Thirty species of protozoa were identified. None of the Actinopoda, Testaceous Rhizopoda, Cryptonionadinae nor Hetero- and Oligo-tricha were found. In every soil tested the hydrogen ion concentration decreased with increase in depth. Also the protozoan population decreased with increase in depth and was absent at 36 inches.

The technique is described under Methods and Discussion.

TABLE OF CONTENTS.

HISTORICAL REVIEW.

SOIL PROTOZOA OF MANITOBA.

HISTORICAL REVIEW
OF
PROTOZOOLOGY.

(Copy Typed by Miss E. Smith.)

INTRODUCTORY NOTE AND REVIEW OF HISTORICAL CONTENT.

Investigators of that branch of science which deals with the microscopic life of the soil have, during the last twenty years, learned to recognize the fact that micro-organisms other than bacteria are of great importance in soil processes. Indeed they are of such importance that they form entirely separate fields for biological studies.

The beginning of this realization was marked by the first researches of Russell and Hutchinson (1) at Rothamsted in 1909 on the effect of partial sterilization of soil. They gained evidence strongly indicating the presence of a factor in their soils, which was inimical to bacteria, and this was more of a biological nature than chemical or physical. The presence or absence of this limiting factor coincided with with the presence or absence of protozoa. It was from this that the protozoa theory of partial sterilization developed.

Other investigators were quick to follow this line of research. Cunningham and Lohnis (2) 1914 gave the theory further support. Meanwhile workers at Rothamsted accumulated more and more data,--confirming the correctness of the pioneer work.

Persuasive proof that protozoa are present in normal soil, not only in the encysted state, was produced by Martin and Lewin (3) 1915 when they discovered a method for demonstrating the presence of active forms; while Cutler (4) 1920 divised a method for estimating the numbers of active and inactive (encysted) forms. Such a mass of data had been

brought forward from Rothamsted and other places, that the protozoa theory furnished, for some types of soil at least, the most probable explanation of the known facts.

Some comparatively recent observations of Cutler, Crump and Sandon (5) 1922 indicate a balance between certain groups of protozoa and bacteria such that inverse rhythmic variations in the respective numbers are determinable by both seasonal and (expressly) daily observations. The exact role of protozoa in soils is yet obscure. A. G. Lochhead of the Ottawa Experimental Station has found the protozoa of Canadian soils to occur and behave decidedly different from those of American soils in U. S. A. Waksman and Starkey (6) 1923 suspect the behaviour of fungi and actinomycetes, and the changes in nature of the bacterial food supply, following partial sterilization, to limit bacterial growth, commonly credited to the presence of protozoa.

During the last decade much progress has been made in systematic study of both aquatic and terrestrial forms of protozoa. Calkins (7) 1926 worked on the biology of the protozoa, while Schaeffer (8) 1926 classified some two-score ameba. A second advanced work was put forth by Doffein (9) 1927-29 and another by Kudo (10) 1931. Then, to facilitate research, some investigators, like Waksman (11) 1932 selected and assembled useful and practical essentials of earlier writings and data. With all this information at hand, the expert of to-day is enabled to progress in this field of research from an advanced vantage-ground of facts.

SOIL STERILIZATION.

Effects of Heat, etc. on Soil as the Earliest Suggestion of the Protozoa Theory of Fertility.

Frank (1888) found that heating of soil caused an increase of soluble matter in soil. Heated moor-soils contained more than twice as much as ordinary soil, and sandy soils not quite twice as much. This was considered in accounting for increased productiveness.

Schmoeger found that from soils heated @ 140°--160° C in an autoclave for several hours, he could extract with 12% HCl almost as much phosphate as from ash of soil; and much more than could be obtained in the same way from unheated soil.

Liebscher concluded that steaming also caused one portion of nitrogen to escape and further made another portion of nitrogen more available for plant growth.

Pfeiffer and Franke steamed soil 3 hours and on it produced a crop of greater dry weight and higher nitrogen content than on unheated soil, even when sterile soil was inoculated with some fresh soil; showing increase in assimilable nitrogen.

Russell and Hutchinson made partial sterilization by (1) heating to 98° C or (2) by adding 4% toluene which at the end of three days was allowed to evaporate by spread-ind. In these soils they found increased ammonia; due to micro-organisms, they believed, since the production curve was typical of bacteria (not of a chemical reaction). Also since in soils heated to 125° C (lethal to all microorganisms)

there is a sudden large production of ammonia and then none. If toluene be left in the soil there is only slow production of ammonia due to enzymes. A rapid period sets in only when soil is sufficiently moist.

Thus Partial Sterilization causes:--

(1) Increased ammonia.
(2) Cessation of Nitrifying process.

Why? because the inertness of bacteria in untreated soils is due to the fact that "The untreated soil contains a factor not bacterial, limiting the development of bacteria, this factor being put out of action by heating or by toluene." For example, if a filtered soil extract (bacterial) of untreated soil is added to toluene soil, there is increase in the rate of ammonia production and in number of bacteria. Again, if untreated soil is added to toluene soil there is no increase, but a reduction.

(a) The factor is not toxin, for it does not affect nitrification bacteria: barley seedlings grown in aqueous extracts of untreated and toluened soils, showed no difference in growth over a period of four weeks.

(b) The factor is probably biological for when untreated soil is added to toluened soil the reduction in the rate of ammonia production is not at once operative.

(c) It is also a large organism since it was not found in filtrate extracts.

(d) Examination of treated and untreated soils showed bacteria in the first, and bacteria and protozoa etc.,in the second.

(e) Direct evidence was furnished by inoculating toluened soil or extract with cultures of large organisms; the result was depression in the rate of ammonia formation. (Russell and Hutchinson (1)). Hence--

THE PROTOZOA THEORY OF SOIL FERTILITY.

"The micro-organic flora of the ordinary arable soil includes a wide variety of organisms performing very different functions, which may be divided roughly into two classes:-"

(1) Saprophytes tending to increase the fertility of the soil, e.g.,producing ammonia, fixing nitrogen, etc.

(2) Phagocytes and large organisms, inimical, or unfavorable to bacteria, which limit fertility.

" Between these two classes of organisms there is an equilibrium under natural conditions, but when partial sterilization takes place the phagocytes are killed but bacterial spores are not; and subsequently the latter develop with great rapidity, since they are freed from the attack of their enemies, and there is an increase not only in ammonia but likewise in crop production."

Researches: Some confirming and others opposing the Protozoa Theory of Soil Fertility.

SEWAGE-SICK SOILS:--

Russell, Golding and Petherbridge (12) and (13) examining Sewage-Sick Soils, found two distinct causes at work:-

(a) Physical causes that lead to retarded percolation.

(b) A factor detrimental to bacteria, which is an abnormal development of the factor in every respect similar to that shown by Russell and Hutchinson to exist in ordinary soils to a lesser extent because of less moitsure and less organic matter.

This "Soil-Sickness" occurs commonly in glass-house soils. It is remedied best by heating soil to above 140° F but not exceeding 212° F. The organisms harmful to bacteria multiply more slowly under normal soil conditions, and possess a lower power of resistance to heat and anticeptics. Because of these organisms the number of bacteria in the soil at any time depends as much on the difference in activity between bacteria and detrimental organisms, as (it depends) on temperature, moisture or any other condition.

Regarding this "Soil-Sickness" or "Fatigue", a phenomenon which Russell and Hutchinson explain as partly due to the presence of protozoa, Loew and Fawcett found that with some sick soils, where conditions are the worst, protozoa are completely absent. Samples of these soils were disinfected with heavy application of CS_2, but from the results

of the study it appears that any benefit derived from the disinfection of the soils, cannot be attributed to the distruction of protozoa.

Lyon and Bizzell found that steaming soil, reduces the nitrates to nitrites and ammonia, but that most of the ammonia was formed from organic nitrogen. After standing uncropped the steamed soil steadily decreased in its content of soluble matter and both ammonification and nitrification were stopped. Recovery varies with productiveness; recovery from the injurious substances produced by steaming is hastened by the growth of plants (wheat). An infusion of unsterilized soil added to steamed soil increased the germination of the seeds and early growth, but retarded later growth. It further hastened decrease of total water soluble matter yet did not increase ammonification or nitrification. Hence we conclude that toxic matter controls the productivity in steamed soils and that the conditions of the organic matter before steaming influences toxicity. That the soil was unable to rid itself of toxic material formed by steaming, indicates that a similar condition causes sterility in the field.

PROTOZOA AND SOIL-FERTILITY:-

It is striking that with few exceptions most investigators fail to make any direct examination of soil protozoa in their attempts to affirm or deny the validity of Russell and Hutchinson's Protozoa Theory of Fertility. Most conclusions were based on indirect results obtained by sterilization of soils, extracts, etc. The evidence thus obtained

goes to explain the important role that bacteria play, rather than that of protoza.

Russell and Hutchinson's conclusion that the absence of protozoa (by treatment with Toluene) was responsible for increased production of ammonia, was tested by Fred (14) who used ether instead of toluene. He subjected a series of soils to 100° C moist heat for several hours, and another series unheated as a check. All soils then received 2% ammonium sulphate, some flasks received 2% and 5% ether, and so as to obtain vigorous nitrifaction 170 cc. of amoeba-free extracts was inoculated into all the flasks, (prepared by leaching 2 Kg. of compost soil plus 4 litres sterile water, and filtering through filter paper. Microscopic examination revealing no amoeba). Analysis for nitrate nitrogen were made at the beginning and end of experiment. Russell showed that heating soil to remove amoebae was not beneficial to nitrate formation,(contrary to Russell and Hutchinson's conclusion)--yet addition of small amount of ether increased nitrification in flasks containing amoeba, and decreased this process in flasks (soils) containing no amoeba. This, Fred believed was due to stimulating effect upon nitrifying bacteria since heated soil, not treated with ether showed no such increase.

Fred did not consider in making his protozoa free extracts that the cysts of protozoa do pass through several thickness of high grade filter paper.

Other investigators who, in testing the validity of Russell and Hutchinson's conclusions, failed to do so were

Laidlow and Price. In their time attention was paid to their conclusions that : (a) Increased fertility of partially sterilized soils was due to the new bacterial flora being a more active decomposing agent than the original one causing an increase in ammonia. A more correct conclusion of theirs was,(b) Protozoa that were killed off by treatment served as food for the new bacterial flora, as well as food for the plant in the form of ammonia; and as some of these large organisms devoured bacteria their destruction allowed rapid development of the new bacterial growth to take place.

Less supporting of the Protozoa Theory of Fertilization, than Laidlow and Price's were the conclusions of Greig-Smith (15). From experiments he concluded that protozoa had little or no action in limiting the number of soil bacteria. He found however, that the larger ciliates such as Colpoda cucullus, were not destroyed when comparatively large amounts of volatile disinfectant were added to the soil. Upon adding suspensions of protozoa there was no evidence of any limitations in the numbers of bacteria. Any enhanced effect was due to the addition of bacteria contained in the suspension. Any phagocytic tendencies that soil protozoa possessed, G.-Smith found to have no influence in limiting the number of bacteria in the soil.

Lipman, Blair, Owen and McLean (16) used soil sterilized at 1.5 atmospheres in the autoclave and found that pasteurization does not increase the ammonifying power of the soil infusion, but sterilization decreases the ammonifying

power of the soil.

Many another indirect evidence for or against the Protozoa Theory of Soil Fertility could be cited.. For instance Jamieson (17) found that increased productiveness following heating of soil or treatment with substances inimical to plant life is "due simply to the riddance of various forms of animal life in soil that prey on plants." and not on any influence of bacterial activity in the soil. Yet all these conclusions did not constitute proof of the validity of the theory. Even at the time that the earlier experiments were made Moore (18) in his "Micro-organisms of the Soil" doubts the various results. He stated that "he could show, as the results of tests over a wide field, that the number of protozoa, including flagellates, ciliates, and rhizopods existing in the soil three days after treatment with various per cents of toluol, carbon bisulphide, etc., may equal or even exceed the number originally present."

"What seemed well established was that the temporary removal (from the soil) of the protozoa, had little bearing on the problem -- Sterilization."

At this time, the strongest support to the Protozoa theory of Fertilization was given by Cunningham and Lohuis (2) 1914 by their studies on soil protozoa, and by the workers at Rothamsted,,Harpenden, England. However, it was realized that the subject of research required a more direct method of investigation when Martin and Lewin (3) 1915 demonstgated the presence of trophic, as well as encysted protozoa in normal soils.

DIRECT STUDY OF THE INDIVIDUAL SOIL ORGANISMS AND THEIR RELATIONSHIPS TOWARD EACH OTHER.

The above pages on Soil Sterilization contain a brief review of the enormous amount of work that has been done on this subject. Kopeloff, Lint and Coleman (19) have compiled a great deal of detailed information on Soil Sterilization. With their publication of 1916, it seems that the ardor for the study of soil processes along the lines of soil sterilization subsided and assumed the nature of curiosity in the fields of soil Bacteriology and Soil Protozoology. Even Kopeloff, Lint and Coleman noticed this, for they remarked that "Soil Protozoology was then in its infancy as a science".

Direct study of individual groups of Protozoa lead to the discovery that certain of the ciliates and amoebae actually injested soil bacteria, used them as food exclusively. Cunningham (20), Purdy and Butterfield (21) 1918, Cutler and Crump (22) 1920, and by making counts, (at daily intervals) of amoeba and bacteria it has been found that an inverse rhythmical relationship, as to relative abundance, exists between these two groups of organisms. For example, Cutler, in 1920, prepared pure cultures of an amoeba, Dimastiganoeba gruberei, and a flagellate, Cercomonas crassicauda. Of each of these he applied an exact number, (about 20,000) to one gram of steril soil each in separate Petri dishes. He then added 11 to 13 millions of bacteria to each. After 15 days he found 9 times as many

amoeba, and 11 times as many flagellates. The bacteria in the dish containing amoeba had first reached a maximum of 178 millions on the third day and then a minimum of 72 millions on the fifteenth day, (59 % decrease. The bacteria with the flagellates increased first to 103 millions on the seventh day, then dropped to 88 millions in 15 days, (14.5% decrease), whereas a pure culture of only bacteria rose from 10 to 214 millions in 6 days, then dropped to 169 millions in 15 days. Thus in case a mutual and symbiotic relativity.

Goodey has pointed out that amoeba lirnax can lead an active existence in the soil and exert a depressing effect upon bacterial numbers. He believes that first a certain high number of about 30,000 per gram soil must be reached by amoeba before depression of bacteria sets in.

Not all protozoa need to have bacteria. Some live in the absence of bacteria (11) and play an important role in the decomposition of plant constituents of the soil with production of ammonia. (23) Some of the most commppolitan protozoa,viz. Colpoda sp.(?), are believed to be such. This was observed very early, and Goodey, Russell and Hutchinson, Rhumbler and many others have made exhaustive study of the composition of the ecto and endocysts of this organism, as well as detailed study of its physiological behaviour. It seems to be one of the hardiest and most generally occurring protozoa that exists.

Many protozoa are credited with being saprophytic and capable of obtaining their food by absorption, and possibly soil flagellates have this same characteristic (according to Thornton and Alexeiev.)

For better information the author would suggest (and refer to) Waksman (11) and a number of other more original works than can be here repeated.

It is of great experimental value that Cutler (2) succeeded to devise a fairly accurate method for estimating the numbers of active and inactive protozoa and other organisms. The method is of such importance that it will be discussed under separate heading.

At Rothamsted, England it had already been found that protozoa occur in far greater numbers in the soil than was formerly believed. Hence Allison (24) applied Cutler's dilution method in making counts of protozoa of some American soils. He found only low numbers. In 1927 Sandon (25) repeated his work. He made a detailed examination of the organisms of various American soils, so as to determine the number and kind of protozoa present, and compare them with those of England. (Some of these counting results are given under "Numbers of Protozoa in Soil"). Through these researches (22), (2), (24), (25), etc., it became evident that a fluctuating equilibrium is maintained between bacteria and protozoa; and that, however cosmopolitan a number of protozoa and bacteria may be, the protozoa of countries

of different latitudes may differ considerably in number, kind and physiology. Lochhead (26), who made microbiological studies of soils of Canada at Ottawa, recorded a depression in protozoal numbers during freezing; different from Sandon's discoveries in America. Such dissimilarity, it is suggested, may be due to additional severity and length of the Canadian winter. Also Lochhead did not obtain any increase in the numbers of actinomycetes, and showed that their optimum temperature was higher than that of the majority of soil Bacteria.

These results corroborate Waksman and Starkey'S (4), who surmise that behaviour of fungi and actinomycetes and physical and chemical changes of the soil limit bacterial growth. This was the standing of the knowledge in Soil Protozoology in the year 1924.

It would be futile to attempt even only a brief survey of the progress made in the systematic study of protozoa, during the last 10 years. Let it suffice to say that, among the authors who had contributed to the advancement of this subject are Calkins (7), 1926, Schaeffer,(8), 1926, Doflein (9), 1927-1929, Kudo (10), 1931, and Waksman (11) 1932.

In regard to the Protozoa Theory of Soil Fertility it is stated at present that, although protozoa are capable of reducing the number of bacteria in soil, due to their phagocytic action, there is very little evidence

that their influence upon the activities of soil micro-organisms, and upon soil processes in general is injurious. The relationship seems to be of mutual benefit to bacteria, protozoa, and fungi, etc., and soil fertility. Bacteria may benefit from the ammonia liberated. Protozoa may and do feed on bacteria. The balanced condition in general appears to be a necessity to normal soil conditions and biological processes. (11)

NUMBERS OF PROTOZOA IN SOIL.

Sandon (25) examining soils from the New Jersey Experimental Station, counted protozoa by Cutler's dilution method, and bacteria etc., by the plate method recommended by Fred and Waksman (27). Sandon found wide differences to occur in the numbers of all the organisms at different times of the year in American Soils; just like Cutler and Crump found them varying at Rothamsted, England.

Sandon's results were as follows:--

TABLE I

ORGANISMS PER GRAM OF SOIL FROM SLIGHTLY DIFFERENTLY TREATED PLOTS OF NEW JERSEY EXPERIMENT STATION.

Month	pH	Bacteria: Million	Actinos: Million	Flagellates	Amoebas	Ciliates
Sept.	7.0	6.4	3.3	196:	222:	0:
Oct.	6.8	4.7	2.7	770:	938:	25:
Nov.	---	12.3	3.8	<115000:	<28770:	<28770:
Jan.	6.9	4.4	8.1	2600:	13600:	135:
March	5.2	13.5	8.4	220000:>	220000:	160:
April	---	36.5	10.4	4795:	81495:	263:

In November even the highest dilutions contained all three types of protozoa; they exceeded the numbers given.

Further, according to these data, protozoa more than double or even triple themselves with the advent of winter frost (see Oct. to Nov.), and bacteria rose from 4.7^{6} to 12.3^{6} while fungi increased from 17300 to 44800. (Data not given on Table). The counts for March were made of thawed soil, and show enormous increase for Flagellates

and Amoeba. Hence, it appears that severe frosts do not reduce the protozoal population, and subsequent thawing acts as a stimulus to multiplication.

Soil of widely differing environment and a distant part of U. S. A. was that from the Experimental Station at Logan, Utah. In this Sandon found protozoa, etc., as follows:--

TABLE II

ORGANISMS PER GRAM OF DIFFERENTLY TREATED SOILS FROM NEAR LOGAN, UTAH EXPERIMENTAL STATION.

Plot No.	Crop and Treatment of soil	pH	Bacteria Millions	Actimos: Million	Flagellates	Amoebae	Ciliates
1	Beets Irrigated Manured	7.7	--	1.7	14380	28770	114.
2	Stubble-fallow dry	7.5	18.9	5.5	450	16000	38
3	Alkaline Pasture	7.7	8.5	2.1	225	14380	56.
4	Alkaline Pasture	8.9	10.4	6.0	225	1798	28
5	Alkaline plowed pasture	8.9	12.8	1.0	57	900	7
6	Alkaline potash soil, plus: Cl. Salt	8.0	7.1	0.9	16	3000	3.4
7	Alkaline pot soil, plus: So_4 salt	8.8	2.5	.0	12.5	225	3.4

Soil No. 1 receiving annually 30 tons manure per acre contained considerably more protozoa than the unmanured soils. Yet heavier application of manure (40 tons annually) seemed to have a lowering effect on the number of protozoa.

Dry farm soil No. 2 was rich in amoebae, and poor in flagellates and ciliates.

In most alkaline soils 4,5,6,and 7 th total number of protozoa was extremely reduced, despite the fact that the nitrogen content in most of these soils was very high, suggesting much organic matter.

Table I illustrates seasonal variation of numbers of organisms, and Table II shows numerical difference due to difference in chemical nature of food supply.

Comparing these results from American soils with those of English soils it was found that,with the exception of counts made just after March thaw, all figures were considerably lower than those found in corresponding plots at Rothamsted, Harpenden, England, by Cutler's method.

TABLE III.

ORGANISMS PER GRAM OF ARABLE SOIL FROM EXPERIMENTAL STATION, HARPENDEN, ENGLAND. (See (28))

	Bacteria	Algae	Flagellates	Amoebae	Ciliates
High Activity	5000^6	100000	770,000	280,000	1000
Low Activity	1000^6	---	350,000	150,000	100

SOIL PROTOZOOLOGY.

Having received the impetus of Russell and Hutchinson's work to awaken it to active life, this new branch of soil biology may rightly be regarded as an outgrowth of the investigations on partial sterilization and soil fertility.

Protozoa have long held an important place in human and animal pathogenesis. The morphology and physiology of the forms concerned have been studied very closely. There are, however, a few other fields, as we have seen, where protozoa play a part, viz. in water and in soil.

Protozoa are unicellular organisms containing chromatin or nuclear substance readily visible from the protoplasmic body, which is either naked at the surface or enclosed by a cell membrane. Organs for locomotion, capture and assimilation of food are usually present although they may be absent entirely. Reproduction is effected by some form of fission to form new individuals, or to form germ cells (gametes). Fission and germ cell production envolves the whole organism, and occurs in the vast majority of protozoa. A process of conjugation or syngamy occurs at some period in the life cycle, the essential feature of the process being fusion of nuclear matter from distinct individuals. The protoplasm of the body is never differentiated into tissues, nor does it exhibit cellular structure. (Exceptional are forms like Rotifers, some of which are grouped with protozoa for the sake of convenience). Under

adverse conditions protozoa are capable of encysting, a state analogous to spore formation of bacteria. The ectocyst may be of calcarious or even siliceous character. Among nuclei we may distinguish a macronucleus concerned with vegetative functions and a sexual division, and micronuclei for sexual production. The number of micronuclei present in an individual is of value in classifying protozoa. The size of protozoa may be from a few microns to 4 centimeters. Now these are the characters and structures of all protozoa in general.

Soil Protozoa are all of a small and hardy type, able to withstand the adverse conditions of their environment. The great majority (with only few exceptions) are microscopic, most of the common ones are not greater than 100 microns and the majority being less than 50u. Their contractile vacuoles and food vacuoles are conspicuous in the ciliates.

This small size of soil protozoa in general suggests that the reducing effect by chemical substances described by Doflein (9) may be a continuous and perpetual factor in the environment of soil protozoa. For example, Doflein states that "the motility of protozoa is affected by chemical substances in solution, salts, alkalis and acids, (all of which are often quite concentrated in soils), cause a decrease in the surface of the pseudopodia, and increase the motility of the ciliates."

For general and detailed study one may turn to the

treatises on protozoa, by Butschli (29), also to (9), (8), (10), and Minchin (30), also to Conn, H.W. Edmondson, C.H. (35) Kofoid (31), Mast, S.O, and a number of others.

From the following two paragraphs it will be seen that it was imperative that certain standard methods of soil protozoology were followed.

Ehernberg and Dujardin (1841) thought of the soil protozoa as being active, but in 1878 Stein discovered the cyst forms and due to this the idea grew that they occurred in soil only accidentally as cysts. Investigations were organized at Rothamsted to ascertain whether there was an active protozoan fauna in the soil. Goodey indicated in 1911 that ciliates occur in the soil only as cysts. Then in 1914-15 Martin and Lewin, by noteworthy methods of separation showed that a trophic population of amoebae and flagellates exists in soils, and that it differs from the population developing in hay infusions. Cutler, Crump and Sandon also found an active or trophic population in the soil, 1920.

Various methods for the detection and differentiation of trophic and cystic forms were used by Fred and Waksman, Martin and Lewin, Whiting and others, and Fellers and Allison devised a kind of standard by which to make a rapid direct examination of soil for trophic forms. These methods will be described later.

METHODS IN SOIL PROTOZOOLOGY:

E.J. Russell states that at Rothamsted the methods developed for studying protozoa are:--

(1) The organisms in the soil are counted by a dilution method, each soil sample being tested,(a) in its original fresh state, (b) after treatment with dilute acid to kill trophic forms and leave only cysts.

(2) Cultures are set up for the isolation and study of different organisms.

COUNTING METHODS FOR PROTOZOA.

Some of the earlier and more crude methods, which most likely developed into Cutler's dilution method, were as follows:--

(a) Direct counting of protozoa in one drop by means of a microscope.

(b) Counting accurately by means of a Blutkor-perzaehl apparat.

(c) Khan's dilution: one cc. of solution was placed in sterile media and diluted 1:10, 1:100, 1:1000, 1:10,000, and thus determining the dilution above which the protozoa are not found by examination at periodic intervals, and below which they do not exist.

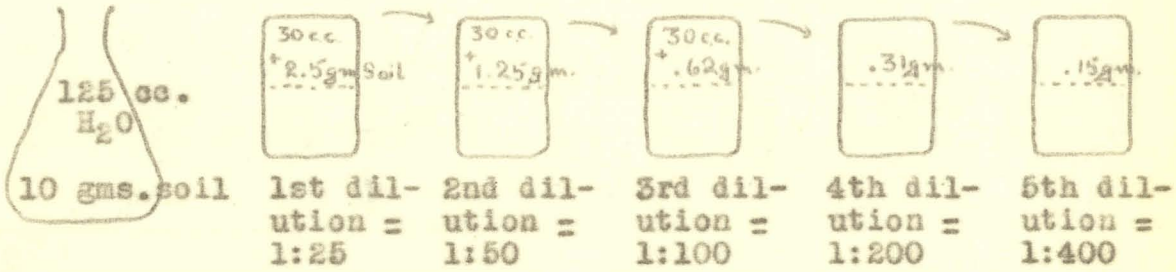
(d) Agar-plating methods used by Killer and others. The difficulty here is to differentiate the protozoa from bacteria and organic matter.

(e) By counting per standard loop, Muller's method.

(f) Cutler's dilution method, which is described in detail, -following.

CUTLER'S DILUTION METHOD APPLIED TO THE COUNTING OF PROTOZOA.

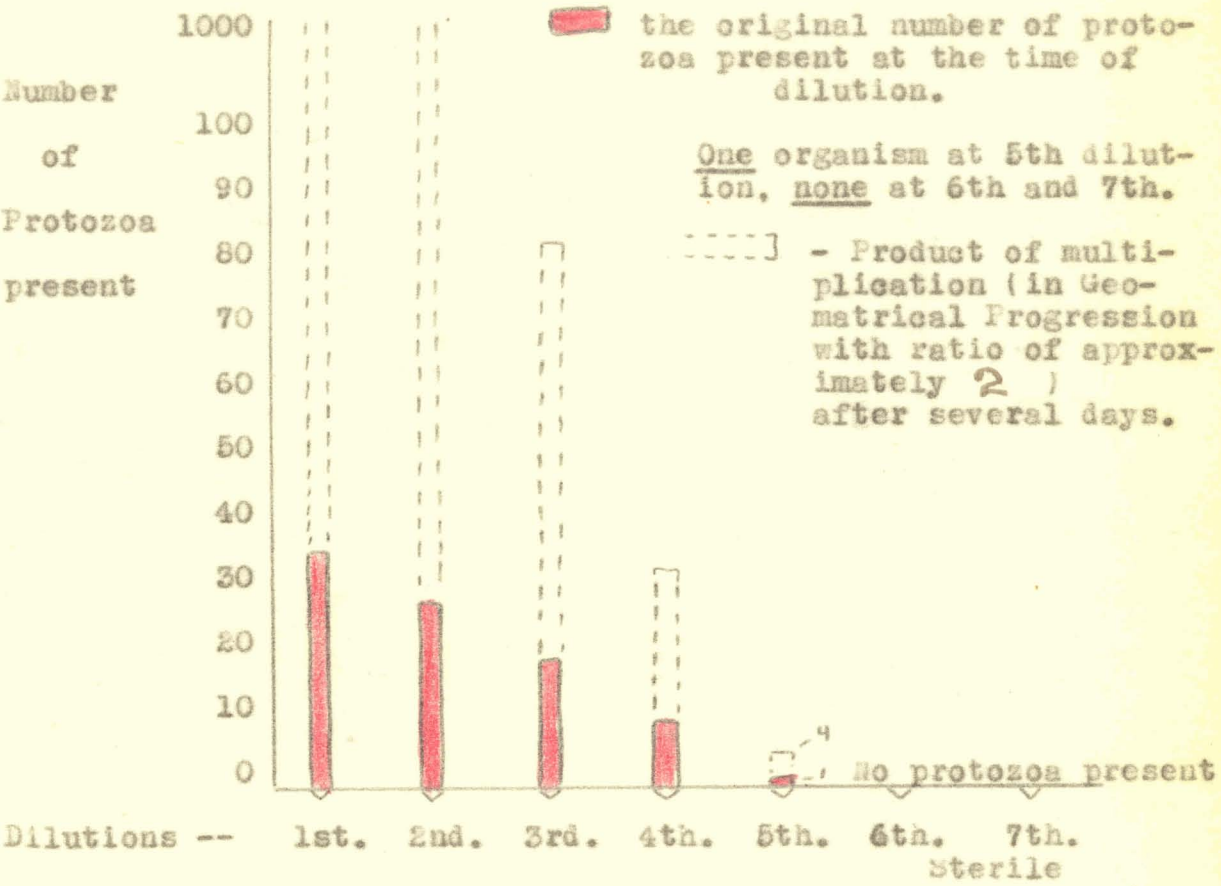
Procedure as follows:-- 10 grams of soil are suspended in 125 cc. of sterile water; this is shaken vigorously for three minutes; 30 cc. of this suspension is then transfered to 30 cc. of sterile water (= first dilution 1:25). This is shaken vigorously and again 30 cc. are transfered to 30 cc. sterile water,(which makes the second dilution 1:50), etc.



Thus the 15th dilution will equal 1:409,600 . The presence of one organism in the 15th dilution indicates 10,000 organisms per gram of soil. Small conical flasks of 125 cc. capacity, are most suitable for this work.

It is very important that the dilutions are all made at once, i.e.,one after the other as quickly as possible, say within an hour. This is necessary because many protozoa are capable of undergoing fission in a few minutes when their environment is suddenly changed. The author has observed Oxytricha pelionella to undergo fission within five minutes due only to the addition of cold water. Many protozoa start multiplication very soon after they are taken into liquid media.

After the dilutions are made to the desired extent, say the 15th dilution, rapid multiplication rather facilitates than retards operations. This can be illustrated graphically thus:--



It is obvious that thus it is easier to detect at which dilution there has been at least one organism present originally, and at which there is none, if the dilutions are left to stand a few days; since time would confirm the sterility as regards protozoa in the sixth dilution (graph). Also in the next step of the procedure, where 1 cc. of suspension medium out of the 60 cc. is removed to the petri dish, the chances are vastly greater that one organism will be with-

drawn into the pipette.

From each of the last (13 say) dilutions of a series prepared as above, one cc. of suspension is put into each of two petri dishes containing sterile nutrient agar. 2-3 cc. sterile water is added and the plates incubated. Thus 26 plates (petri dishes) would be involved. These are then examined microscopically and the number of sterile plates noted.(See Fisher's table of calculations..From such tables the number of protozoa per gram soil can be read directly by noting the number of sterile plates. (See next page.)

Knowing the number of sterile plates in a standard series of dilutions, say 50:204,800, 2nd column, enables us to read the number of protozoa; for example nine sterile plates would indicate approximately 7400 protozoa per gram of original sample. The number of organisms per gram of moist soil is then converted to its equivalent value for dry material according to the moisture content of the original sample.

FISCHER'S TABLE OF CALCULATIONS.

The Number of Negative or Sterile Plates Indicating Number of Protozoa per Gram of Soil.

Series 1, (Dilution 25:102400		Series 2, (Dilution 50:204800		Series 3, (Dilution 50:409600	
Number of sterile plates:	Protozoa per gram	Number of sterile plates	Protozoa per gram	Number of sterile plates	Protozoa per gram
1	110,000	1	220,000	1	420,000
3	36,000	3	72,000	3	140,000
5	16,000	5	32,000	5	64,000
7	7,600	7	15,200	7	30,000
9	3,700	9	7,400	9	15,000
11	1,800	11	3.600	11	7,300
15	450	15	900	15	1,800
17	230	17	460	19	450
19	110	19		21	220
21	56	21		23	
23		23		25	
25	6.8	25	13.6	27	14

Fischer's complete table however contains all the odd and the even numbers of plates and their corresponding numbers for protozoa.

POSSIBILITY OF ERRORS WHEN USING CUTLER'S AND FISCHER'S COUNTING METHODS.

(1) The error becomes greater towards either end of the series; and commonly a 2-plate difference is ignored being often insignificant. E.g.,9 negative plates (2nd column) equal 7400 organisms per gram; not significantly different from 7--11 negative results.

(2) The method of removal of the 1 cc. culture (for the agar plate) may be faulty. Taking 1 cc. out of 30 cc.

the worker stands one chance in thirty that the pipette will draw the one organism present in the 30 ccs. of the critical dilution, (say the 8th.)

(3) Failure to agitate the mixture sufficiently thoroughly to make a homogeneous suspension, in each instance, may lead to great irregularity in the numbers of protozoa occurring.

DETECTION AND DIFFERENTIATION OF ACTIVE FROM CYSTIC FORMS.

Fellers and Allison divised a simple standard method by which to make a rapid direct examination of soil for trophic protozoa;. They dampened the soil with water on a slide, examined this for five minutes microscopically, then, if no living cells were detected in that time, the sample was called negative. Of over 35 samples of soil collected only two samples were positive, i.e., contained trophic or actively feeding forms.

Allison employed another method whereby cystic forms were counted directly, and the active forms thus determined by difference between total and cystic numbers. Cystic forms were cultured and counted by the above dilution method after first treating the soil sample with 2% hydrochloric acid, (sp.gr. 1.15) over night. With soils high in lime it is necessary to determine, first, the carbonate present so that the acid may be added in such excess that the final strength will be sufficient to accomplish the desired effects. This treatment was found effect-

ive in destroying all active forms without injuring the cysts.

Fred and Waksman employed the above method in a much simplified way.

Lewin and Martin developed a direct method of detecting protozoa in the soil. They stirred soil into a solution of picric acid and picked up the organisms floating on the surface. The method is tedious and was later improved by Koffman (32 ?), who vigorously stirred soil and water together in a thin paste. The supernatant liquid was then examined microscopically.

Whiting (33) placed soil in an evaporating dish, and added through a funnel with neck-opening at the bottom of the soil mass, enough picric acid or corrosive sublimate to completely cover the soil. After thorough shaking, a cover slip,marked to indicate film side, was placed (floated) on the surface to obtain the film. This is an efficient method for fixing and mounting.

These elaborations are necessary since (as Cutler showed in 1920) the organisms rigidly adhere to the soil particles from which they can be dislodged only with difficulty. Fixation by acids, dilution with water and vigorous shaking removes the protozoa which are then buoyed to the surface of the liquid, sometimes only after centrifuging.

CULTURE MEDIA FOR PROTOZOA.

Unlike higher organisms of the soil such as the Helminthes, etc, it is impossible to make exhaustive study of soil protozoa in a mounted, preserved or other dead condition. In order to have protozoa available for observation and experimentation in the living condition, it is necessary for certain investigations of soil protozoa, to remove them from their natural environment and culture them in suitable media, liquid or solid. Fred and Waksman (27) have recommended media 1 to 4.

Medium (1) Nutrient or Beef Extract Agar.

Agar	15.0	grams.
Beef Extract	3.0	"
Peptone	10.0	"
Sodium Chloride	5.0	"
Dist. Water	1000.0	cc.
Hydrogen ion conc. pH.....	7.0	

In the preparation of this medium the author used Oxo cubes,(in place of commercial Beef Extract), with good results. After sterilization for two hours, the pH was adjusted by means of calculated quantities of HCl and NH_4OH and a Quinhydrone electrode apparatus. pH is more generally adjusted by a colorimetric method. The agar medium was then poured while hot into 20 cc. test tubes so as to make slants. The soil water suspension was then added to the cool medium. This allows free movement of active organisms in the water and a large feeding surface on the agar slant.

Although this is an unusual manner of using the medium, it was found to serve extraordinarily for ciliates, and not so well for amoeba and flagellates. The main objection to this medium is that, due to the organic nature of the beef extract and the proteolytic enzyme of the peptone, decomposition sets in usually after a couple of weeks.

MEDIUM (2). HAY INFUSION.

Meadow hay (finely chopped)..... 50.0 grams.

Tap water..... 1000.0 cc.

Boiling for 2 hours.

Standing and cooling over night.

Filtering.

Investigators of 1900-1912 use a one per cent hay infusion quite widely. Kopeloff and Coleman found 10% very desirable, whereas my own cultures yielded best results with a 40% solution. One per cent was found to be decidedly too weak. This medium is very readily infested with hay bacilus, perhaps from adhering spores which are hard to kill. Goodey (34) made the infusion slightly alkaline to litmus and added egg albumen and 0.75% NaCl. Zaobitzer, Fine and Woodruff, Russell and Hutchinson, all employed this medium.

MEDIUM (3) HAY AGAR NUTRIENT.

Hay..... 40 grams. Water..... 1000 cc.

This is boiled for 30 minutes and is then filtered.

15 grams agar and a little meat extract are then added and the liquid left to solidify.

MEDIUM (4). MANNITOL SOIL EXTRACT.

Stock Soil Extract..... 100.0 cc.

Mannitol..... 10.0 grams.

Tap water..... 900.0 cc.

Francois Perey, J. (1925) obtained good results and high counts in France, using Soil Extract.

MEDIUM (5). HORSE DUNG EXTRACT.

3 lumps of horse dung.

500 cc. of water are boiled for 1½ hours, filtered through very fine cloth.

The author found this medium most satisfactory of all; the development of protozoa was enormous, and it does not develop mould at the surface as easily as hay infusion. Martin and Lewin added 6 grams agar to the above and a little water to the culture plates, to get strong growth. S. Waksman (11) recommends a more elaborate preparation of this kind.

MEDIUM (6). SALT SOLUTION. (NaCl).

This has been found satisfactory for the preservation of protozoa during a few days or a week. Its merit lies in the fact that it is clear, not readily contaminated and free of obnoxious odors. Doflein (9) states that distilled water kills protozoa, whereas salt increases the motility of ciliates.

The above media are used for all classes and types of protozoa together. More elaborate media, prepared with the intention of meeting precisely the needs of particular

individual genera of organisms were employed by some investigators. e.g.:--

NUTRIENT MEDIUM FOR EUGLENA OR FLAGELLATES.

Doflein (9) and Killer, according to Coleman and Kopeloff, found the following medium especially adapted for the growth of Euglena.

Peptone..... 0.5 grams.

Grape Sugar..... 0.5 "

Citric Acid..... 0.2 "

$Mg.SO_4.7H_2O$..... 0.02 "

K_2HPO_4..... 0.05 "

Water.....100.00 cc.

This is the "Zumstein" solution for Flagellates.

NUTRIENT MEDIUM FOR AMOEBAE.

Celli and Fioca in 1894 were the first to make pure cultures of amoeba from a 5% water solution of Fucus crispus with and without addition of bouillon on alkaline potato etc. Other workers on this were Miller, Behla, Schardinger, etc.

Turtox News, October 1933, published by the Biological Supply House, Chicago, contained a table of cultures and culturing conditions for protozoa, which are very simple and practical. With other information this table is as follows:--

PROTOZOA	Type of Container.	Type of Media.	(1) Optimum Temperature (2) Light (3) Life of Culture.
AMOEBA	Shallow (Finger-bowl) dish, etc.	(a) Boiled timothy hay (b) Hay of wheat (c) Pond weeds	Cool, 68°F = 20°C. Dull light 2-6 weeks
PARAMOECIUM	Battery Jar.	(a) Boiled wheat (b) Hay infusion (c) Pond weeds	Average 65-78°F Average 2-4 weeks.
EUGLENA	Battery Jar	(a) Boiled rice (b) Pond weeds	Average 65-80°F Bright (in sun light) Several months.
VARTICELLA	Battery Jar.	(a) Hay infusion (b) Pond weeds	Average 65-75°F Bright (not in direct (sun light) 2 days to 2 weeks.
STENTOR	Battery Jar or Aquarium Tank	(a) Pond weeds living in clear water	Average 65-75°F Bright. Several months, intermittantly
MIXED PROTOZOA	Battery Jar or Aquarium tank	(a) Hay infusion (b) Pond Debris	Average 65-80°F Average light Duration is many months.

Forms change,

Some disappear,

others arise.

DURATION OF LIFE OF THE CULTURE.

Some of the peptone and beef extract media are extremely particular as to conditions, if the quality of the nutrient is to be preserved. They decompose very readily. Again, hay infusion and dung extract seem to be much more stable in composition--in retention of their original composition. Turtox Live Material Laboratory reports one culture of Paramoecium aurelia which is over a year old and is still in good condition. This was originally a hay infusion, and was fed every few weeks with a few kernels of wheat and a few small pieces of timothy. Another hay infusion contained Euglena for six months and then gave way to rotifers.

Generally, in cultures containing soil and its organisms, either the life (protozoan sequence) or the nutrient quality of the culture seems to be exhausted after one to two and a half months, unless the medium is fed by the addition of fresh material. Fellers and Allison, however, found some forms of protozoa in soil extract medium after two years incubation.

SOIL PROTOZOA

of

MANITOBA.

1933 ------ 1934

METHODS.

In a preliminary survey of the protozoa of Manitoba soils samples were obtained from localities stated on Tables I to 7. Ninety-six samples of soil were cultured in duplicate and examined during 1½ to 2 months. Sixteen of these samples were repeated thrice; once in each of three seasons, i.e., samples were taken in fall (October, 1933) in mid-winter (Jan. 1934) and again in spring (May 1934), so as to note seasonal changes in fauna. Surface samples were taken at a depth of from one to six inches, and where possible cultures were made while samples were still moist. However, samples sent in from distant places were dry, being put up in paper bags. Deep samples were taken either by the pit or by the auger method (described separately several pages on) and different levels one inch to forty-two deep. Of this kind 80 samples were obtained from 16 different places. No sample was taken where there was standing water or a stream in close proximity, except when study was made of water bottom fauna. The degree of acidity or basicity of all these soils was compared by the Quinhydrone Electrometric method, (described subsequently).

Three gram portions of the soil sample under consideration were placed into 20 cc. test tubes containing 10 cc. of nutrient media. The Erlenmyer flasks commonly used for this purpose were found to be too large and bulky for such a large number of samples. The duplicate cultures were made one of hay infusion and the other of horse dung

or Beef extract-agar as nutrient material. The test tubes were plugged with cotton batting so as to maintain atmospheric pressure within, and good aeration of the media surface. These cultures were then incubated at room temperature (20o-21° C), in specially built incubator ovens with automatic thermostatic adjustment, and also quite successfully in an open room where the temperature varied considerable. Examinations were made every second day for the first three weeks, then bi-weekly for a period of 1½ months. After this period not a single new species of any class of protozoa was found. Hence it is feasible that all the protozoa existing in the soil as cysts or spores excyst within 30 days.

In preparing a soil culture Whiting recommends three minutes of thorough and vigorous shaking, but during incubation and examination of cultures care must be exercised <u>not</u> to agitate the soil to a suspension as this is liable to kill the trophic organisms in the upper liquid. Organisms may exhibit preference in the liquid levels, e.g., Vorticella microstomata was invariably found attached to the surface film or scum. Colpod,cucullus, C. striatum, C. saprophila and other ciliates are commonly found immediately under the surface liquid, whereas many other forms, particularly Rhizopods, prefer the area just above the soil-surface. The culture solution was withdrawn for examination by means of a fine specially made eye dropper. Wide plain glass slides are very suitable for routine obser-

vations, with or without cover slips. Ordinarily, ciliated organisms are satisfactorily seen with 250-500 magnification. This also is suitable for rhizopods, but for the small flagellates and for stained preparations 1200 magnification is often found necessary.

Good fixing and staining of protozoa on glass slides is difficult and requires patience and skill. Distortion of the body of the organisms is almost unavoidable. Bacteriological stains used with the dry or flame method of fixing are applicable only to minute flagellates and even here good results are not common. Slow killing was found to result in distortion and shrinking, whereas too fast killing often brings about disorganization of the structure. Corrosive Sublimate was most commonly employed although Osmic vapor (organism being subjected to its action by inverting the slide, on which it is in a drop, over the acid surface), or a 2% solution of Osmic acid is very satisfactory. Similarly KI, Potassium Iodide, may be used. Intra vitamine staining with dilute solutions of methyl green, methylene blue, or congo red give good results. Cilia are brought out visibly by tannic acid, whereas nuclei show dark with Iron Haematoxin.

Very active organisms had to be retarded for observation. This was effected by placing a few strands of cotton on the slide under the cover slip to trap the larger ciliates. Dilute agar solution was very difficult to adjust to the correct fluidity, hence it was unsatis-

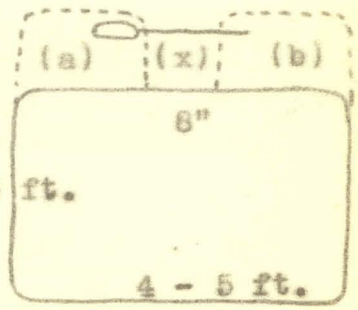
factory in this case. The most effective retarding agent was found to be "extraction of heat". The slide containing the organism in a drop of water was laid, drop upwards, on metal (sheet or plate) lying on snow or ice. The metal serves as a conductor of heat and further also prevents the bottom side of the slide from getting wet. Active ciliates commonly become entirely static. Some assume a spherical form as if in the process of encysting.

Direct examination of soils for protozoa was found to be entirely futile in the soils gathered in fall and winter. Likewise the dry soils taken in spring seemed free of trophic forms.

SAMPLING OF SOILS.

(1) Sampling at Different Depths:--

(a) By the Open Pit Method--Pits,3 feet wide and 4 feet long were dug 4 to 5 feet deep. On one of the long walls two columns (a b) of soil were removed as as to leave about 8 inches of soil exposed on three sides as a middle column (x). The samples were taken from this middle column by means of a long knife, the handle held in one hand in the open space (a) and the tip of the knife with the other hand in space (b). The soil (x) could thus be conveniently taken out by cutting down. This vertical column of soil was removed in



Top view of hole

parts at points corresponding with obvious changes in the appearance of soil composition, i.e.,the different horizons, A,B,and C Since A, the uppermost layer or horizon, is removed first it is not contaminated. Then by slicing the sides of the B horizon all contamination from A is removed, and next B can be taken out and so on.

The ditch method described by Waksman (11) serves equally well.

(b) By the Auger Method. --Three to five borings were made, 4 paces apart on each plot. The augers were marked 6", 12", 24", 36", 48" along their shafts, and all the 3--5 borings at 0--6" were mixed to make up one representative composite sample. Similarly all 6"- 12" made one sample, etc., so that 5 samples at successive depths resulted. These arbitrary depths seldom or never agree with horizons, and contamination of a lower sample with an upper is almost unavoidable.

(2) Sampling the Surface Soils.

A small amount of soil (¼" deep) is scraped away from the surface. The exposed soil can then be sampled. This soil of one-quarter inch down to six inches deep is generally called surface soil.

CONTAINERS.

The best containers are such as do not allow complete dessication, nor stagnation by air-tight sealing. Protozoa are dependent on atmospheric oxygen, and prolonged sealing seemed to kill them.

DETERMINATION OF HYDROGEN ION CONCENTRATIONS IN THE SOILS.

The p.H. value, which technically is the logarithm of the reciprocal hydrogen ion concentration, was determined electrometrically by means of the Quinhydrone electrode, fully described by Billman Tovborg Jensen (36). About 10 grams soil passed through ½ mm. sieve were shaken with 20 cc. of distilled water until the mass was homogeneous. Three electrometric determinations were then made, 1st, immediately after shaking, 2nd, 2 minutes later, and the 3rd at equilibrium. The resistance readings from the apparatus were then substituted in the formula, (See Jensen and Billman) and the p.H. calculated for the 128 soils. Also the temperature of the mixtures was noted.

Other factors important in the development of protozoa of the soil, ~~is~~ are moisture content and Organic Matter.

MOISTURE EQUIVALENT (M.Eq.)

The methods of Briggs and McLane (37) were followed:-- Centrifuge boxes lined with blotting paper and filled with ½ mm. sieved soil to a depth of 1 cm. were set in water for one hour. They were then removed to a container, covered with moist cloth and left to stand over night. Next day they were centrifuged at 2440 revolutions per minute for forty minutes, then weighed in the weighed containers, dried in an oven at 105°- 108° C over night. The third day cooled and weighed and the necessary calculations made. The moisture lost was calculated in per cent of dry soil. The per

cent loss being equal to the (M.Eq.). As is obvious the method is tedious and of several days duration. Only freshly sampled, or air tight sealed soils can be determined. Lengthy sealing is not permissable, e.g.,samples sent in from distant places. Hence the writer could not complete these determinations.

SPECIES OF PROTOZOA FOUND IN MANITOBA SOILS.

The following species were identified by their form, size, activity and habits, and later from stained preparations which revealed cilia, nuclei and micronuclei. Measurements were made by a graduated glass disk, used on the diaphragm of the ocular. These measurements were later checked by means of tracings made with a special Leitz "Zeichenocular".

Classification is chiefly according to Waksman (11) and partly after Edmondson (35) and several others.

CLASS - Sarcodina

Sub-class - Rhizopoda. (pseudopodia without axial filaments)

Amoebea (naked without tests)

Amoebida (naked-pseudopoda blunt or pointed, but never filamentous)

1. Hyalodiscus (amoeba) limax (Dvj.) ++
2. Hyalodiscus (amoeba) gutulla (Dvj.) +++
3. Amoeba radiosa (Ehrbg.) +
4. Amoeba proteus (Ehrbg.) (rare)
5. A form very similar to Amoeba cucumis (Martin and Lewis) (rare)

CLASS - Mastigophora.

Sub-class - Flagellidia.

Group - Pantostomatinae. (naked,-colorless,-food injested, often amoeboid, 1 or more flagella)

1. Cercomonas crassicauda (Dvj.) +++

Group - Protomastiginae. (Small flagell, usually
(more or less amoeboid,
(having a fine periplast,
(no chromatophores, pseudo-
(podia, when present, never
(serving for locomotion)

2. Bodo ovatus. (S.K.) ++
3. Bodo globosus (Stein) +
4. Pleuromonas jaculaus (Pevty) +

Group - Chrysomonadinae. (Small flagellates;
(thin cuticle generally
(present, yet they are
(capable of amoeboid
(movement, 1 or 2 flagella,
(cysts endogenous)

5. Oikomonas sp.(?) (Kent) ++
6. Monas gutulla (Ehrbg.) ++
7. Monas termo (Ehrbg.) +
8. Physomonas elongata (Stokes) ++

Group - Euglinidae. (A complex vacuole system
(anteriorly, green chromato-
(spheres, enclosed in a mem-
(brane, 1 or 2 flagella)

9. Euglena sp.(?) (rare)
10. Heteronema acus. (Ehrbg.) +

Group - Phytomonadinae. (Solitary, enclosed in cellul-
(ose wall chlorophyll and
(stigma present, contract,
(vavuole anterior, 2 flag-
(ella)

11. Polytoma uvella (Edmondson) +

CLASS - Infusoria

Sub-class - Ciliata

Group - Holotricha. (Body uniformly covered with
(cilia, sometimes slightly
(lengthened about the mouth;
(no adoral spiral zone)

1. Holophrya sp. (?) (Conn) ++
2. Holophrya sp.(?) (Edmondson) +
3. Urotricha farcta (Edmondson) (rare)
4. Prorodon teres (?) (Ehrbg.) +

Group - Holotricha. (Continued)

5. Loxophyllum flexilis (Stokes) +
6. Trichoda pura (Ehrbg.) +
7. Colpidium striatum (Stokes) ++++
8. Colpodium colpoda (Stein) ++
9. Colpoda campyla (Edmondson) +
10. Colpoda saprophila (Stokes) +++
11. Colpoda cucullus (Stein) ++
12. Colpoda steinii (Goodey) +
13. Cyclidium glaucoma (Ehrbg.) (rare)

Group - Hypotricha. (Body flattened dorso-ventrally, cilia often fused to form cirri on ventral surface. Adoral zone of membranellae, comparatively large.)

14. Oxytricha pelionella (Mull) +
15. Oxytricha sp.(?). +

Group - Peritricha. (Body cup-like, stalked and sedentary when full grown, cilia mostly limited to adoral zone, membranellae leading down to vestibule into which phraynx and contractile vacuoles open. Young often with posterior ring of cilia free swimming.)

16. Vorticella microstomata (Ehrbg.) +

A number of species of both Flagellates and Ciliates, which I was unable to identify were observed. If more of the existing literature on classification and identification had been available, I believe they could have been identified as previously listed and named organisms, for they occur quite commonly. Some of these are illustrated on the plate and described with it.

The occurrence of species is indicated as follows:-

Rare ---- (rare). Occasional ---- + Common ++

Abundant ------ +++

DISCUSSION.

Thus it seems that ciliates stand highest in number of species, followed by the flagellates, rhizopods being least. Yet in actual numbers of individuals in the soil flagellates rank first, followed by ciliates and rhizopods. This is not so obvious due to the large size and activity of the ciliates. Many of the flagellates approach the size of bacteria and are confusing for that reason.

Now, grouping those species which have been found abundant in the soils of Manitoba, we find that there are 14 ciliates, 10 flagellates, and 3 rhizopods. The number of species of flagellates is much greater than stated, but owing to their minuteness, identification was impossible. Several nematodes and also insect larvae were found in various soils.

Further, it was found that most aquatic or fresh water forms were missing in the Manitoban Soils sampled in January. For example, Vorticella microstomata and Paramoecium were found only in samples taken in summer. Also in winter the species of flagellates were fewer than in summer, Monas guttilla and Bodo ovatus, etc., being apparently absent in winter. Most remarkable was the large number of Colpidium striatum both in winter and summer. This species was found to dominate every other protozoa in all the soil cultures. Next to this in abundance was Colpoda saprophila, and this was strongest in fall and winter.

Comparing the Manitoba protozoa with those obtained by Fellers and Allison in New Jersey soils we find strong similarity. They found 17 ciliates, 6 flagellates, 1 rhizopod and 1 algae to constitute the commoner species. Over a wide range of territory many more species were identified but the above numbers comprise the abundant ones.

Considerably different results were obtained by Kofoid (31) 1908 in his study of Plankton in the Illinois Rivers. Of these aquatic forms he found 55 ciliates, 62 mastigophora, 30 rhizopods, 4 heliozoa and 5 suctoria in the Illinois River. Bearing in mind that these were fresh water forms one still finds fair semblance in species-number between soil microfauna and that of fresh water. The absence of rotifers and suctoria from soil fauna is noteworthy.

Aquatic fauna seems to be much more varied than soil fauna.

Soil microorganisms have been found to be in the following general proportions:
Algae 1:, Protozoa 4:, Fungi 40:, Actinos 80, Bacteria 200, (Fred and Waksman (27)). No wonder Starkey suspects the influence of Fungi and Actinos upon protozoa to be pronounced.

In soil taken from adjacent fields, and physically practically identical, increase in alkalinity appears to cause decrease in number of protozoa. (See Tables 3,4,5,6)

GROUP RELATIONSHIP AND DISTRIBUTION OF PROTOZOA IN SURFACE SOILS OF THIRTY DIFFERENT LOCALITIES.

TABLE I

Locality from which soil was taken	Soil texture	p.H. values	Flagellates	Ciliates	Rhizopods	Total number of species
Manitoba Agri. College Farm						
Plot A-33-1	Black					
No Manure	Clay	8.08	8	8	3	19
Plot A-33-2	"					
Phosphate	"	8.03	2	8	2	12
Plot A-33-3	"					
Potassium	"	8.20	4	5	0	9
Plot A-33-4	"					
Nitrogen	"	8.20	5	5	2	12
Plot A-33-13	"					
Manured	"	7.56	2	6	2	10
Plot 1-29-8	"					
Sweet Clover	"	8.05	1	6	1	8
Plot 1-29-10	"					
Manured	"	7.80	2	5	3	10
Plot 3-24-1	"					
Wheat, not manured	"	7.60	1	3	0	4
Plot 3-24-2	"					
Wheat, Manured	"	7.70	3	3	2	8
Plot 3-24-3	"					
Oats, Not manured	"	7.65	2	4	3	9
Plot 3-24-4						
Oats Manured	"	7.90	4	3	3	10
Plot of Soy Beans	"	7.30	2	5	2	9
Soils from seven different sections of the dried out areas of South Western Manitoba	1, Sand	7.96	1	2	1	4
	2, medium sandy loam	8.26	3	2	2	7
	3, Sandy loam	7.60	2	2	1	5
	4, "	8.30	1	2	0	3
	5, "	8.50	1	1	1	3
	6, "	8.00	3	2	0	5
	7, "	8.30	1	4	2	7

TABLE I (CONT'D)

Locality from which soil was taken	Soil texture	p.H. values	Flagellates	Ciliates	Rhizopods	Total number of species
Brought forward			48	76	30	154
Winnipeg Bush Soil	Fine sandy loam	7.56	4	4	2	10
Winnipeg Prairie Soil	Silty Clay	7.70	3	5	1	9
Brandon Exp.Farm	Sandy Loam	8.75	1	3	0	4
Hainstock	Clay Loam	7.60	2	4	1	7
Thunderhill	Clay Loam					
	Richly Organic	7.20	1	6	2	9
Minitonas (North of Bowsman	Sandy Loam	6.90	2	4	1	7
	Silt Loam	7.90	2	5	0	7
Altona	Clay Loam	7.50	4	6	1	11
Roland	Heavy Silty Clay	8.19	5	4	2	11
Swan River	Sandy Loam	7.60	3	3	3	9
Winnipeg, Tuxedo Park	Sand	8.45	1	4	0	5
Totals in Manitoba Soils	=		76	124	43	243
Average numbers of species per soil	=		2.5	4.	1.3	8

VERTICAL DISTRIBUTION OF PROTOZOA IN VARIOUS SOILS.

TABLE 2.

BUSH SOIL (Winnipeg, Fort Garry)

Depth, Soil Horizon and p.H.	Organisms	Species of Protozoa
0-3" Horizon A_0 Brown leaf mat Porous and Humified Neutral, p.H.7.3	Aquatic ciliates, Larger Soil Ciliates No flagellates No Rhizopods	(1) Tachelophyllum tachellastum (2) Paramoecium etc. (1) Colpid striatum (2) Colpoda saprophila
3½-8" Horizon A Grey leached clay Friable, granular: Slightly alkaline: p.H. 7.7	Zone of Soil ciliates (abundant) Zone of Soil Flagellates Zone of Soil Rhizopods	(1) Holophrya (2) Colpid striatum (3) Colpoda saprophila (4) Colpoda cucullus (1) Monas termo (2) Physomonas elongata (3) Bodo (4) Arcomonas crassicanda (1) Amoeba gutrella (2) Amoeba climax
8½-18" Horizon B_1 Black clay Tough, nutty, Acid, p.H. 6.4	No Protozoa (?) (Soil Bacteria) (present)	

(Continued)

TABLE 2

(Continued)

Depth, Soil Horizon and p.H.	Organisms	Species of Protozoa
18½-30" Horizon B_2 Olive grey clay Cloddy, waxy and stained. Acid to Neutral p.H. 6.6	No Protozoa	
	Some Bacteria	
30½-48" Horizon C Drab clay over parent material, i.e., Lacustrine clay Neutral to alkaline, p.H. 7.5	No Protozoa	

Table 2, Bush soil, and Table 3, Prairie soil, were sampled by the pit method; hence depths are separated by horizons, i.e., changes in composition of soil. But Table 4 was sampled by augers; hence the depths are arbitrary (not by horizons) and standard that is 6", 7", 12", 24", 36". Table 4 is representative of 12 others, series of samples taken after a similar manner.

TABLE 3.

PRAIRIE SOIL (Winnipeg, St.Norbert)

Depth, Soil Horizon and p.H.	Organisms	Species of Protozoa
0-1½" Horizon A_o	Soil Algae	(1) Protococcus sp.(?)
		(2) Nostoc sp.(?)
Black soil sod mat with Blue Grass roots		(3) Anaboena
	Aquatic Ciliates	(1) Vorticella
		(2) Paramoeten
	Few Soil Ciliates	(1) Colpoda saprophila
Neutral to Alkaline p.H. 7.6		(2) Colpodium striatum
	No Flagellates, etc.	
1½-5" Horizon A	Few small soil ciliates	(1) Colpoda saprophila
		(2) Colpoda striatum
Black clay, granular, friable	Many Rhizopods of--	sp. Amoeba gutulla
Alkaline p.H. 7.7	Few Flagellates	(1) Monas
		(2) Cercomonas crassicanda
	Soil Bacteria	
5½-8" Horizon B_1	Zone of Soil Ciliates	(1) Colpoda striatum
		(2) Colpoda cucullus
Grey brown clay		(3) Colpoda saprophila
		(4) Holophays
Fragmental plastic.		(5) Common (Unidentified)
	Zone of Soil Flagellates	(1) Monas gutulla
		(2) Physomonas elongata
Alkaline, p.H.7.9		(3) Many Bods.
	Few Rhizopods	(A. gutulla)
	Soil Bacteria	

(Continued)

(Continued

TABLE 3.

Depth, Soil Horizon, and p.H.	Organisms	Species of Protozoa
8½-18" Horizon B_2	No Ciliates	
Greyish Drab clay:	Small minute Flagellates only! 8u-10u--	
"Effervescing" with carbonate accumulation		(1) Physomonas elongata (2) Monas termo, etc.
Basic p.H. 8.25	No Rhizopods Soil Bacteria	
18½-30", Horizon C_1		
Drab clay Compact, with large, soft concretions of carbonate ("Effervescing") Basic p.H. 8.15	No Protozoa	
30½- Horizon C_2		
Yellow clay, Compact and plastic in Parent material, i.e. (Lacustrine Clay) Basic p.H. 8.40	No Protozoa	

TABLE IV

CULTIVATED SOIL (Winnipeg, M. A. C. Plot 3-24-4) (Manured)

Depth, Soil and pH	Organisms	Species of Protozoa
0--6"	Algae	
	Moulds	
Black clay loam (Structure) finely granular. (Consistency)- moderately plastic	Flagellates	(1) Monas termo (2) Bodo globosus (3) Cercomonas crossie (4) Polytoma uvella
Slightly alkaline: p.H. 7.7	Ciliates	(1) Colpoda saprophila (2) Colpidi striatum (3) Oxytricha pelionella
	Rhizopods	(1) Hyaludiscus (Am.) limax (2) Hyaludiscus (Amoeba) gutulla (3) Amoeba radiosa
	Bacteria	
7--12" Black clay Structure granular Consistency plastic	Flagellates	(1) Monas termo (2) Bodo globosus (3) Cercomonas crossie
	Few Ciliates	(Colpid striatum (2) Colpoda saprophila
Alkaline, pH 8.0	Bacteria	
13-24" Structure compact with carbonate concretions Yellow clay soil (p.H.) 8.2 Basic	Flagellates	(1) Monas termo (2) Bodo globosus
	Few Ciliates	(1) Colpoda saprophila
	Bacteria	
25-36" Yellow clay, Structure compact with soft concretions of CO_3 (p.H.) 8.2 Basic	No Protozoa	
	Bacteria	

Table 4 is typical of fourteen depth-investigations made on different plots at the Manitoba Agricultural College and several points in South Western Manitoba, (see Tables 5 and 6).

It is notworthy that the larger ciliates, e. g., Oxytrich (various sp.) and forms like Trachelopyllum tachyblastum were found to occur only in surface soils. Smaller ciliates like Colpidium saprophila were found at a depth of 24 inches and more in Manitoba. (Different from Waksman's report on other soils). Minute flagellates were commonly found deeper than any other protozoa. (See Tables 5 and 6).

The pit method of sampling is by far the most accurate. Here contamination of soil by upper horizons is quite avoidable, whereas the auger method has this fault almost inevitably.

In Tables 5 and 6 (following) an attempt has been made to show a possible correlation between Protozoa, depth and Hydrogen Ion concentration of 50 different samples of prairie soil. (Over)

CORRELATION OF PROTOZOA WITH HYDROGEN ION CONCENTRATION AND DEPTH OF SOIL.

F. indicates presence of Flagellates, C, Ciliates, R, Rhizopods and 0, no Protozoa.

TABLE V

WINNIPEG, MAN. (Manitoba Agricultural College Farm)

Precipitation for 1933 was 19 inches. Soil from Block 3, Range 24, Plots 1--5

Depth in inches	Plot 1. p.H	Plot 1. Proto-zoa	Plot 2 p.H	Plot 2 Proto-zoa	Plot 3 p.H	Plot 3 Proto-zoa	Plot 4 p.H	Plot 4 Proto-zoa	Plot 5 p.H	Plot 5 Proto-zoa
0--6"	7.6	FC	7.7	FC	7.65	FCR	7.9	FCR	7.7	FCR
7-12"	8.1	FC	8.17	FCR	7.9	FCR	8.0	FC	8.1	FCR
13-24"	8.3	F	8.26	F	8.3	FR	8.2	FC	8.2	F
25-36"	8.18	0	8.17	F	8.45	0	8.2	(?)R	8.3	F
37-48"	8.55	0	8.26	0	8.45	0	8.3	0	8.54	0
Number of Species of Protozoa	4		8		9		10		7	

TABLE VI

SOUTH WESTERN MANITOBA. (South of Hartney, Lauder, Etc.)

Precipitation for 1933 was 10 inches, Depth samples from five different localities.

Depth in inches	Plot 1 p.H	Plot 1 Proto-zoa	Plot 2 p.H	Plot 2 Proto-zoa	Plot 3 p.H	Plot 3 Proto-zoa	Plot 4 p.H	Plot 4 Proto-zoa	Plot 5 p.H	Plot 5 Proto-zoa
0--6"	7.96	FCR	8.26	FCR	7.6	FC	8.38	FC	8.5	FCR
7--12"	8.2	FC	7.9	FCR	8.00	FCR	8.40	F	8.7	0
13-24"	8.6	F	8.17	0	8.1	0	8.3	0	8.6	F
25-36"	8.66	0	8.5	0	8.2	F	8.6	0	8.6	0
37-48"	8.5	0	8.75	0	8.3	0	8.48	0	8.9	0
No. of species of Protozoa	4		7		5		3		3	

Thus it appears that with the increase of alkalinity of the soil, the presence of Protozoa becomes less pronounced; and where the surface soils become alkaline, to a p.H. 8.3 or more, (as shown in Table 6, column 4 and 5), the number of Protozoa and their species becomes small. Other chemical and physical factors varying with increase of depth undoubtedly play an important part in the decrease of Protozoa. The writer would like to refer to one of the earliest experiments on Soil Sterilization (paragraph 1). Frank 1888 pointed out the lack of soluble matter and lack of productiveness of sandy soil. All the soils in Table 6, were sandy soil. However it should be noted that the precipitation during 1933 was about 9 to 10 inches lower for the soils of Table 6, than those of Table 5, Winnipeg.

TABLE VII

GENERAL OCCURRENCE OF PROTOZOA FOUND IN MANITOBA SOILS.

Common Species of Protozoa found in Manitoba Soils	Spitbergen: Cutler & Sandon	England: Goodey, Martin & Lewin	America: Allison & Sandon	New Jersey: Waksman, Fellers & Allison	South Africa: Fantham	Russia: Yakimoff Nowikoff	Germany: Wolff: France	Sweden: Koffman	France: Perey
Hyalodiscus (Amoeba) limax			x	x					
Hyalodiscus (Amoeba) gutulla		x	x	x	x	x	x		
Amoeba radiosa			x	x		x		x	
Amoeba proteus			x	x	x			x	
Amoeba cucumis		x	x	x				x	
Bodo ovatus	x			x			x		
Bodo globosus				x		x		x	
Cercomonas crassicanda		x		x		x		x	
Monas gutulla				x		x	x	x	
Pleuromonas joculans			x	x	x				
Physomonas elongata				x				x	
Oikomonas (sp.?)		x		x		x		x	
Heteronema acus				x		x			
Euglena (sp.?)				x		x			

TABLE VII

GENERAL OCCURRENCE OF PROTOZOA FOUND IN MANITOBA SOILS.

Common Species of Protozoa found in Manitoba Soils	Spitbergen: Cutler: Sandon	England: Goodey: Martin & Lewin	America: Allison & Sandon	New Jersey: Waksman: Fellers & Allison	South Africa: Fantham: Nowikoff	Russia: Yakimoff	Germany: Wolff: France	Sweden: Koffman	France: Perey
Polytoma uvella						x	x		
Holophrya			x	x		x	x		
Uroleucha faveta			x			x		x	
Loxophyllum flexilis				x	x				
Prorodon teres			x	x		x	x		
Colpidium colpoda				x					
Colpidium striatum				x	x				
Colpoda cucullus		x	x	x		x	x	x	x
Colpoda saprophila			x	x					
Colpoda steinii		x	x	x		x	x		
Colpoda campyla				x					
Cyclidium glaucoma			x	x		x		x	
Oxytricha pelionella			x	x	x			x	
Vorticella microstomata		x	x			x		x	

SUMMARY

(1) Different effects of small variations in hydrogen ion concentration of various surface soils appear to be either wanting or else obscured by the diversified combinations of other chemical and physical properties of the soil. (Table I.)

(2) The depth at which the greatest number of protozoa occur is 2 to 8 inches. Representatives of the three major classes are commonly found as deep as 12 inches. Small Flagellates often reach a depth of 24 inches. In compact soils, which are not drought-cracked, protozoa are seldom found below 25 inches. (Table 2 and 3).

(3) Sandy soils with low hydrogen ion concentration contained the least number of protozoa, while cultivated black soil was found to contain the greatest. (Tables 1,5,& 6).

(4) The effect of soil treatment by manure and various chemical fertilizers was not detectable in these investigations probably due to the fact that the plots were small and therefore all in close proximity to each other. (Table 1 and 5).

(5) The comparatively high number of species found in bush soil is partly accounted for by the fact that the soil sample was taken from an area of "Wood-land invasion".(Table 2)

(6) Three common fresh water protozoa were found on dry land, and here only in late spring and summer in the upper three inches.

(7) As far as conclusions are justifiable from these brief investigations, the number of species of protozoa in Manitoba soils is considerably smaller than the number of protozoa species found in soils farther south, New Jersey, U. S. A.

(8) Of the protozoa found in Manitoba, 26 have according to reports, been found in U. S. A. (America), 16 in Russia, 13 in Sweden, 8 in Germany, 7 in England, and 6 in South Africa. (See Table 7).

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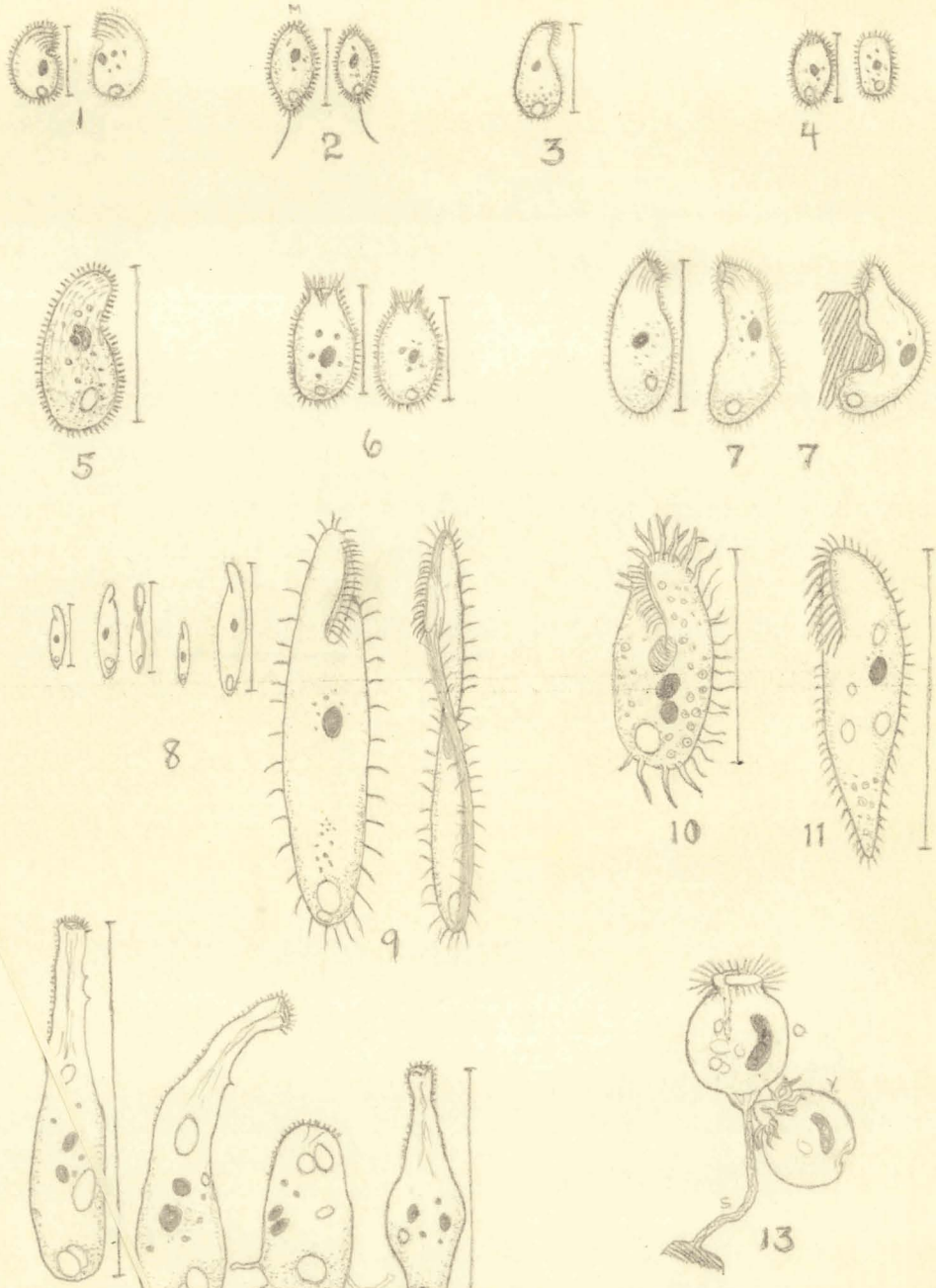
ILLUSTRATIONS ON PLATES.

The illustrations on the following plates were made from both living organisms and fixed, stained preparations on microscope slides. All were drawn by means of the same high power objective and a Leitz Wetzlar Zeichen okular. They are therefore all of the same magnification, (except one, No. 9, Plate I), and their proportionate relation in size is further shown by indicator lines. For instance, on Plate I, number I is 30 u (mu) long, whereas number 12 A. is 130 u long; the symbol mu u being the unit of measurement. Of these, 1000 u are equal to one millimeter, which is equal to one sixteenth of an inch.

Comparison of the flagellates described on Plate 2 with the ciliates, Plate I, makes obvious the fact that soil flagellates are generally less than half the size of soil ciliates.

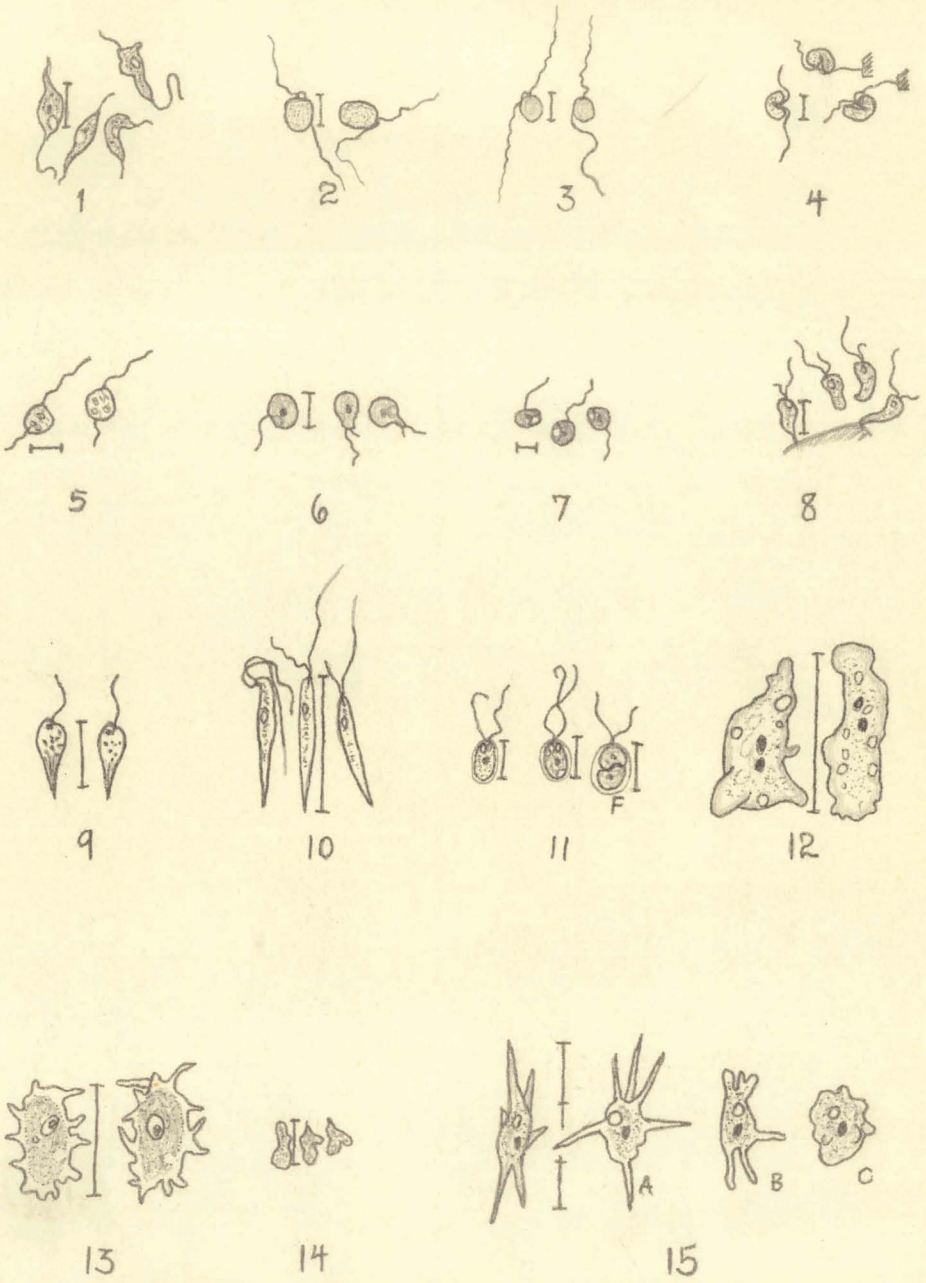
(Over)

Plate 1.



Commonly occurring Ciliates.

Plate 2.



Commonly occurring

Flagellates & Rhizopods.

DESCRIPTION OF PLATE I.

CILIATA.

(1) Colpoda saprophila. (Stokes).

Group Holotricha, 30u, abundant. Ventral border anteriorly notched, recurved pharynx, wide ventral groove.

(2) Urotricha farcta (Edmondson)

Group Holotricha, 40u, rare. Posterior springing hair, prominent apical aperture oral.

(3) Colpoda cammyla (Edmond.)

Group Holotricha, 45u, occasional. Cilia lengthened circumorally anteriorly, body pear-shaped, ptp granular.

(4) Holophrya (various species). (Edmond.etc.)

Holotricha. 30 u, common, shape eliptical or cylindrical, cilia short and uniform, nucleus central.

(5) Colpidium striatum (Stokes)

Holotricha, 67u, abundant. Nucleus central, posterior contract vacuole, pharynx ventral, stria visible.

(6) Genus unidentified, Group Holotricha.

45u, occasional, resembling Prorodon teres but is smaller, has only one contractile vacuole posterior. Found in soils (plot A-33-2). (plot 1-29-8)

(7) Unidentified, Group Holotricha.

65u, common, very flexible, thin pelliole, mouth anterior, long circumoral cilia, oval central nucleus, one posterior contractile vacuole. Found in soils from, plot (1-29-8), (A-33-1)

(8) Unidentified, Group Hypotricha.

25u to 50u (30u), abundant, small, elongated, often flattened and spiral, posterior contract vacuole, pharynx ventral, stria visible. (over)

(8) Unidentified, Group Hypotricha (Continued)

Found in soils from : plots (1-29-8) (3-24-4), (A-33-1), Hainstock, Man., Brandon, Man., Swan River, Man., Thunder Hill, Man., South-Western Manitoba.

(9) Same as No. 8. More Magnified.

to show long and sparse cilia, adoral zone of membranellae, elongated body dorso-ventrally compressed, spiral, single small nucleus, movements steady and uniform.

(10) Oxytricha pelionella. (Edmond.)

Group Hypotricha, 80u, occasional, 2 oval nuclei, one contractile vacuole, dorso-ventrally flattened, anterior and anal cilia enlarged.

(11) Unidentified, Group Hypotricha.

90 to 110u, occasional, resembles Uroleptus, but is smaller and has several contractile vacuoles, single oval nucleus, no styles. Found in soils: (plot A-33-13) and Thunder Hill.

(12) Unidentified.

90 to 130u, rare, ordinary form A and B resembling Trachylophylum tacheblastum but has a broader flexible neck, broad posterior, transparent, mouth anterior, cilia short and apparently local, contractile vacuoles several, two macro-nuclei, and three or more micronuclei. Fig. C. form assumed upon cooling to 3° C on ice, note pseudopodia. (Plot A-33-13 in two different samples)

(13) Vorticella microstomata. (Stein)

Group Peritricha. 60u, occasional, body spherical, stalk very thin, cilia limited to adoral zone, vestibule 3/7 length of body, basal thickening, where stalk and body conjoin, is prominent. Macro-nucleus curved. Habit sedentary, ciliary movement extremely swift. Contraction of stalk instantaneous, (see Fig.13,o.), relaxation slow. Daughter organism, product of longitudinal fission commonly seen attached at base, (See 13,y.)

(13) Vorticella microstomata (Continued)

reaches size of mother organism, and grows a temporary posterior ring of thick slowly undulating cilia, before it breaks free.

(14) Cyclidium glaucoma (Ehrbg.)

Group Holotricha, 30u, rare, transparent body covered with long rigid cilia, hoodlike membrane covers oral aperture, movements darting and eccentric.

(15) Colpoda cucullus (Stein)

Group Holotricha, 35u, common, nucleus central contractile vacuole posterior mouth ventral, notch midways between anterior and posterior.

PLATE 2

FLAGELLIDIA AND RHIZOPODA.

(1) Cercomonas crassicauda (Duj.)

Group Pantostomatinae, 18u, abundant, naked colorless, often amoeboid, one flagellum at either extremity.

(2) Bodo ovatus (S. K.)

Group Protomastiginae, 14u, common, one flagellum trailing, one propulsive, body oval.

(3) Bodo glabosus (Stein)

Group Protomastiginae. 9u, occasional, small almost spherical, two long flagella, one trailing.

(4) Pleuromonas jaculans (Perty)

Group Protomastiginae. 8u, occasional, small oval to kidney shaped, one side rivoginated, two flagella arise from indenture. Often attached by one.

(5) Oikomonas sp.?

Group Chrysomonadinae. 14u, common, thin cuticle irregularly spherical, granular, one or two flagella.

(6) Monas gutulla (Ehrbg.)

Group Chrysomonadinae. 15u, commonm irregular in form, nucleus and vacuole posterior.

(7) Monas termo (Ehrbg.)

Chrysomon. 7u, occasional. Shape globular to ovoid, changeable, one flagellum.

(8) Physomonas elongata (Stokes)

Chrysomon. 12u, common, body generally twice as long as broad. Shape changeable, often attached by posterior pedial.

(9) Euglena (sp.?)

Group Euglinidae. 29u, rare, body elongated, posterior end pointed and dark, eye-spotpresent, one flagellum in anterior notch, greenish in color.

(10) Heteronema acus (Ehrbg.)

Euglinidae. 55u, occasional, body long narrow plastic, longer flagellum trailing.

(11) Polytoma uvella (Edmond.)

Group Phytomonadinae. 19u, occasional, one or two contractile vacuoles near base of two flagella, which cross each other characteristically in fixed state, form solitary enclosed in cellulose wall, chlorophyll and stigma present.

(12) Unidentified, Group Amoebida.

70u, common, strongly resembling Naegleria - gruberi, but is larger, and has a number of contractile vacuoles.

(13) Unidentified, Group Amoebida.

45-50u, rare, naked pseudopodia pointed, ectosarc strongly differentiated from coarsely granular inner cytoplasm. Resembling Amoeba cucumis (Martin and Lewin), but is smaller.

(14) Hyalodiscus (amoeba) gutulla. (Duj.)

Amoebida, 20u, very common, very minute rhizopod usually club shaped, endoplasm clear.

(15) Amoeba radiosa (Ehrbg.)

Amoebida, occasional, contracted 30u, spikes extended 70u, has generally five to six radia spikes A, which, when disturbed contract and change to pseudopodia, B. C.