AN
INVESTIGATION OF SOIL ALGAE
IN MANITOBA.

A THESIS.

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of the University of Manitoba

by

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ABSTRACT.

An investigation of a number of different soils from various districts in Manitoba was carried out by means of nutrient cultures, for the purpose of determining what species of algae, if any are present. Twenty-five species of algae were identified: a characteristic group consisting of the species Chlorococcum humicola, (Naeg.), Chlamydomonas communis, (Snow), Nostoc commune, (Vauch.), Ulothrix subtilis, (Kutz.), Phormidium autumnale, (Ag.), and Phormidium tenue, (Menegh.), were found to predominate. Certain species of algae normally submerged aquatics, were recorded for the first time in Manitoba.

A possible correlation between zonal distribution and algal species and texture and algal species was suggested.

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1. HISTORICAL AND GENERAL INTRODUCTION.

During recent years soil microbiology has become an important subject for scientific research, the activities of certain organisms, both in the fauna and flora, having been found to play an important role in the ecology of the soil.

The seventh and eighth decades of the last century may be given as the beginning of the development of this science, although as far back as the 15th century agriculturalists realized that decomposition of vegetable and animal matter took place in the soil, and that the process was intimately connected with soil fertility. How this was accomplished, however, was an absolute mystery to them, so that it presented an immediate problem for investigation. The efforts of early workers such as Glauber, 1655, Mayow, 1674, and Liebig, 1840, in this field brought to light three ideas on decomposition: humus formation, nitrate formation, and chemical oxidation. It was Boussingault, in France, who first recognized the connection between nitrifaction and soil fertility, but it was not until the valuable discoveries of Pasteur on bacterial fermentation that the explanation of nitrification as a biological action was realized.

Between 1880 and 1910, soil microbiology advanced rapidly in its development. The outstanding contributions of this period began with the brilliant studies of Schloesing and Muntz, who, inspired by the wonderful discoveries of Pasteur, 1862, definitely established the biological nature of the process of nitrifaction. Berthelot, in 1885,

established the fact that the building up of nitrates in the soil is due to the activities of certain micro-organisms. The work of Warington and numerous other investigators finally culminated in the isolation, by Winogradsky, (1890), of organisms active in the oxidation of ammonium salts to nitrites, and of nitrites to nitrates. The bacteriological investigations of Hellriegal, Welfarth, (1888), Beijerinck and Omelianshy, (1902), are worthy contributions to the development of soil microbiology in this period, as are those of Hiltner, Chester and Meyer of recent years. As a result of these investigations a new conception of our understanding of the microbial population in the soil in relation to soil economy was established toward the end of this later period. Up to 1910, therefore, the bacteria were the only soil organisms believed to be of any importance and which were studied in detail; other organisms were looked upon as occasional soil invaders rather than permanent members of the soil population capable of taking part in some of the most important soil processes. Although the activities of the nitrogen-bacteria of the soil were definitely established, and those of the soil protozoa investigated, as well as the pathogenic effect on higher plants of Myxomycetes, Nematodes, and Fungi, the recognition of Algae as an important constituent of the soil population is comparatively recent, proceeding largely from the investigations of Esmarch, (1910), France, and Madeburg, in Germany; Robbins, Moore and Schramm in the

United States; Petersen in Denmark, and Bristol-Roach in England.

The publication of Esmarch's paper, (1910), on the Blue-Green algae of some of the soils of the German-African colonies, marked the beginning of the study of a new group of soil organisms. The results of his experiments showing their distribution in cultivated and uncultivated soils, (Table 1), proved interesting from an agricultural point of view, and led to further research by other workers.

TABLE 1. FREQUENCY OF OCCURRENCE OF CERTAIN COMMON SPECIES IN ESMARCH'S SOIL SAMPLES. (5)

SPECIES	Percentage of Samples containing given Algae.					
	Uncultivated damp sandy soil			Cultivated soils		
	Shores of Elbe	Shores of Lakes	Sea shore	Sandy land	Clay	Marsh land
Anabena-variabilis	46 %	43 %	9 %	10.3 %	60 %	46 %
Anabena-torulosa	31 %	14.3%	65.6%	27.6 %	34.3%	56.4%
Cylindrospermum musicicola	23 %	28.6%	0	24 %	48.6%	59 %
Cylindrospermum majus	0	14.3%	0	38 %	40 %	33.3%
Nostoc Sp.III	7.7%	0	0	38 %	37 %	48.7%

In 1912, Robbins published a paper on the "Algae of some Colorado Soils", in which twenty-one species of Algae were described. The results of his investigations also led to further research in this field in the United States.

Petersen in his "Danske Aerofile Alghe" (1915), made a valuable contribution to our knowledge of soil algae, especially of soil diatoms.

Schramm (1914), in the United States, confined his research more especially to methods of growing the various algal species in pure-cultures, carrying out the technique developed by Chodat in Switzerland (1902), in the latter's physiological investigations of certain algal species.

The systematic work of Moore (1), on the occurrence of these organisms in the soil is of great value, and together with Karrer, a co-worker, demonstrated the existence of a subterranean algo-flora, of which Protoderma-viride, the most constantly occurring species, was shown to multiply at a depth of one meter.

More recently in England, at the Rothamstead Experimental Station, the work of Dr. Muriel Bristol-Roach has been an inspiration to many workers in soil algae. Through her investigations, valuable contributions have been made to our present knowledge of the occurrence, distribution, activities, and influence of these organisms in the soil, especially in relation to soil fertility.

Previous to 1910, however, the researches on algae

of habitats, other than the soil were investigated, by workers interested in their physiological characteristics. The names of Dillwyn (1809), Cooke, (1882-1884), Wolle, (1887), stand out in the history of the "Fresh-water Algae". The economic value of some lower algae was completely overlooked until about a quarter of a century ago, when the Swedish scientist Henseen, demonstrated their importance in marine plankton as the producers of the organic substances upon which the whole of the animal life of the ocean is dependent.

In more recent years the investigations of Chodat, and W. G. West, Fritsch, and others have contributed greatly to our literature on the occurrence of these organisms both in the soil and in other habitats. The classification of the Algae by Fritsch having been adopted by numerous present-day algologists. Since the work of Esmarch, therefore, in 1910, our knowledge of the occurrence and distribution of the alga-flora of the soil has increased considerably, large numbers of algae having been recorded as inhabiting the soil; including species of about 28 genera of Myxophyceae, 12 genera of Chlorophyceae, (Isokontae), 11 Conjugateae, (Isokontae), and 4 Heterokotae, so that there is little doubt that there exists a definite algal flora living vegetatively in the soil.

Although our knowledge of soil algae is extremely limited, and our conception of the role they play is largely based on speculation, it is believed that eventually they

will be shown to play a significant part in the economy of the soil.

In Canada there is a paucity of literature to be found dealing with the microflora of the soil, and little or none on the alga-flora of the soil. It offered thus a new field which seemed worthy of investigation in view of the recent wide-spread interest in soil microbiology in relation to soil fertility.

The present undertaking is a beginning to determine what species, if any, are more or less regular constituents of the various soils in the Province of Manitoba in Canada.

2. SOIL SAMPLES UNDER INVESTIGATION.

Samples, seventy-five in all, representative of soils in widely separated districts in Manitoba, were for the most part collected at random, with the possible exceptions of those obtained from the Dominion Government Experimental Farms at Brandon and Morden and from the Agronomy Department of the Manitoba Agricultural College. Those from Brandon and Morden were taken by means of a soil auger, while those from the Agricultural College were obtained by "horizon-depths" from freshly dug open pits.

The majority were collected in the Autumn of 1932, while others had been stored in a dry condition for longer periods. (See Table 2). In nearly every case the samples were air-dried before culturing.

The soils obtained were all "surface" samples. The

conditions under which they were taken varied somewhat as did the depths for a number of the samples. Sample number 5 was taken as a soil suspension from a depression where melted snow had accumulated in the spring of 1932. Samples 17--20 were obtained after a rain storm and so were in a somewhat "muddy" condition. The samples from Riding Mountain were taken according to the "horizon depth" of the soil profile; viz., 0--3", 0--6", etc. Those from Brandon had the dimensions, 2"X2"X2", 2"X2"X4", 6"X6"X6". All the samples therefore, were taken from the surface eight inches, none having been obtained below this depth.

The samples thus collected were air-dried, crushed if necessary, and placed in labelled glass jars for future use.

The nature of the soil samples under investigation varied considerably, and is best expressed in Table No.2 which gives the following data for the majority of the samples, the complete data for some of the samples being unobtainable.

(1) Geographical Location from which sample was obtained. A Map 1/ showing the location of the various samples under investigation is shown on Page 9 together with a glossary of same.

1/ The Map was obtained from unpublished data through the courtesy of Professor J. H. Ellis, M. A. C.


LAKE
WINNIPEG
Podzol and degraded
Rendzinas
8
Podzols
76 Beaches
and
Escarpment
Local Soils
Woodland
Podzolic
Invasion
8
Podzols
Northern Chernozem
on Drift
PRAIRIE
4. Normal Chernozem
Drift
Assini brine
Delta
Light-brown
Sandy
Dune
Sand
Chernozems
BELT
1a
Rendzinas and
High-Lime Peats
Woodland Invasion
1. Red River Valley
Chernozem and
Meadow
Associates
Escarpment
Drift
Podzols
Rock Outcrop
Podzols
and
Acid
Peats
6. Podzolic
Zone on
Morainic
Drift

(2) Approximate date of Collection.

(3) Moisture Equivalents 1/ of samples. These were calculated from as many of the samples as possible.

(4) Common soil type: prairie, woodland or forest, etc. Cultivated or uncultivated.

(5) Texture of soil: clay, loam, sand, etc.

(6) Soil Reaction--that is, whether the soil is "acid" or "alkaline" to indicators.

With this information, certain conclusions can be drawn as to the "growth" of the algae in these various soils.

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1/ Moisture Equivalent: (Briggs and McLane (4)), define it as: "The amount of water in per cent of dry soil that a soil will retain against a definite constant centrifugal force, for a given time". The force developed in the machine used, is 3000 times gravity which equals 2440 revolutions per minute for forty minutes.

TABLE 2. DATE OF SAMPLING, FIELD NOTATIONS, MOISTURE EQUIVALENT, TEXTURE AND REACTION of the SOILS STUDIED; AND THE NUMBER OF ALGAE SPECIES OCCURRING.

Sample No.	Date sampled	Field Notations	Moisture Equivalent.	Texture of Soil	Soil Reaction	Number of species identified.
1	May 1932	Garden soil--sod	39.80	clay-loam	Neutral	Four
2	May 1932	Cultivated soil	40.05	clay	Neutral	Five
3	May 1932	Leaf mold	---	Humus	Acid	Five
4	May 1932	Plowed field	39.82	clay-loam	Neutral to Alkaline	Three
5	April 1932	Snow-water on lawn-soil	---	clay-loam	Neutral	Five
6	April 1932	Garden soil	38.89	clay loam	Neutral	Four
7	May 1932	Unplowed field	39.19	clay loam	Neutral	Three
8	May 1932	Plowed field	38.49	clay loam	Neutral	One

Sample No.	Date sampled	Field Notations	Moisture Equivalent	Texture of Soil	Soil Reaction	Number of species identified
9	May 1932	Bush soil	39.52	clay loam	Neutral to Alkaline	One
10	May 1932	Cultivated soil	39.11	clay loam	Neutral	One
11	May 1932	Open field	38.49	clay loam	Neutral to acid	Six
12	June 1932	Cleared forest land	44.93	clay loam	Neutral to Alkaline	Five
13	June 1932	Cleared woodland	5.36	Sand	Neutral to Alkaline	Five
14	June 1932	Sand ridge with Jack Pine	7.61	sand	Acid	Two
15	June 1932	Forest	55.82	clay	Acid	Four
16	June 1932	Farm meadow	34.91	loam	Neutral	Two
17	June 1932	Field	56.34	clay	Alkaline	One
18	June 1932	Cultivated field	39.77	clay	Alkaline	Six

Sample No.	Date sampled.	Field Notations	Moisture Equivalent	Texture of Soil	Soil Reaction	Number of species identified
19	June 1932	Cultivated farm	14.52	sandy loam	Neutral to Acid	Three
20	June 1932	Road	40.64	clay loam	Neutral to Alkaline	Three
21	June 1932	Cultivated garden	33.62	loamy	Alkaline	Three
22	June 1932	Small bluff	---	sandy loam	Neutral to acid	One
23	June 1932	Cleared woodland	---	loamy	Neutral to Acid	Five
24	Sept. 1932	Meadow soil	62.72	clay	Neutral	Three
25	Sept. 1932	Top of prairie ditch	39.67	clay	Alkaline	Seven
26	Oct. 1932	Valley soil	30.17	clay loam	Very Alkaline	Two
27	Oct. 1932	Valley soil	30.17	clay loam	Alkaline	Four
28	Oct. 1932	Highland soil	28.91	sandy loam	Alkaline	One
29	Oct. 1932	Highland soil	28.94	sandy loam	Alkaline	One

Sample No.	Date sampled	Field Notations	Moisture Equivalent	Texture of Soil	Soil Reaction	Number of species identified
30	Oct. 1932	Garden soil	---	sandy loam	Alkaline	Three
31	Oct. 1932	Cultivated soil	17.33	silty loam	Neutral	Two
32	Oct. 1932	Cultivated soil	17.04	silty	Alkaline	Three
33	Oct. 1932	Cultivated soil	34.64	Loam	Neutral	----
34	Oct. 1932	Cultivated soil	34.38	loamy	Neutral	One
35	Oct. 1932	Cultivated soil	32.11	loamy	Neutral	Three
36	Oct. 1932	Cultivated soil	18.88	sandy loam	Neutral	Four
37		Bushland	31.93	sandy Loam	Alkaline	Two
38		Cultivated soil	15.43	fine sandy loam	Alkaline	Two
39	June 1932	Garden soil	27.70	loam	Alkaline	Four
40	June	Leaf Mat	---	Humus	Acid	Six

Sample No.	Date sampled.	Field Notations	Moisture Equivalent	Texture of Soil	Soil Reaction	Number of species identified
41	Oct.	Forest	12.76	Sandy loam	Neutral to Alkaline	Four
42	Oct. 1932	Soil beneath trees	---	sandy	Neutral	Two
43	Oct. 1932	Garden soil	---	loam	Alkaline	One
44	Oct. 1932	Prairie sod	12.92	sandy loam	Neutral to Alkaline	One
45	Oct.		35.63	clay loam	Acid	----
46	Sept. 1932	A_0 Leaf mat	121.72	Humus	Acid	----
47	Sept. 1932	A_1	48.60	clay	Acid	One
48	Sept. 1932	A_1	23.55	Sandy loam	Acid	One
49	Sept. 1932	A_2	11.54	sandy	Acid	One
50		Surface	31.77	sandy loam	Neutral to Acid	One

Sample No.	Date sampled.	Field Notations	Moisture Equivalent	Texture of soil	Soil Reaction	Number of species identified
51		Surface	15.16	sand	Acid	One
52	1927	Surface	6.88	fine sandy loam	Neutral	Two
53	1927	Surface	6.10	Coarse sandy loam	Neutral	One
54	10/7/32	Surface	23.50	loam	Neutral	One
55	10/7/32	Surface	31.86	silty clay loam	Neutral	One
56	10/7/32	Surface	23.66	sandy loam	Neutral	One
57	July 1932	Surface ← Fertiliz er	27.83	fine sandy loam	Alkaline	Two
58	Aug. 1932	Surface	42.16	Clay	Neutral	One
59	Fall, 1932	Surface ← (Fertil- (izer)	49.78	silty clay loam	Alkaline	One
60	Fall, 1932	Surface	42.13	fine sandy loam	Neutral	Two

Sample No.	Date sampled	Field Notations	Moisture Equivalent	Texture of Soil	Soil Reaction	Number of species identified
61	Aug. 1932	Surface	54.85	clay	Neutral	Two
62	July 1932	Surface	38.99	loam	Neutral	One
63	June 1932	Surface	34.13	sandy ? loam	Acid	One
64	June 1932	Surface	51.06	clay loam	Acid	One
65	June 1932	Surface	31.11	sandy loam	Alkaline	One
66	June 1932	Surface	25.09	loamy with very fine sand	Neutral to Acid	One
67	Oct. 1932	Northern Chernozem: Phytomorphic	37.67	sandy loam	Alkaline	One
68	Oct. 1932	Meadow Hydromorphic	55.91	clay loam	Acid	One
69	Oct. 1932	Degraded Chernozem: Podzol	23.49	clay loam	Neutral	One

Sample No.	Date Sampled	Field Notations	Moisture Equivalent	Texture of Soil	Soil Reaction	Number of species identified
70	Oct. 1932	Prairie Meadow	34.18	clay loam	Neutral to Acid	One
71		Surface	5.5	sand		
72		Surface	42.17	clay		One
73		Surface	37.11	gravelly loam		
74		Surface	27.51	clay		
75		Surface	35.12	clay		

SKETCH MAP OF MANITOBA SHOWING LOCATION OF SOIL SAMPLES UNDER INVESTIGATION. 1/

LEGEND:

Podzols--Ash-grey soils of forest belt.

Rendzina--Soils on lime-stone parent material.

Chernozem--Black-earth--loamy soils of prairie Belt.

The numbers refer to the sample number.

The dots mark the approximate location of the soil sample.

1. Manitoba Agricultural College. Site--Fort Gerry, Manitoba.
2. Manitoba Agricultural College. Fort Garry, Manitoba.
3. Manitoba Agricultural College. Fort Garry, Manitoba.
4. Manitoba Agricultural College. Fort Garry, Manitoba.
5. North Winnipeg, Manitoba.
6. North Winnipeg, Manitoba.
7. St. Vital, Manitoba.
8. St. Vital, Manitoba.
9. St. Vital, Manitoba.
10. St. Vital, Manitoba.
11. River.
12. The Brokenhead River, Manitoba.
13. Cook Falls, near Whitemouth, Manitoba.

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1/ Obtained from unpublished data from the Soils Department of the Manitoba Agricultural College.

14. Reynolds, Manitoba.

15. Sandilands, Dawson Trail, Manitoba.

16. Dufresne Station--two miles North, Manitoba.

17. Five miles east of Carman.)
) Adjacent to High-
18. Five miles east of Brunkild.) way No. 2.
)
19. Eight miles east of Morden.)
)
20. Five miles east of Manitou.)

21--22. Plympton (Meadowvale), Manitoba.

23. Ponemah Beach, Manitoba.

24. Half-mile east of Beaudray Station, Manitoba.

25. One mile west of Headingly, Manitoba. (Ditch).

26--29. Brandon Experimental Farm, Manitoba.

30--36. Morden Experimental Farm, Manitoba.

37. Burnside, Manitoba.

38. Bird's Hill. Manitoba.

39. St. James, Manitoba.

40. Between Victoria and Hillside Beach. (Near R. R. tracks).

41. Pine Ridge, Manitoba.

42--44. Aweme, Manitoba.

45. St. Norbert, Manitoba.

46--49. Riding Mountain, Manitoba.

50. Gilbert Plains, Manitoba.

51. South-west Portage la Prairie, Manitoba.

52. Sand Hills North of Glenboro, Manitoba.

53. Five miles north-east of Bird's Hill, Manitoba.

54. Killarney, Manitoba.

55. Minga, Manitoba.

56. Boissevain, Manitoba.

57. Altamont, Manitoba.

58. Teulon, Manitoba.

59. Kelwood, Manitoba.

60. Ochre River, Manitoba.

61. Dauphin, Manitoba.

62. Gilbert Plains, Manitoba.

63. Thunder Hill, Manitoba.

64. Harlington, Manitoba.

65. Roaring River, Manitoba.

66. Bowsman River, Manitoba.

67--70. Moline, Manitoba.

71. Prison Farm, Headingly, Manitoba.

72. Whitewater Lake near Deloraine, Manitoba.

73. Le Pas, Manitoba.

74. Hudson Bay Railway, Mile 37, Manitoba.

75. Mile 185. (Much farther North than indicated on Map).

3. CULTURAL METHODS.

In view of the fact that algae occur in the soil in close association with other micro-organisms such as bacteria and fungi, for the purpose of identification and particularly for the study of their physiological reactions, they must necessarily be isolated. This is carried out by their cultivation on artificially prepared media, i.e., under controlled conditions of temperature, moisture, and nutrient supply, etc. The methods developed by early workers for this purpose, and adopted by more recent algologists, will be described in connection with the work carried out for this paper.

Methods of Study of Soil Algae:

As already referred to, soil algae studies are based primarily on pure-culture methods, suggested by earlier workers, but developed as a laboratory technique and brought into common practice by Chodat of Geneva. The methods of "counting" so commonly used in the study of other groups of soil organisms, have been found by Bristol-Roach (8) to be of little value when applied to the study of soil-algae on account of the gelatinous sheath common to so many of the species.

The Media:

For this purpose artificial culture media are used, or at least artificial conditions are created. In many cases, therefore, no direct evidence is procured as to what is actually taking place in the soil under natural conditions.

Beijernick,[31] (1890), was the first to isolate algae in pure culture, using a medium consisting of ditch water to which 10 per cent gelatin had been added.

The media used in connection with this problem were either mineral (inorganic) salts solutions in the required proportions as recommended by Moore, and Bristol-Roach, or agar-nutrient media. The agar medium was found to be the most satisfactory for "plating" the algae, while the nutrient salts solution was most suitable to the growth of the algae. The "agar" medium was prepared by dissolving in a litre of distilled water the specified amount of agar, filtering and sterilizing; after which the inorganic salts desired were added. This on cooling solidifies. The nutrient salts solution is usually made up to a litre of distilled water and carefully sterilized. For example: 10 grams of melted agar added to Moore's nutrient solution was found by Schramm (23) to be an excellent medium for the growth of soil algae on plates. This was used with good results for a number of algae in this work.

Technique:

This was carried out with the utmost of care; cleanliness and sterilization of the vessels used being absolutely essential for good results. All the culture vessels were heated three separate times to a temperature of about 140 degrees C. and kept at that temperature for about three hours, to insure sterilization. The vessels were always plugged with

cotton-wool. The culture media were heated in a steam sterilizer for similar periods of time.

Cultivation of Algae:

All of the soil samples investigated were taken from various districts in Manitoba; as many as five samples being taken from a location. These were examined by means of cultures in mineral salts solutions set up with different degrees of dilution of well shaken soil suspensions, growth of algal vegetation taking place in from ten to fourteen days.

Methods of Isolation of Impure Cultures of Algae (30)

The methods of isolation of algae from the soil fall into methods for obtaining enrichment, crude, pure, and single cell cultures. Crude cultures were obtained from the original soil suspensions. It was sufficient to place the latter in the sunlight to obtain in a short time an abundant algal vegetation.

The "enrichment" culture methods consisted in making conditions favorable for normal development of the algae, which for the purpose of identification proved satisfactory. The pure culture methods deal with the processes of obtaining the algae free from contaminating organisms as well as from other species of algae, the enrichment culture being a preliminary step in the isolation of the pure culture.

The "Enrichment" Culture Method.

The cultures were set up in very carefully sterilized Erlenmeyer flasks and filled to their greatest diam-

eter with ground quartz, (15--20 grams) previously washed free from all suspended matter. The flasks were then plugged with cotton-wool and sterilized. The soil inoculum, consisting of 5 grams of soil to 15 cc of distilled water, was shaken in a test tube for 3 minutes and allowed to settle, after which 1 to 3 cc of the suspension was carefully transferred to the flasks by means of a sterile pipette. Various amounts of a nutrient salts solution were added to the flasks depending on the dilution desired. The nutrient solutions used were those suggested by Moore and by Bristol-Roach, having the following compositions:

Moore's Nutrient Solution:

NH_4NO_3----------0.5 gm.

$MgSO_4$----------0.2 gm.

K_2HPO_4----------0.2 gm.

$CaCl_2$----------0.1 gm.

$FeSO_4$----------trace.

Distilled water------1000 cc

Bristol-Roach's Nutrient Solution:

KH_2PO_4----------1.0 gm.

$NaNO_3$----------1.0 gm.

$MgSO_4$----------0.3 gm.

$CaCl_2$----------0.1 gm.

NaCl----------0.1 gm.

$FeCl_3$----------0.01 gm.

Distilled water------1000 cc

The nutrient solution was used in the following proportions: one cubic centimeter of the soil inoculum to 50, 100, 150, 300 cc, etc. of the solution. For purposes of comparison however, certain of the cultures were made with soil suspensions of different dilutions, and others with nutrient solutions diluted to 0.5 or 0.25 the original strength. In some instances too, more than one cubic centimeter of the soil inoculum was transferred to the culture. Table 3 shows some of the variations which were carried out in the culturing of samples 1 to 4:

TABLE 3. VARIATIONS CARRIED OUT IN CULTURING SAMPLES 1 to 4.

Sample No.	Amounts of soil suspension to water used in dilution.		Amount of diluted suspension used.	Amount of Nutrient Culture used.
	Soil suspension	Distilled water		
1 (a)	5 cc	50 cc	2 cc	50 cc
1 (b)	5 cc	50 cc	5 cc	100 cc
2 (a)	5 cc	100 cc	1 cc	300 cc
2 (b)	5 cc	100 cc	5 cc	100 cc
3	no dilution		1 cc	100 cc
4	no dilution		3 cc	100 cc

The cultures were placed in a north-east window in a room of maximum temperature (70 to 80 degrees), the bright light stimulating the growth of the algae and retarding the growth of other organisms. The first signs of growth were observed in the majority of the cultures at about the twelth day; (See Table 4 §, p.34 variations occurring in some cases, depending on a number of factors such as the degree of dilution, the condition of the soil, texture of soil, and its location, besides variations of the environmental conditions. Table §4 § shows the relative time taken for the growth of algae in the samples cultured. The enrichment culture method proved the most satisfactory for the growth of algae in a mixed culture.

Isolation of Pure-Cultures--The Separation of Species in Mixed Culture.

The separation of one form of algae from another was not a very difficult task, but to obtain these species free from other contaminating organisms such as bacteria and fungi proved almost impossible. Some of the species were isolated readily from the soil, and others only with difficulty. For morphological studies and classification it was only necessary to separate the various forms from each other and to cultivate them under artificial conditions. In this way a culture consisting solely of one species was obtained, but in nearly every case bacteria and fungi developed along with it so that a pure culture did not result. Since this

work, however, is mainly a study of the morphological characteristics of the algae for the purpose of identification, absolutely pure cultures of the algae were not necessary.

Methods of Separation of Species in Mixed Cultures:

The first attempt to separate the various algal forms was by the use of a sterilized platinum loop and the microscope. This method however, proved to be somewhat tedious and the results were not always certain to be successful. The most satisfactory results were obtained by culturing on solid media, the "agar-plate" method for the isolation of pure cultures 1/ of algae. This was carried out in several ways.

(1) In the case of samples 7 and 8 the algae were obtained as separate colonies directly from the soil by inoculation of the soil suspension into a sterile agar medium in a flask.

(2) The plate-method, suggested by Schramm (23) was used with some success. It consisted of procuring a suitable medium, one which would remain liquid down to a temperature at which delicate algal cells would not be injured, (34.5 to 35 degrees C), favorable to the growth of algae whilst being unfavorable to the growth of contaminating organisms. Schramm used the nutrient mineral salts solution recommended by Moore

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1/ Pure-culture (Waksman) (30) not necessarily free from bacteria.

with the addition of 10 grams of agar, (see page 15). This was carefully prepared, and sterilized in an autoclave, after which it was poured into sterile test tubes, plugged with cotton-wool and covered with wax-paper, for future use.

The alga to be plated was taken with as little adhering matter as possible by means of a sterilized platinum needle and carefully introduced into the tube of nutrient agar (6 to 8 cc) while the latter was still a few degrees above its congealing point (34.5 to 35 deg. C), thoroughly shaken for a few minutes and then poured into a Petri dish. The plates were allowed to cool and then turned upside down, so as to prevent the moisture from spreading the bacteria over the surface, and then placed in the light of a north-east window, and covered with a Bell-jar.

The first appearance of the algal colonies varied, usually taking from two to five weeks. This method was adopted in the isolation of species from cultures of samples 7, 12, 4, 5 and 41, the results of which are given in Table No. 5

TABLE 5 TO SHOW GROWTH OF ALGAE ON NUTRIENT AGAR PLATES.

Sample No.	Species	Date plated	Date of first appearance of algal colonies
7	Chlorococcum sp. Microthamion-strictissimum	Jan.9th /33	Jan.30th/33
12	Chlorococcum sp.	Feb.10th/33	Feb.29th/33
4	Nostoc sp.	Jan.9th /33	Jan.30th/33
5	Chlorococcum Nostoc	Jan.9th /33	Jan.30th/33
41	Chlorococcum	Mar.15th/33	Mar.24th/33

(3) The "streak" or "inoculation" method was carried out with some success. The broken-up mass of algal material was streaked out several times by means of a sterile platinum needle upon the surface of a solidified agar plate, so that each streak carried less of the inoculum than the preceding one. Growth here took from two to three weeks, depending on the dilution of the medium and quantity of inoculum used. This method was followed in the isolation of species from cultures of samples 1 to 4.

(4) The dilution method: The inoculum was placed in a test tube of melted and cooled nutrient agar, a series of successive transfers being made into other tubes, in this way obtaining a series of dilutions, and then poured into sterile Petri dishes. This method provides for the devel-

opment of deep algal colonies, while the "streak" method allowed for the development of surface colonies. Sub-cultures for isolation purposes were made by transferring the well-developed colonies to liquid media.

(5) Another pure-culture method suggested by Chodat, adopted and described by Bristol-Roach (6), was tried for experimental purposes, in the case of samples 7 to 11. None of the species so cultured however, were obtained free from contaminating organisms. The method consisted chiefly in growing the algae in dilute mineral salts solution, in flasks, exposing the cultures to bright sunlight, sub-culturing for enrichment purposes, by making dilute suspensions of the algae, well shaken in order to separate any bacteria or fungi from the algal cells, until the proportion of contaminating organisms to the algae is very low. Varying quantities of the suspension were than inoculated into flasks containing melted and cooled agar (34.5 to 35 deg. C), the colonies being allowed to develop in the solid agar in bright sunlight. The developing colonies were in this way separate from one another, facilitating the sub-culturing of the desired alga on a fresh medium. Sub-culturing was done by cutting out aseptically, the desired alga and transferring to a fresh medium.

The above methods were used in connection with the present problem, namely, the identification of species of algae occurring in the soils examined from various districts in Manitoba.

EXPERIMENTAL

One of the first experiments to be carried out in connection with this work was the culturing of a stone picked up on the edge of a flower-bed on the University Site, Fort Garry. The stone was place in 15 cc of Moore's nutrient solution and placed in the sunlight of a south window on May 11th, 1932. The first signs of growth were visible on May 15th, by the appearance of a thin film on the surface of the solution. By May 23rd, an intense green coloration was observed in the form of a ring around the sides of the test tube as well as throughout the solution. Macroscopically blue-green algae were seen to be present as well as green algae. Microscopically species of the Oscillatoriaceae, as well as the Chlorococcales were found to be present.

"Streak" culture methods were carried out in connection with the culturing of samples 1, 2, 5, and 23, the data for which is given in Table (6)

TABLE 6 RESULTS OF "STREAK-CULTURING" OF SAMPLES 1, 2, 5, and 23

Sample No.	Soil Type	Species	Date of inoculation of alga	Date of appearance of algal colonies
1	Garden soil (grassy)	Chlorococcum humicola	Sept.16th/32	Oct.12th/32
2	Garden soil (cultivated	Chlorococcum humicola	Sept.16th/32	Oct.12th/32
5	Soil in suspension of snow-water	Nostoc commune	Sept.16th/32	Oct.18th/32
23	Cleared woodland	Chlorococcum humicola	Sept.16th/32	Oct.20th/32

The table shows the relative length of time taken for the appearance of the various algal colonies on nutrient agar plates, by the "streak" method. A sub-culture of alga Chlorococcum humicola from sample 6 was made on January 9th, 1933 unto a fresh medium, the growth of the algal colonies being first observed on January 23rd, definite algal colonies appearing by February 11th.

On November 18th, 1932, "enrichment" cultures of samples 7 to 11, were set up as described, but nutrient agar) was used in place of the mineral salts solution.

An opportunity for observing the effect on the "growth" of the algae of nutrient agar as compared to nutrient salts solution was made in this way. The cultures were placed in the light of a north-east window and the algal colonies allowed to develop.

Examined with the unaided eye by transmitted light there was no growth visible until after two weeks when colonies appeared in all cultures except one. In this case the soil inoculum was added to the agar before it had cooled sufficiently, in all probabilities killing the algae introduced. The experiment was repeated, using the nutrient salts solution instead of agar and algal growth was clearly visible within ten days.

On January 9th, 1933, algae from the enrichment culture of sample 7 were plated by the "dilution" method, and on January 30th, the first appearance of the algal colonies were observed, scattered throughout the plate, and by February 11th, the colonies were well defined, when observed under the 16 mm objective. Various algal forms were noted, but one in particular stood out because of its branched form. It was later identified as Microthamnion strictissimum, a species which hitherto has not been recorded as occurring in the soil, nor has it been previously recorded in Manitoba. It was first described by L. Rabenhorst, (19), in 1851, as an aquatic form. See Page (61a), Plate 1. Fig. 1

Samples of soil from Brandon Experimental Farm were cultured on January 23rd., 1933. Growth of algae in these

soils varied somewhat, samples 26 and 27 developing more vigorously than the others. The first signs of growth were visible in cultures of samples 26 and 27 on February 11th, growth in samples 28 and 29 on February 15th. The results here as in other soils are dependent upon a number of factors found by early and recent workers to influence the "growth" of algae. These factors are related to the "nature" of the soil, and include texture, (clay, sand or loam, etc.), soil reaction, (acid or alkaline), depth, moisture content, and nutrient supply. Looking at Tables 2+4 it will be seen that those soils in which the algae developed most rapidly are more alkaline than the others, and that their moisture equivalents are higher. It was noted also that these samples were taken at different depths: samples 26 and 28 being 6"X6"X2" up to two inches in depth, and samples 27 and 29, 6"X6"X4" up to four inches in depth.

Observations upon the growth of soil algae in the samples from Riding Mountain show that the distribution of algae in the soils are uniform within the upper few inches, there being more in the top inch, than in the second, decreasing in the third, and increasing again in the fourth inch after which they decrease rapidly. This agrees with the observations made by Bristol-Roach (5) at Rothamstead, and can be attributed to the biochemical activity of algae both on the surface of the soil, in the sunlight, where they synthesize their protoplasm from the carbon dioxide of the air; and from

the water containing inorganic nitrogenous and mineral compounds, adding considerable organic matter to the soil; and below the surface in the dark, where conditions of temperature and moisture are more uniform and where they are capable of utilizing the organic matter present for their metabolism, adding nothing either to the energy or the food content of the soil. Beyond this depth, where there is only a trace of organic matter, they were found to decrease rapidly.

Robbins (20), believes that the depth to which algae will extend, is largely dependent upon the texture of the soil, its ventilation and methods of cultivation; irrigation also playing a part in their distribution. This was found to be the case with the majority of the samples examined. Algae were found to occur more abundantly in cultivated, artificially aerated soils, than in virgin undisturbed soils. This was brought out especially in the culturing of samples 67 to 70, which offered an interesting group for the observation of the relative length of time required for the development of algae in soils of different types, taken at different depths. These samples were all from the same district, but of different "associates" 1/ (13), collected in October

1/ "The Associate"--(13) (determined by relief and local environment). Associate--the "soil types" found in association on a given parent material.

of 1931, and cultured on March 6th. 1933. Table (7) gives the data for each of the samples, column 6 giving the relative length of time required for the development of the algae in these soils.

TABLE 7 COMPARISON OF DEVELOPMENT OF ALGAE IN SAMPLES 67 to 70.

(1) Sample No.	(2) Horizon depth	(3) Soil Zonal Type	(4) The "Associate" 1/	(5) Soil Reaction	(6) Length of time for development of algae
67	0---3"	Chernozem (black-earth) loam	Phytomorphic (normal)	pH--7.5	25 days
70	0---7" Transition	Meadow-Prairie Chernozem	Phyto-hydromorphic	pH--6.9	11 days
68	0---8"	Northern Chernozem Meadow	Hydromorphic (locally) (humid)	pH--6.6	24 days
69	2½"-7"	Meadow-Podzol (degraded Northern Chernozem)	Hydromorphic	pH--6.8	18 days

1/ "The Associate" (13)--determined by relief and local environment). Associate--the "soil types" found in association on a given parent material.

Samples from Riding Mountain were taken in September, 1932; those from Brunkild, Morden and Manitou in June, 1932. The cultivated series were cultured on January 23rd, 1933, Moore's nutrient solution being used, the first appearance of algal growth being visible in the cultures after 17 days. Those of the uncultivated series, (Nos. 46, 48, and 49), were cultured on February 20th, 1933, and showed no visible signs of growth until March 13th. A glance at Table ⟨ 2 ⟩ shows the cultivated series of soils to be alkaline in reaction, while the uncultivated soils are more or less "acid". This confirms the point brought out by early workers that soil algae thrive much more vigorously in a somewhat alkaline medium. This would also account for the belief that the presence of algae in the soil has something to do with the fertility of the soil. Although no definite proof has been as yet established, it is believed, (Kossowitch), (15), that the algae in their gelatinous sheaths, provide easily available carbohydrates, from which the bacteria derive the energy essential to their work, and that nitrogen-fixation in nature is due to the combined working of a number of different organisms rather than to the individual action of a single species. Thus it was interesting to note the presence of moss protonemae in association with the algae in sample 38, since it has been demonstrated by Servettaz,(24) Von Ubisch, (29) Robbins(20) and others that the protonemae of some mosses can make use of certain organic substances especially the sugars,

and grow vigorously in the dark. It has been shown however, that further development of the moss must take place in the presence of sunlight. (5)

Nutrition of Algae:

Many workers have shown that the nutrition of the higher algae is essentially that of the green land plants. The surface layer of soil is a habitat having all the physiological conditions necessary for algal growth, and it is not surprising to find that these organisms have a fairly wide distribution in this habitat. The introduction of bacteriological technique to algal cultures led such workers as Beijernick(2), Artari(1), Chodat(11) and Bristol-Roach(5-9) to the introduction of bacteriological technique to algal cultures. This led to the investigations of the physiological behaviour of some of the lower algae in the hope of understanding some of the fundamental problems underlying the nutrition of organisms containing chlorophyll in their protoplast. Microbiological studies have shown that certain other unicellular and multicellular micro-organisms such as Euglena viridis, and moss protonemae, etc., are related to the algae by virtue of their possession of chlorophyll. Table A, adopted from Bristol-Roach (5), gives a brief classification of the chlorophyll-bearing micro-organisms found in the soil:

TABLE A. A BRIEF CLASSIFICATION OF THE CHLOROPHYLL-BEARING MICRO-ORGANISMS FOUND IN THE SOIL.

	GROUP		COLOR	PIGMENT
		Euglenineae		
I.	Flagellata	Cryptophyceae	green	Chlorophyll
	Algae	A. Isokontae		
II.	1. Chlorophyceae	i. Chlorococcalee	green	Chlorophyll
		ii. Ulothricales		
		iii. Volvocales		
		B. Heterokontae	yellow-green	Chlorophyll-xanthrophyll
	2. Bacillariales (Diatomales)	Mostly Pennatae and Naviculoidae	yellow or golden-brown chromatophores	carotin Chlorophyll xanthrophyll
	3. Myxophyceae (Cyanophyceae)	Mostly filamentous Oscillatoriaceae and Nostocaceae	blue-green	Phycocyanin chlorophyll
III.	Bryophyta	Moss protonema	green	Chlorophyll

"Soil Algae" therefore, Bristol-Roach states, (5), are, "those micro-organisms of the soil which have the power, under suitable conditions, to produce chlorophyll", and further she believes that this definition is wide enough to include other chlorophyll-bearing micro-organisms of the soil and suggests that the name "chlorophyll-bearing protophyta" be given to this whole group.

The study of soil algae has been approached from two very different view-points according to the environmental conditions under which the organisms may be living. According to the first, the soil is merely a special case of a much wider class of habitats; including the surface of rocks, palings, trees, roofs and walls of buildings, etc. Algae growing in these habitats have been named "terrestrial" by Fritsch (14), and are directly subjected to climatic conditions: rain, dew, wind and changes in temperature. Algalogists taking this point of view emphasize the importance of water supply for their growth. Those taking the second point of view, on the contrary, regard true soil algae as regular inhabitants of the soil itself, capable of growth both on the surface and below the ground. On the surface exposed to sunlight the algae are capable of fixing the energy of the sunlight, and of assimilating carbon dioxide by means of their chlorophyll, producing complex organic matter. In this capacity the algae supply energy material to the soil. Below the soil, in the absence of light, photosynthesis

ceases, even though chlorophyll is present in the protoplast, the organisms obtain their energy and carbon from pre-formed organic matter, (humus, dung, excreta, etc.), in the soil, and so live saprophytically. Here moisture supply is not so important a factor as carbon nutrition in the absence of light. Dr. Bristol-Roach's experiments (6) have revealed the fact that soil algae always retain this property of assimilating complex organic matter, even when living in light, as on the surface of the soil, in which case the chlorophyll apparatus is regarded as supplementary, only coming into play in the presence of light. The soil therefore, as a suitable culture medium for the development of algae is an important factor in considering the occurrence and distribution of these organisms in it and accounts for their apparent abundance in some types of soil as compared to others. Esmarch, (1910), (5), in his investigations observed that in cultivated soils, the algae were not confined to the surface but occurred at lower depths, this he ascribed to the presence of resting spores carried down by soil cultivation and seepage of surface water, earthworms, and other soil organisms, since samples from uncultivated soils at the same depth were unproductive. He also observed that in general cultivated soils contained a greater number of blue-green algae than uncultivated soils. From his observations he concluded that the two chief factors governing distribution of algae on the surface of the soil were moisture, and the

availability of mineral salts. What Esmarch found to be true for his soils was found to hold for Manitoba soils in comparing the growth and species of algae in samples 18, 19 and 20, (cultivated), with those of 46, 48 and 49, (uncultivated), the results of which are given in Table (8).

TABLE 8 COMPARISON OF GROWTH AND SPECIES OF ALGAE IN CULTIVATED AND UNCULTIVATED SOILS IN MANITOBA.

Cultivated Soil from Brunkild, Morden, and Manitou, Manitoba			Uncultivated Soil from Riding Mountain, Manitoba.		
Sample No.	Species	No. of days	Sample No.	Species	No. of days
18	Chlorococcum-humicola Arthrospira-jenneri Lyngbya sp. (Nostoc commune Navicula mutica	17	46	No growth	Feb. 20--Mar. 31
19	Chlorococcum-humicola Hantzschia amphioxys Navicula mutica	17	48	Chlorococcum humicola	22
20	Ulothrix subtilis Chlorococcum-humicola Phormidium tenue Phormidium-autumnale	17	49	Chlorococcum humicola	22

TABLE 4. RESULTS OF CULTURAL EXPERIMENTS.

Sample No.	Location	Date cultured	Date of growth	Species found
1	M. A. C.	May 23/32	June 6/32	Phormidium autumnale Chlorococcum humicola Nostoc sp. (1) Phormidium tenue
2	M.A. C.	May 23/32	June 6/32	Nostoc commune Chlorococcum humicola Phormidium autumnale Oscillatoriaceae sp. Phormidium tenue
3	M. A. C.	May 23/32	June 9/32	Chlorococcum humicola Hantzschia amphioxys Phormidium autumnale Phormidium tenue Navicula mutica
4	M. A. C.	May 23/32	June 16. 1932	Nostoc commune Chlorococcum humicola Phormidium tenue
5	North Winnipeg	May 23/32	June 12 1932	Chlorococcum humicola Nostoc sp. Dactylococcus bicaudatus Phormidium autumnale Phormidium tenue
6	North Winnipeg	May 23/32	June 16 1932	Chlorococcum humicola Nostoc sp. Stichococcus bacillaris Microthamnion strictissimum
7	St.Vital	Nov.18/32		Gongrosira terrocola Chlorococcum humicola Microthamnion strictissimum

Sample No.	Location	Date cultured.	Date of growth	Species found.
8	St.Vital	Nov.18/32		Chlorococcum humicola
9	St.Vital	Nov.18/32		Chlorococcum humicola
10	St.Vital	Nov.18/32		Chlorococcum humicola
11	St.Vital	Nov.18/32		Chlorococcum humicola Phormidium autumnale Phormidium tenue Moss protonema Hantzschia amphioxys Navicula mutica
12	Broken-head	Dec.1/32		Chlorococcum humicola Chlamydomonas communis Ulothrix subtilis Stichococcus bacillaris Oscillatoriaceae sp.
13	Cook Falls near Whitemouth	Dec. 1/32	Jan. 4/33	Stichococcus bacillaris Ulothrix subtilis Chlorococcum humicola Chlamydomonas communis Phormidium tenue
14	Reynolds	Dec. 1/32	Jan. 4/33	Ulothrix subtilis Chlorococcum humicola
15	Dufresne Sandilands	Dec. 1/32	Jan. 4/33	Navicula mutica Chlorococcum humicola Young Nostocace colonies. Hantzschia amphioxys

Sample No.	Location	Date cultured	Date of growth	Species found
16	Dufresne Station	Dec. 1/32	Jan. 4/33	Ulothrix subtilis Chlamydomonas communis
17	Carman	Jan. 23/33	Feb. 9/33	Chlorococcum humicola
18	Brunkild	Jan. 23/33	Feb. 9/33	Chlorococcum humicola Ulothrix subtilis Arthrospira jenneri Lyngbya sp. (sheath yellow) Nostoc commune Navicula mutica
19	Eight miles East of Morden	Jan. 23/33	Feb. 9/33	Chlorococcum humicola Hantzschia amphioxys Navicula mutica
20	Five miles East of Manitou	Jan. 23/33	Feb. 9/33	Ulothrix subtilis Phormidium autumnale Phormidium tenue
21	Meadow-vale	Feb. 20/33	Mar. 6/33	Chlorococcum humicola Nostoc commune Moss protonema
22	Meadow-vale	Feb. 20/33	Mar. 6/33	Nostoc sp. (2)
23	Ponemah Beach	Feb. 20/33	Mar. 6/33	Navicula mutica Chlorococcum humicola Nostoc commune Gomphonema sp. Ulothrix subtilis

Sample No.	Location	Date cultured	Date of growth	Species found
24	Beaudray Station	Jan.23/33	Feb. 9/33	Phormidium autumnale Phormidium tenue Euglena sp.
25	West Heading-ly	Jan.23/33	Feb. 9/33	Scenedesmus obliquus Chlorococcum himicola Chlamydomonas communis Ulothrix subtilis Nostoc ellipsosporum Hantzschia amphioxys Moss protonema
26	Brandon Exp.Sta.	Jan.23/33	Feb.11/33	Chlorococcum humicola Chlamydomonas communis
27	Brandon Exp.Sta.	Jan.23/33	Feb.15/33	Chlorococcum humicoal Nostoc sp. Navicula mutica Navicula sp.
28	Brandon Exp.Sta.	Jan.23/33	Feb.15/33	Chlorococcum humicola
29	Brandon Exp.Sta.	Jan.23/33	Feb.11/33	Chlorococcum humicola
30	Morden Exp.Sta.	Jan. 6/33	Jan.23/33	Chlamydomonas communis Chlorococcum humicola Nostoc muscorum
31	Morden Exp.Sta.	Jan. 6/33	Jan.23/33	Chlamydomonas communis Chlorococcum humicola
32	Morden Exp.Sta.	Jan. 6/33	Jan.27/33	Chlorococcum humicola Chlamydomonas communis Nostoc commune

Sample No.	Location	Date cultured	Date of growth	Species found
33	Morden Exp. Sta.	Jan. 6/33	Jan. 30/33	---------- ---
34	Morden Exp. Sta.	Jan. 6/33	Feb. 1/33	Chlorococcum humicola
35	Morden Exp. Sta.	Jan. 6/33	Jan. 23/33	Chlamydomonas communis Oscillatoriaceae sp. Ulothrix subtilis
36	Morden Exp. Sta.	Jan. 6/33	Jan. 23/33	Chlamydomonas communis Phormidium tenue Ulothrix subtilis Chlorococcum humicula
37	Burnside	Jan. 9/33	Feb. 9/33	Chlamydomonas communis Ulothrix subtilis
38	Bird's Hill	Dec. 1/32	Jan. 10/33	Ulothrix subtilis Moss protonema Chlorococcum humicola
39	St. James	Jan. 23/33	Feb. 9/33	Chlamydomonas communis Chlorococcum humicola Phormidium tenue (definitely constricted) (form) Hantzschia amphioxys
40	Between Victoria and Hillside Beaches	Jan. 23/33	Feb. 13/33	Phormidium autumnale Ulothrix subtilis Phormidium tenue Chlorococcum humicola Chlamydomonas communis Dactylococcus bicaudatus

Sample No.	Location	Date of culturing	Date of growth	Species found
41	Pine Ridge	Jan.23/33	Feb. 9/33	Chlamydomonas communis Chlorococcum humicola Nostoc sp. Like muscorum, larger type. Phormidium tenue
42	Aweme	Feb.20/33	Mar. 2/33	Chlorococcum humicola Ulothrix subtilis
43	Aweme	Feb.20/33	Mar. 1/33	Chlorococcum humicola
44	Aweme	Feb.20/33	Mar. 6/33	Chlorococcum humicola
45	St. Norbert	Feb.20/33	Mar. 9/33	-- ----------
46	Riding Mountain	Feb.20/33		- ----------
47	Riding Mountain	Feb.20/33	Mar.13/33	Chlorococcum humicola
48	Riding Mountain	Feb.20/33	Mar.13/33	Chlorococcum humicola
49	Riding Mountain	Feb.20/33	Mar.13/33	Chlorococcum humicola
50	Gilbert Plains (1)	Feb.20/33	Mar. 6/33	Chlorococcum humicola
51	S. W. Portage la Prairie	Feb.20/33		Chlorococcum humicola

Sample No.	Location	Date cultured	Date of growth	Species found
52	Sand - hills North of Glenboro	Feb.20/33	Mar.10/33	Chlorococcum humicola Chlamydomonas communis
53	Five miles N.E. of Bird's Hill	Feb.20/33		Chlorococcum humicola
54	Killarney	Mar. 6/33	Mar.22/33	Chlorococcum humicola
55	Minga	Mar. 6/33	Mar.24/33	Chlorococcum humicola
56	Boissevain	Mar. 6/33	Mar.24/33	Chlorococcum humicola
57	Altamont	Mar. 6/33	Mar.24/33	Chlamydomonas communis Chlorococcum humicola
58	Teulon	Mar. 6/33	Mar.27/33	Chlorococcum humicola
59	Kelwood	Mar. 6/33		Chlorococcum humicola
60	Ochre River	Mar. 6/33	Mar.22/33	Chlamydomonas communis Chlorococcum humicola
61	Dauphin	Mar. 6/33	Mar.17/33	Chlorococcum humicola Chlamydomonas communis

Sample No.	Location	Date cultured	Date of growth	Species found
62	Gilbert Plains (2)	Mar. 6/33		Chlorococcum humicola
63	Thunder Hill (Swan River)	Mar. 6/33		Chlorococcum humicola
64	Harlington (Swan River)	Mar. 6/33	Mar.24/33	Chlorococcum humicola
65	Roaring River (Swan River)	Mar. 6/33	Mar.24/33	Chlorococcum humicola
66	Bowsman River	Mar. 6/33	Mar.24/33	Chlorococcum humicola
67	Moline	Mar. 6/33		Chlorococcum humicola
68	Moline	Mar. 6/33		Chlorococcum humicola
69	Moline	Mar. 6/33	Mar.24/33	Chlorococcum humicola

Sample No.	Location	Date cultured	Date of growth	Species found
70	Moline	Mar. 6/33	Mar.17/33	Chlorococcum humicola
71	Stone-wall	Mar. 6/33	Mar.20/33	---------- -
72	Delor-aine	Mar. 6/33	Mar.20/33	Chlorococcum humicola
73	Le Pas	Mar. 6/33	Mar.20/33	
74	Le Pas Mile 37	Mar. 6/33		
75	Le Pas Mile 185	Mar. 6/33		

4. CULTURAL RESULTS.

Algae were found to be present in the soil both in the vegetative form and in the form of resting spores. Bristol, 1927, (8), devised a method based on the known fact that resting cells can withstand conditions of desiccation, that are fatal to vegetative cells, by which she showed that soil algae are actually present in large numbers in the vegetative condition. This important contribution has increased the status of these organisms as members of the soil population to a position comparable with that of the other groups of soil organisms. (8).

In examining the algae, in following out their life-history and in identifying them, they were carefully drawn to scale with the aid of a camera-lucida. This was found to be the surest way, after determining the magnification, of obtaining accurate measurements of the alga, and in a great many instances proved to be the deciding factor in the identification of a particular species.

A certain amount of difficulty was experienced in the identification of the algae found in the cultures of the soils studied. This may have been due to a number of reasons. Firstly, to the condition of the soils which varied greatly, one having been cultured while still in a moist condition, others having been air-dried over a period of months, while some had been stored over a period of years, and so could contain only resting spores in a viable con-

dition. The length of time taken for the germination of resting cells varied in individual species, and observation of their development from day to day in a hanging-drop culture was necessary before the algae could be identified with any certainty, especially in distinguishing between the unicellular forms in a mixed culture, since a number of the lower algae pass through stages in their life-histories in which they closely resemble one another. In identifying the unicellular alga Chlorococcum-humicolum, (Naeg.), in culture one and two, its development under a Van-Teighm cell was necessary. At the time of preparation of the hanging-drop culture, (Oct.12th, 1932), the cell-walls of the organism were clearly visible, starch was present, and two to four pyrenoids were observed. Division of the cells into two, and then into four, etc, was also noted. Two days later, reddish colored cells were found to be present, the red color being due to the presence of reserve food material in the form of reddish oil.

A second factor which made identification of the various forms difficult was the somewhat artificial conditions under which the algae were growing, excessive moisture and nutrient supply, variations in temperature and sunlight tending to produce abnormal forms in some cases. It was therefore necessary to decide whether these variations were the result of the conditions or whether they were characters of a new species. The organism giving the most

difficulty was Chlorococcum humicolum, (Naeg.), (Rabenh.), which before its identification was complete had to be isolated. This was carried out by carefully picking out individual cells with a capillary pipette, under the low power objective, and placing them in a hanging drop culture in a damp chamber. In this way the complete life-history of the organism was observed, and its identification finally determined. It was interesting to note that all stages in the life-history of this alga were observed in the majority of the cultures examined, so that it would seem that this species is more or less a regular inhabitant of Manitoba soils. The occurrence of unhealthy forms of Chlorococcum humicolum in a few of the crude cultures examined made recognition increasingly difficult. When isolated however, from other contaminating organisms, normal development of healthy colonies made identification possible.

For identification of the diatoms the method adopted by Bristol-Roach, (6) was followed. This consisted in fixing the material in a permanent condition and then carefully studying the markings on the walls under an extremely high magnification.

Another factor, mentioned by Bristol-Roach, (6), which might prevent immediate identification of a algal species is "competition" between various species in mix-cultures, resulting in the development of abnormal forms

which must necessarily first be isolated before identification could be definitely established.

Examination of the cultures of the soils under investigation showed a characteristic algal flora for Manitoba. Twenty-five species of algae were identified. Of these eleven were species of Myxophyceae, (blue-green), nine Isokontae, (Chlorophyceae), and five Bacillariales (Diatomales). Besides these, species of Euglena, (Flagellata), and moss protonema, (Bryophyta), were found in association with the algae. It was observed that although more species of Myxophyceae were identified, they were less universally distributed than the Chlorophyceae, the latter occurring in ninety per cent of the soils examined, while the Diatoms appeared the least frequently, being more or less restricted to damp "habitats."

A characteristic group of algae consisting of the species Chlorococcum humicolum, (Naeg.), Chlamydomonas communis, (Snow), Nostoc commune, (Vauch.), Ulothrix subtilis, (Kutz.), Phormidium autumnale, (Ag.) and Phormidium tenue, (Menegh.), were found to predominate. It was also noted that in a number of the samples, associations between two or more species prevailed. Phormidium autumnale and Phormidium tenue, (Myxophyceae), were found occurring together in eight of the eleven cultures in which one or the other were observed, likewise Ulothrix subtilis was found in association with Chlamydomonas communis in a num-

ber of the cultures. The typical soil diatoms Hantzschia amphioxys and Navicula mutica occurred together in four of the nine cultures in which one or the other was identified.

It is a feature of the cultures that certain algal species regarded as typically of the soil were either completely absent or else occurred in relatively few of the samples. On the other hand certain species developed in the cultures which formerly have not yet been recorded as occurring in the soil. Of the twenty-five species of algae identified, two nommally submerged aquatics were recorded for the first time in Manitoba. These were the species Dactylococcus bicaudatus and Microthamnion strictissimum, the latter being recorded for the first time in Canada. Another organism, Gongrosira terricola, (Bristol), is also recorded for the first time in Canada. This unusual form was obtained in a culture of the soil from an unplowed field in the vicinity of the St. Vital Sanatorium on the east bank of the Red river. Microthamnion-strictissimum, (Rabenh.), also having been obtained from this locality. It was isolated on an nutrient agar plate, well defined colonies appearing within a month's time.

See Plate 3 Page 61a

The blue-green Nostocaceae were found to be common to the cultivated soils examined, more species of Nostocaceae being identified than in any other one family.

To facilitate the classification of soils, for soil survey purposes, Manitoba has been divided into "Zones" and "Sub-zones", (See Map Page 9), the information for which was obtained from the Soils Department of the Manitoba Agricultural College. To demonstrate the relative distribution of the algal species identified in the various soil sub-zones, Chart No 2 has been drawn up. Looking at the Chart we find that there seems to be an indication towards a "zonal" distribution of the species found. More species were found in those soils of the Eastern zone than in those of the Northern zones, while some species found occurring in one were absent in others. The vertical lines in the Chart mark off divisions in which the species occur, in this way showing the relative distribution of the algal species in the particular soils studied. The number at the foot of each division indicates the total number of different species occurring in each of the sub-zones. From these we may conclude that the occurrence of algae in soils show an inclination towards a zonal distribution. However, until further data can be obtained, concerning this possibility, these observations should not be considered as conclusive.

Cultural results would also seem to indicate that there is a correlation between the distribution of algal species found occurring in the soil and the soil texture, this latter being dependent on the moisture equivalent of the soil. To show this possibility, Chart No.3 has been

CHART NO. 2

RELATIVE DISTRIBUTION OF ALGAL SPECIES
IDENTIFIED IN THE VARIOUS SOIL ZONES OF MANITOBA.

ALGAL SPECIES	A. PRAIRIE ZONE																								B. TRANSITION ZONE			C. FOREST ZONE																
	1. Red River Valley								2. Assiniboine Delta						3.								4		5. Woodland Invasion of Prairie			6.											7.			8.		
	Clay. Meadow-Prairie.								Sandy Chernozem						Normal Chernozem								Northern Chernozem					Eastern Podzolic											Grey forest			Northern Podzols.		
Sample No =	24	25	39	1	45	18	17	19	51	37	42	26	64	70	30	57	52	20	54	55	56	72	67	68	63	64	65	40	12	3	13	53	38	7	16	21	14	15	59	46	50	73	74	75
1. Chlamydomonas communis		•	•							•		•			•	•	•											•	•		•				•									
2. Chlorococcum humicola		•	•	•		•	•	•	•		•	•	•	•	•	•	•		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•		•	•	•	•		•			
3. Dactylococcus bicaudatus																												•																
4. Scenedesmus obliquus		•																																										
5. Ulothrix subtilis		•				•				•	•							•										•	•		•		•		•		•							
6. Ulothrix sp.																																												
7. Stichococcus bacillaris																													•		•													
8. Microthamnion strictissimum																																		•										
9. Gongrosira terricola																																		•										
10. Navicula mutica						•		•																						•								•						
11. Gomphonema sp.																																												
12. Hantzschia amphioxys		•	•					•																						•								•						
13. Oscillatoriaceae sp.																													•															
14. Arthrospira Jenneri						•																																						
15. Phormidium autumnale	•			•														•										•		•														
16. Phormidium tenue	•	•		•		•												•										•		•	•													
17. Lyngbya sp.																																												
18. Nostoc sp (1.)				•																																		•						
19. Nostoc commune						•									•																					•								
20. Nostoc muscorum																																												
21. Nostoc ellipsosporum (major)		•																																										
22. Nostoc sp. (2)																																												
No of Species occuring =	12.								3.						6.								1.		1.			14.											2.			0.		

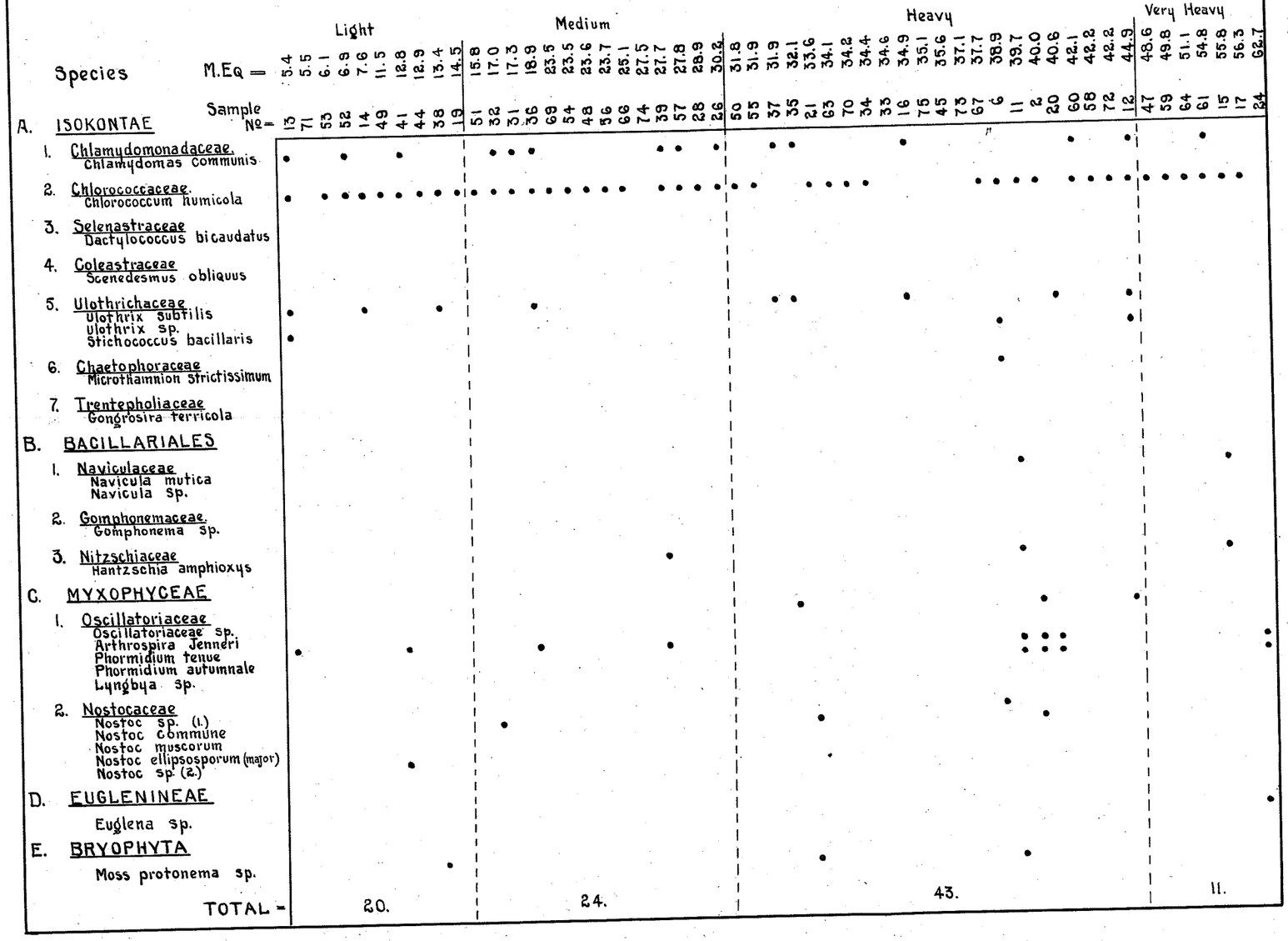
drawn up, in which the soil samples are arranged in order of increasing moisture equivalents, so that the soils may be: light, medium, heavy and very heavy; or sand, sandy loam, loam, clay loam and clay. The chart shows that from the soil samples studied, indications are that the majority of the species occur throughout the various textures, but five different species occurs in soils of heavy texture which did not appear in the light textured soils; another alga occurs both in the light and the heavy soils, while one particular form occurs only in the light textured soils. These observations however, are not conclusive because of the variations in time which elapsed between sampling in the field and culturing in the labofatory.

Another interesting comparison between the occurrence of algal species in the samples in the sub-zones, (Chart 2), arranged according to texture, (i.e.,within the zone,) shows that there exists no decided correlation between the texture of the soil within the zone and the occurrence of algal species; so that the general trend of algal distribution in the soil would seem to be zonal rather than textural.

An examination of these results leads to several interesting hypotheses in relation to the occurrence and distribution of the algae in the soils studied. Until further research in this field is carried out,however, no definite conclusions can be drawn.

CHART Nº 3.

RELATIVE DISTRIBUTION OF ALGAL SPECIES FOUND IN SOILS OF DIFFERENT TEXTURE.



5 DESCRIPTIVE NOTES ON SPECIES OF ALGAE FOUND IN SOME MANITOBA SOILS.

A. (ISOKONTAE).

CHLOROPHYCAE.

1. Chlamydomonadaceae (Volvocales).

Chlamydomonas communis. (Snow) Plate 1 Fig. 1 Page 59a

This species was found in eighteen of the soils cultured in widely distributed localities. In its most vigorous and normal motile condition it resembles the typical Chlamydomonad cell. Its shape is ovoid to ellipsoid, rounded at the posterior end and narrower at the anterior end. Length 10.5 to 13 mic., breadth, 5 to 6.5 mic. Bright green in color. Protoplasmic flagella of equal length continuous with protoplasm of cell and attached at the anterior end. Prominent pyrenoid body near centre of cell; stroma, starch, pigment spot an inconspicuous rod; a single bell-shaped chloroplast lining the membrane of the cell except for a very small area just at the anterior end at which point two vacuoles can be seen. The nucleus occupies a position between the centre and the anterior end of the cell, observed by staining with picro-caramine.

11 Chlorococcaceae.

Chlorococcum-humicola. (Naeg.) Rabenh. Plate 1 Fig. 2 Page 59a

This species occurred in the cultures of every soil. Cells: spherical--massed to form a stratum. Breadth, 3 to

20 mic. sometimes larger. Vegetative cells, 6 to 8 mic. in diameter were observed; resting cells, (akinetes), 10 to 12 mic. were also seen. A single parietal chloroplast bell-shaped to spherical with an aperture on one side usually occupying practically the whole periphery of the cell, with a median pyrenoid; (several observed in older cells); membrane relatively thin; reproduction variable; nuclear division into two, four, eight, etc., cells. Akinetes with a thick wall and filled with a reddish-orange colored oil, and aplanospores were also observed.

III Selenastraceae.

Dactylococcus bicaudatus. (Hansg.) Plate 1, Fig. 3 Page 59a

This species was found in two of the soils cutured. The cells were typically lunate with acute apicies, prolonged into spine-like processes, either solitary or in loose colonies with two to five cells; cell-wall thin. Chloroplast single; parietal with more or less distinct pyrenoid body. Length 13 to 19 mic. Breadth 2.5 to 5.8 mic. Multiplication by oblique longitudinal division of protoplast with aplanospore development was observed.

Locality: Between Victoria and Hillside Beach, Manitoba, beside ditch along railroad track.

IV Coleastraceae.

Scenedesmus obliquus. (Turp.) Kutz. Plate 2, Fig. 1, Page 60a

This species was found in one soil Cells were ob-

served in groups of four or eight; ellipsoid to fusiform in shape and arranged with their longitudinal axes parallel and generally in one plane; either in a single row or in two alternating rows, forming a coenobium. Length, 10 to 15 mic. Breadth, 5 to 7 mic. Chloroplast, parietal, cup-shaped, often occupying the whole length of the cell, usually with a single pyrenoid body. Cells with delicate muscilaginous envelopes outside cellulose wall. This organism was found in soil from the top of a prairie ditch in Headingly, West of Winnipeg.

V Ulothrichaceae (Ulothrichales)

Ulothrix subtilis (Kutz.) Plate 2 Fig. 2 Page 60a

This species, next to Chlorococcum-humicola, was the most commonly occurring green algae, having been identified in fourteen of the soils cultured. The plant body was found to consist of simple unbranched filaments.--9 mic. thick, with cells as long as broad; cells 4 to 7 mic. in diameter, filaments encased in muscilaginous sheaths. Cells uninucleate, with one or more pyrenoids. Reproduction by fragmentation of filaments. Reproductive stages observed showed formation of aplanospores and akinetes by the enlargement of certain of the vegetative cells of the filament.

Stichococcus bacillaris. (Naeg). Plate 2 Fig. 3 Page 60a

This species was found in the cultures of three soils.

The condition of the cultures was such that the filamentous form of this species was very rarely observed. Filaments very short, consisting of not more than three cells, readily fragmenting into individual cells; cells somewhat rectangular in general, with rounded ends and thin walls, but may be irregular in form; about 3 to 6 mic. long and 2.7 to 3.8 mic. broad, with a parietal chloroplast occupying only half the cell. Reproduction by fragmentation was observed. This species was found in soils from the Brokenhead River district and Cook Falls near Whitemouth, Man.

VI Chaetophoraceae.

Microthamnion strictissimum. (Raben.) Plate 3 Fig.1 Page 61a

This species was found in two of the soils cultured. The plant body, which is macroscopic, was observed as a branched, filamentous thallus, composed solely of the projecting system--threads extensively branched--filaments not more than 1 mm in height--hairs absent; branches were found to arise immediately beneath a septum--these were either long or short. Cells thin-walled, cylindrical, three to seven times as long as broad. Length, 10 to 12 mic. Breadth 3.5 to 4 mic. Terminal cells obtuse; chloroplast, a long parietal plate occupying 2/3 of cell,--pale green in color without pyrenoids. Reproductive stages not observed.

This species which is typically aquatic in habit was found in soil from an unploughed field near the St. Vital Sanatorium on the East Bank of the Red River.

VII Trentepholiaceae.

Gongrosira terricola (Bristol) Plate 3 Fig. 2 Page. 61_a

A species greatly resembling the one described by Bristol (1920) was found in the culture of a soil from an unplowed field. The stratum is expanded, and creeping filaments are closely interwoven to form a pseudo-parenchymatous disc or mass of distended cells. The upstanding filaments are comparatively short and tapering with obtuse apicies; they are irregularly branched with false dichotomy. The basal cells are swollen, 5 to 7 mic in diameter in the young forms, and 11 to 16 mic. in diameter in older forms. The cells of the branches are sub-cylindrical, distended or irregular on account of a tendency to branch. They are smaller in dimensions, (10 to 18 mic. long and 6 to 14 mic. wide), than described by Bristol, with a single band-shaped or irregular chloroplast containing one pyrenoid or sometimes none. No zoogonidangia were observed.

B. BACILLARIALES (DIATOMALES)

1. Naviculaceae.

Navicula mutica. (Keutz.) Plate 4 Fig. 1 Page. 62_a

This extremely variable diatom was observed in the cultures of six different soil samples, and appears to be one of the commonest soil-diatoms. A number of different forms were found, the chief distinction between them being the size of the markings making up the transverse striations. Variations in the size of the valves was also noted,

the width of the valve varied from 5 to 7.5 mic. and the length from about 10 mic. to 30 mic.

This species was found in association with Hantzschia amphioxys in damp habitats.

11 Nitzschiaceae

Hantzschia amphioxys. (Ehr.) Plate 4, Fig. 2. Page 62_a

The forms of this common soil diatom which occurred in the cultures of six different soils, were usually small, about 30 to 40 mic. in length, by 6.5 to 7 mic. in width, but occasionally larger forms were observed. The valve-view is somewhat curved with rostrate apicies, and the girdle-view is straight and linear-rectangular. A row of carinal dots are clearly visible along the concave margin of the valve. The keels of the two valves are displaced to the same side of the solitary frustule.

This species was found in soil from the top of a prairie ditch West of Winnipeg, as well as in some cultivated soils.

C. MYXOPHYCEAE.

1. Oscillatoriaceae.

Arthrospira jenneri (Kuetzing.)

This species was only found in one soil, in the cultures examined, in association with other algae. Plant mass thin; trichomes 5 to 8 mic. in diameter; fragile, forming a loose spiral 9 to 15 mic. in diameter; slightly con-

stricted at joints; apex of trichome not tapering; cells quadrate, shorter than the diameter; 4 to 5 mic. long; transverse walls somewhat granular, dark blue-green in color.

Phormidium tenue, (Menegh.)

This species, found in eleven different samples of soil, is the commonest blue-green alga in the soils examined. Plant mass thin, membraneous, expanded; pale blue-green; filaments simple, elongate; somewhat straight, densely entangled; sheaths thin; trichomes one to two mic. in diameter; straight, slightly constricted at joints; trichomes tapering and bent; apical cell acute; conical calyptra absent; cells 2.5 to 5 mic. in length; transverse walls somewhat indistinct; cell contents homogeneous; pale blue-green.

Phormidium sp.

Very like Phormidium tenue, but a definitely constricted form. Apex of filament is rounded and a sheath is present. Cells 3 to 5 mic. in diameter, and somewhat cylindrical.

Phormidium autumnale. (Ag.)

Next to Phormidium tenue, this species of blue-green algae occurred most frequently in the soils examined, having been found in cultures of eight samples. The filaments were interwoven to form an expanded plant mass; fragile, dark blue-green; filaments straight; sheaths narrow

and distinct; trichomes 4 to 7 mic. in diameter; not constricted at joints. Ends of trichome were slightly tapering; the apical cell rounded to capitate; calyptra present; cells 2 to 5 mic. in length; transverse walls granulated; cell contents blue-green.

Lyngbya sp.

A species very closely resembling Lyngbya major was found in the culture of one soil. Filaments free, unbranched, forming an expanded mass, yellow rather than colorless; sheaths firm and thick, trichomes 11 to 16 mic. in diameter; apex of trichomes slightly tapering; somewhat capitulate; cells 2 to 3.4 mic. in length.

11 Nostocaceae.

Nostoc ellipsosporum (Desmazieres)

A blue-green alga very closely resembling this form was identified in one of the soil cultures. It agreed in all characters except in the size of the gonidia, which were much larger, being 15 to 25 mic. long and 10 to 12 mic. wide and oblong-cylindrical in shape. They possessed a definite thick cell wall and were brownish-yellow in color. Plant mass gelatinous, expanded; filaments flexuous, laxly entangled; trichomes four mic. in diameter; pale blue-green to olive green; cells alike, cylindrical; 6 to 14 mic. in length. Heterocysts oblong; 6 to 7 mic. wide, 6 to 14 mic long.

Nostoc muscorum (Kuetz.) Plate 5 Fig. 2 Page 63_a

This species was obtained from one of the soils cultured. Plant mass gelatinous to membranaceous; dull olive-green; filaments flexuous, densely entangled; trichomes 3 to 4 mic. in diameter; cells similar, cylindrical; almost twice as long as broad; heterocysts somewhat globose, 6 to 7 mic. in diameter; gonidia, 4 to 8 mic. in diameter, 8 to 12 mic. in length; oblong; in a catenate series, numerous; wall of gonadium smooth, yellowish.

Young colonies were globular, with densely matted filaments coiled in loops toward the periphery of the colony; where the sheaths of the individual filament could be distinguished. The whole colony was surrounded with a mucous sheath. Young colonies in most cases were terminated at either end by heterocysts. The colonies later split to form an expanded leaf-like stratum in which the characters of the filaments could be distinguished.

Nostoc commune (Vaucher.) Plate 5 Fig. 1 Page 63_a

This species, and forms of this species, were found in a number of cultivated soils.

The plant mass was observed as a gelatinous, somewhat leathery mass, blue-green, olive to brown in color; filaments flexuous, entangled; sheaths colorless to yellow; trichomes 4.5 to 6 mic. in diameter. In one form the vegetative cells were barrel-shape; in others depressed or spherical. The former were 4 to 6 mic. in diameter, and the heter-

ocysts sub-spherical, 7 to 9 mic. in diameter; the heterocysts being smaller than the vegetative cells; in some cases the general dimensions being 4 to 6 mic. in diameter. These variations probably represent different stages in the development of the alga, the latter form most likely being an older form. On the other hand, the reduced size of the heterocyst rather indicates that it may be somewhat abnormal form due to cultural conditions.

Chart No.1 Illustrates the relative distribution of the algal species found occurring in the soils investigated.

CHART Nº 1.

OCCURENCE OF ALGAE IN VARIOUS SOILS OF MANITOBA

Species.

Sample Nº 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75

A. ISOKONTAE

1. Chlamydomonadaceae
   - Chlamydomas communis
2. Chlorococcaceae
   - Chlorococcum humicola
3. Selenastraceae
   - Dactylococcus bicaudatus
4. Coleastraceae
   - Scenedesmus obliquus
5. Ulothrichaceae
   - Ulothrix subtilis
   - Ulothrix sp.
   - Stichococcus bacillaris
6. Chaetophoraceae
   - Microthamnion strictissimum
7. Trentepholiaceae
   - Gongrosira terricola

B. BACILLARIALES

1. Naviculaceae
   - Navicula mutica
   - Navicula sp.
2. Gomphonemaceae
   - Gomphonema sp.
3. Nitzschiaceae
   - Hantzschia amphioxys

C. MYXOPHYCEAE

1. Oscillatoriaceae
   - Oscillatoriaceae sp.
   - Arthrospira Jenneri
   - Phormidium Tenue
   - Phormidium autumnale
   - Lyngbya sp.
2. Nostocaceae
   - Nostoc sp. (1.)
   - Nostoc commune
   - Nostoc muscorum
   - Nostoc ellipsosporum (major)
   - Nostoc sp. (2.)

D. EUGLENINEAE

- Euglena sp.

E. BRYOPHYTA

- Moss protonema sp.

PLATE 1.

Fig. 1. Chlamhdomonas communis. (Snow)

Motile cells X 900

Fig. 2. Chlorococcum humicola (Naeg.) Rabenh.

1. Vegetative cells.

2. Resting cells "akinetes".

Magnif. X 650

Fig.3. Dactylococcus bicaudatus, A. Br.

Vegetative cells X 650

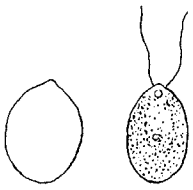


Fig-1

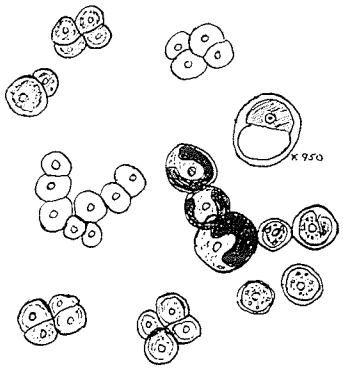


Fig-2'

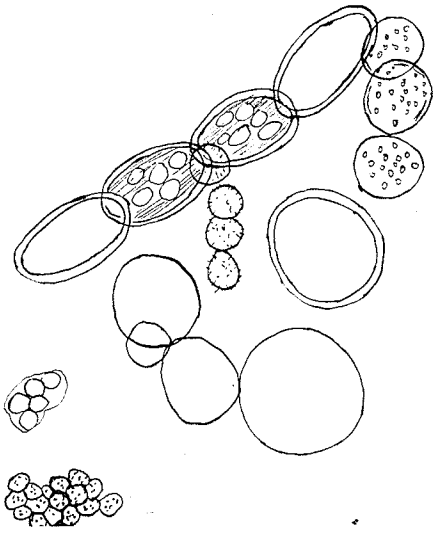


Fig-2²

Fig-3

PLATE 2.

Fig. 1. Scenedesmus obliquus, (Turp.) Kutz.

Vegetative cells X 650

Colonies of vegetative cells.

Fig. 2. Ulothrix subtilis, (Kutz.)

Vegetative filaments X 650

Fig. 3. Stichococcus bacillaris, (Naeg.)

Vegetative cells X 650

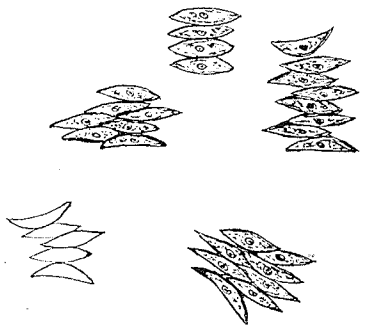


Fig-1

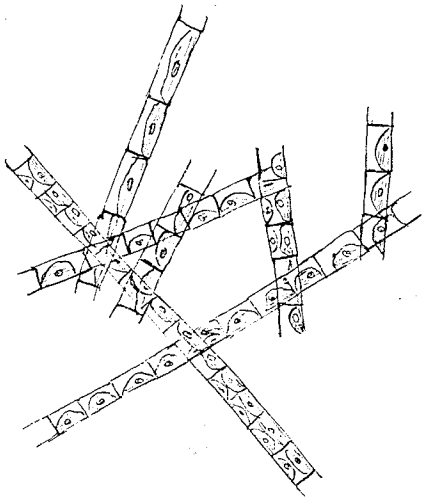


Fig-2

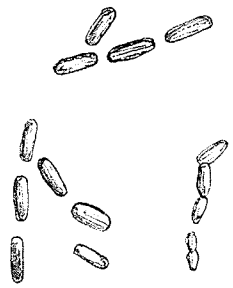


Fig-3

PLATE 3.

Fig. 1. Microthamnion strictissimum (Raben.)

1. Photograph of Petri dish showing development of Microthamnion colonies on nutrient agar.

2. Part of thallus X 900

Fig. 2. Gongrosira terricola (Bristol)

Part of pseudo-parenchymatous thallus showing false dichotomous branching and tapering apical branches.

FIG-1'

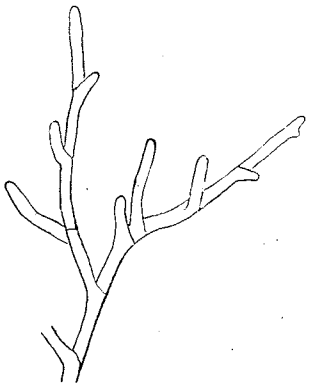


FIG-1²

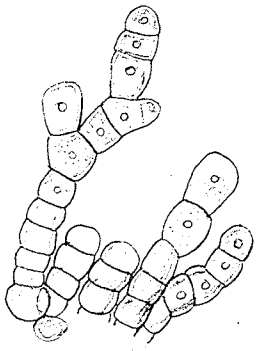


FIG-2

PLATE 4.

Fig. 1. Navicula mutica. (Kuetz.) (After Bristol)

1. Valve-view
2. Valve-view
3. Girdle-view

X 1435

Fig. 2. Hantzschia amphioxys. (Ehr.) (After Bristol)

1. Valve-view showing striae and carinal dots.

X 1435

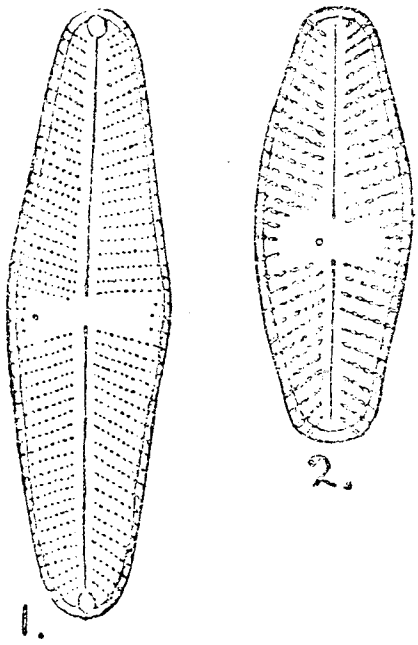


FIG-1

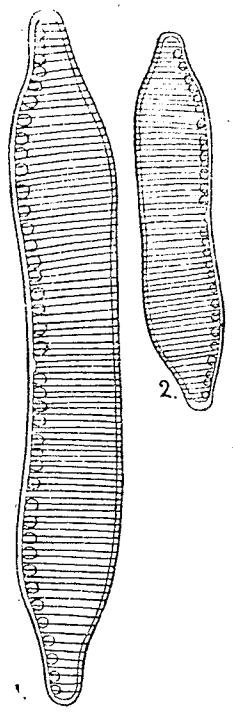


FIG-2

PLATE 5.

Fig. 1. Nostoc commune, (Vaucher.) (After Bristol) X 825

h.--heterocyst

sh.--seriate heterocysts

S^A.--spores of form "A"

S^B.--spores of form "B"

g.s.-germinating spores of form "B"

f.--young filament from germinated spore

v.--vegetative cells of form "A"

Fig. 2. Nostoc muscorum, (Kuetz.) (After Bristol)

F.-a-d, successive stages in germination, X 825

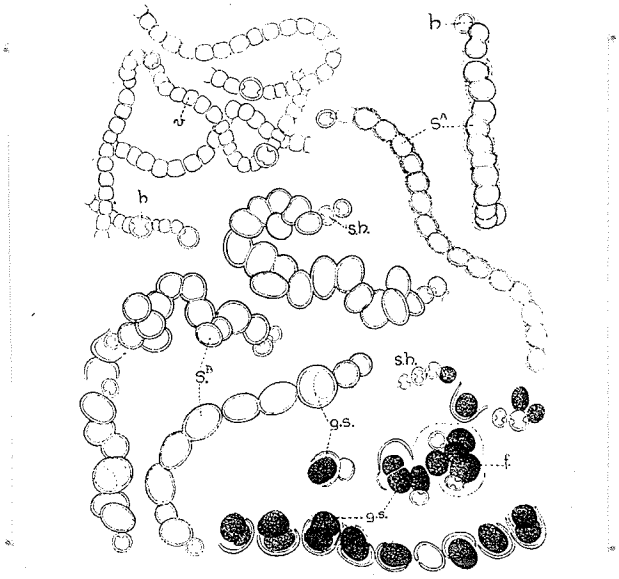


FIG-1

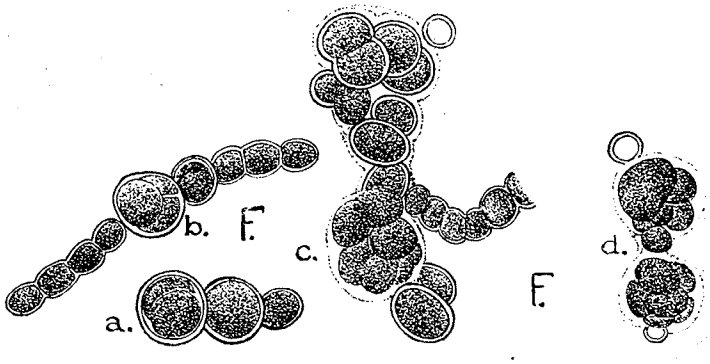


FIG-2

6. SUMMARY OF RESULTS OBTAINED.

1. Algae were found to be present in the soil both in the vegetative condition and in the form of resting spores.

2. The dilution method showed the algae to be distributed quite uniformly within the upper few inches of the soil. (8").

3. Soil samples which were dried over long periods of time took longer to culture and were not so productive. See Tables 2 &4(Samples 46 to 75) Pages 8a, 34

4. Agar-plate cultures were for the most part successful in separating the algal species for purposes of identification.

5. The algae were found to develop equally well in either of the nutrient solutions used, (Page 15), while Schramm's (23) nutrient agar medium proved very satisfactory for "plating" algae.

6. Heat was found to be injurious to the algal spores, preventing their normal development.

7. Optimum light and temperature also were necessary for algal development in artificial media.

8. Twenty-five different species were identified.

9. Chlorophyceae (Isokontae) were found to be more widely distributed in the soils examined than either the Myxophyceae or the Diatomales, (Bacillariates).

10. More species of Myxophyceae were identified in cultivated soils than in uncultivated soils.

11. In nearly every culture examined, stages in the life-history of the unicellular alga Chlorococcum humicola were observed.

12. No definite correlation between soil "texture" and algal species was established, although distribution appeared to be wider in heavy (clay) soil than in lighter soils.

13. Results showed indications of a zonal distribution of the algal species identified in the various soils studied.

14. No great variation of species in "acid" and "alkaline" soils was observed, although growth was found to be more vigorous in the latter soils.

15. In the carrying out of this problem, some species, not generally considered as typical soil forms, were identified.

The results of this thesis therefore would seem to show that there exists in Manitoba a characteristic algae-flora living vegetatively in the soil.

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