# A CONTRIBUTION TO OUR KNOWLEDGE OF THE ALGAL FLORA OF LAHORE SOILS

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## Introduction.

In the present work an attempt has been made to describe those forms which grow in different types of soils found in Lahore and its vicinity. On account of the fact that only those algae have been described which could grow from the beginning of October, till the middle of May next, *i.e.*, mostly during the winter months, the number of forms studied has necessarily been a very limited one.

## Method and Technique.

Samples of soils from different localities in Lahore representing gravel, sand, loam, and clay were collected. Gravel was collected from Shalamar, sand from Bahawalpur road, loam from gardens and fields and clay from Ravi bank and canal bank. Humus soil was collected from manure pits in Government College Botanic Garden and from the jungle on the left bank of river Ravi.

As a general rule composite samples were taken from a thorough mixture of at least 3 borings at 3 different places, to nearly the same depth in the same locality.

Most of the soil samples were alkaline in reaction, though a few were neutral.

Soil is collected after the whole apparatus and the culture media prepared for the cultivation and isolation of soil algae are sterilized. The culture media are sterilized in an autoclave at 1½ lbs. pressure for 15 minutes.

Into each of the sterilized flask a few grams of the soil to be examined is introduced by means of a sterilized spatula. The vessels are closed and placed under glass cases in the North window and left for some weeks to develop. The culture solution used for the purpose are the aqueous mineral salt solutions, a complete composition of which is given in the appendix. Evaporation from the surface of cultures takes place only slowly and if at all any water is needed sterilized distilled water is used.

Culture flasks were also set up in the manner of More and Karrer. Half an inch of burnt sand is placed in the conical flasks sterilised and plugged in the manner already described. A few grams of the soil to be investigated is introduced.

The culture solution is added before the introduction of the soil or a suspension of the soil in the mineral salt solution is added.

The sand in the flasks is slanted so as not to be wholly submerged, giving various moisture conditions.

According to the methods followed by Esmarch, a few petri-dishes were set up, but these did not yield any good result. Soil is packed in a petri-dish to a depth of 1 cm. well-moistened with sterile distilled water and the surface is covered with a piece of pure filter paper. These cultures are kept in diffuse light at a temperature of 20° to 25° C. The filter paper is moistened with sterile distilled water from time to time.

## Growth in Culture.

The first sign of growth is observed after different intervals, in different soils, and even in the same soil, and in different periods of the year. On the surface first a scum gradually appears, which consists of bacteria. This scum next assumes a green tinge while at the same time on the soil surface many small tufts of green patches grow and become larger and larger every day. The whole culture medium assumes a light green colour owing to the presence of free floating unicells or filaments. The greenish scum also thickens gradually and the sides of the flask as well become covered with a green or dark green, gelatinous mass. As a result of continued growth, specially in culture-flasks in which Oscillatoria grows, the green mass becomes very sticky and difficult to be separated from the substratum. The culture-flasks which are kept moist show patches here and there of green velvety tufts which gradually increase in area. The soil becomes covered with a gelatinous mass and in it are entangled the filaments, etc. In flasks in which Diatoms develop the surface assumes a dull brown colour.

The first growth except of the uni-cellular algæ, when examined, reveals only a slight development of filaments very difficult to be identified. The abnormal condition of excessive moisture under which algæ grow in these cultures must tend to produce forms different from those that grow under natural conditions.

There is a good growth in culture flasks which are left undisturbed. Those which are frequently handled for observations or which are taken out of the glass house to be placed in direct sunlight, growth was very slow. The growth is impeded as it were, by

disturbance. It is also noted that the growth is quicker and more well-marked in culture flasks in which the culture medium covers the soil completely, than in flasks which are kept simply moist and wet. Throughout November and December, there was no growth at all in culture flasks. The mean temperature of glass house during this time was 22° C. There was slight growth of Algæ in January and the first half of February when the mean temperature was 29° C. Growth was plentiful after mid-February. Up to this time the result seemed to be disappointing. Only six or seven species appeared. After mid-February the temperature ranged from 30° C. to 40° C.

It is also noted that growth is quicker in culture flasks when they are placed in the South window than when they are placed in the North window. In April, however, the flasks had to be placed in the North window as the direct rays in the South window proved very hot and impeded the growth. Four flasks were inoculated using the same soil and the same culture medium. Two of these were placed in the South window and two in the North. Growth was first observed in flasks which were placed in South window.

Different genera and even the different species are observed never to grow simultaneously in culture flasks having the same type of soil. In the floating scum various unicellular forms, for example, Chlorococcum, Protococcum, etc. appeared interspersed a little later with undeveloped filaments of Ostillatoria. Phormidium faveolarum and Nostoc filaments come out next and grow in almost all the flasks. Three weeks after that Anabaena and other species of Phormidium, Scytonema, Cylindrospermum sp. make their appearance in succession. Still later more species of Nostoc, Anabæna, Calothrix and Scytonema make their appearance. Next appear Hapalosiphon and Fischerella sp. After that long, broad, circular colonies of Nostoc appear which develop to form a large net-work of Nostoc filaments. At the same time some green algæ make their appearance at this stage, e.g., Ulothrix, Hormidium sp. etc. Diatoms appear quite early and continue to remain there for a considerable long time.

## Isolation of Pure Cultures.

An attempt has also been made to isolate pure cultures of algae, by following Waksman (1) and Ward (6). Only in the former, pure colonies of Pleurococcus, Chlorococcus and Oscillatoria developed.

## Cultures from Desiccated Soils.

A few soil samples were placed in sterilised papers (sterilized with absolute alcohol) for desiccation in the class room, and left there for about 12 weeks. The soils were then inoculated in culture flasks

following Bristol (5). The following table indicates the locality from where the soil sample was obtained, the date on which it was placed for desiccation, the date on which culture flasks were set up and the date on which first growth was observed.

| Locality | Date of desiccation | Date of inoculation | Date of first growth observed |
|---|---|---|---|
| Old garden ... | 7-11-1931 | 13-3-1932 | 28-3-1932 |
| Garden ... | 9-11-1931 | do. | 26-3-1932 |
| Cultivated field ... | 15-11-1931 | do. | 26-3-1932 |
| Uncultivated field ... | do. | do. | 3-4-1932 |
| Canal turf ... | do. | do. | 25-3-1932 |
| Grassy plot ... | 15-11-1931 | do. | 30-3-1932 |

Growth has been found to be more plentiful in cultivated field soil than in any other type of soil. The number of species, however, is not the same as in fresh sample of soil. Oscillatoria, Anabaena, Phormidium, Nostoc and Diatoms only appeared in desiccated soils.

For the culture of diatoms, a modification of Miquel's medium (see Appendix) was used but not with very good results. The diatoms growing in this medium are not in any way larger in size than they ordinarily occur in the culture flasks and also the number of species does not show any increase in number.

A systematic list of the algæ recorded from 15 soil samples is given below. The classification followed is that of West and Fritsch (2).

*Chlorophyceæ*

Isokontae—Chaetophoraceæ.

Pleurococcus Naegelii (Chodat) from 4 samples.

Chlorococcum sp.—from 4 samples.

Ulotrichales.

Ulothrix sp. from 2 samples.

Hormidium sp. from 1 sample.

*Cyanophyceæ*

Chroococcales—Chroococcaceæ.

Aphanotheca naegelii, Wartm. from 3 samples.
Eucapsis minuta Fritsch. from 4 samples.
~~Aphanocapsa virescens (Hans) Rabh. from 2 samples.~~
Chroococcus minutus (Kutz) Nag. from 2 samples.
C. macrococcus (Kuetzing).
C. limneticus and
C. multicoloratus. from 3 samples.
Oscillatoria tenuis from 2 samples.
O. brevis and O. irrigua Kutz from 9 samples.
Isocystis sp. from 7 samples.
Phormidium foveolarum (Mont) Gom. from 7 samples.
P. unicinatum (Ag) Gam. and
P. ambiguum. from 2 samples.

*Nostocaceæ*

Nostoc verrucosum Linn.
N. ellipsosporum.
N. sphaericum.
N. pruniform.
N. spongiformæ from 7 samples.
Anabæna variabilis.
Anabæna sp. and
Anabæna inequalis (Kuetzing) from 7 samples.
Cylindrospermum majus from 2 samples.

*Scytonemaceæ.*

Scytonema sp. from 3 samples.

*Rivulariaceæ.*

Calothrix sp. from 3 samples.

*Stigonemataceæ.*

Hapalosyphon sp. from 2 samples.
Fischrella sp. from 1 sample.

DIATOMS—Bacillariaceæ.

1. Cyclotella Meneghiniana.
2. Synedra Ulna.
3. Navicula sp.
4. Navicula (Pinnularia) Gibbs
5. Navicula (Calonies) sp. ?

6. Gyrosigma scealproides, Rabh.
7. Amphora minutisima.
8. Cymbella sp.
9. Rhopalodia gibberula.
10. Nitzschia Kutzingiana.
11. Nitzschia Subtilis.
12. Nitzschia sp.

## Summary.

Fifteen samples of Lahore Soils were collected and inoculated in culture flasks containing special enrichment culture media, favouring the growth and development of algæ. Four species of green algæ, 12 species of Diatoms and as many as 28 species of blue green algæ have been recorded.

Isolation of pure culture in Agar media was tried but only three species grew in the solid media.

A few soil samples were placed for desiccation for 12 weeks. Their algal growth was also studied, which was similar to that of the fresh soil forms.

Before concluding the writer takes this opportunity to express his sense of deep gratitude to Dr. Chaudhuri of the Punjab University for generously permitting him to work under him on Algal-flora of Lahore Soil, and for his constant help all along the course of study. The writer also feels indebted to Dr. S. L. Ghose of the Government College for giving every sort of help whenever needed.

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## Appendix.

Media used for the cultivation and isolating of algæ:—

1. Moor.

| | | |
|---|---|---|
| $NH_4\ No_3$ | ... | 0.5 gram. |
| $KH_2\ PO_1$ | ... | 0.2 „ |
| $MgSo_4\ 7H_{20}$ | ... | 0.2 „ |
| $CaC_{12}$ | ... | .1 „ |
| $FeSo_4$ | ... | Trace. |
| Distilled water | ... | 1,000 cc. |

2. Bristol.

| | | |
|---|---|---|
| $NaNo_3$ | ... | 0.5 gram. |
| $KH_2 PO_3$ | ... | .5 „ |
| $MgSo_4$ | ... | 0.15 „ |
| $CaC_{12}$ | ... | .05 „ |
| $NaC_1$ | ... | .05 „ |
| $FeC_{13}$ | ... | .005 „ |
| Distilled water | ... | 1,000 cc. |

3. Detmer.

| | | |
|---|---|---|
| $Ca(NO_3)_2$ | ... | 1.0 gram. |
| $KH_2PO_4$ | ... | 0.25 „ |
| $KC_1$ | ... | 0.25 „ |
| $MgSo_4\ 7H_{20}$ | ... | 0.25 „ |
| Tap water | ... | 1,000 cc. |

4. Pringshiem.

| | | |
|---|---|---|
| $NH_4\ Mg\ Po_4$ | ... | 1.0 gram. |
| $K_2\ So_4$ | ... | 0.25 „ |
| $Fe_2\ (PC_4)_2$ | ... | Trace. |
| Water | ... | 1,000 cc. |

5. Benecke.

| | | |
|---|---|---|
| $Ca\ (No_3)_2$ | ... | 0.5 gram. |
| $Mg\ So_4\ 7H_{20}$ | ... | 0.1 „ |
| $K_2\ HPO_4$ | ... | 0.2 „ |
| $Fe\ C_{13}$ | ... | Trace. |
| Water | ... | 1,000 cc. |

6. Knops.

| | | |
|---|---|---|
| $Mg\ So_4$ | ... | 0.25 grams. |
| $Ca\ (NO_3)_2$ | ... | 1.0 „ |
| $KH_2\ PO_4$ | ... | 0.25 „ |
| $KC_1$ | ... | 0.12 „ |
| $Fe\ C_{13}$ | ... | Trace. |
| Water | ... | 1,000 cc. |
| Strength = | ... | 0.172 per cent. |

7. Klebs.

| | | |
|---|---|---|
| $Ca\ (NO_3)_2$ | ... | 4 parts. |
| $KH_2\ PO_4$ | ... | 1 part. |
| $Mg\ SO_4$ | ... | 1 part. |
| $KNO_3$ | ... | 1 part. |

Made up in strengths from 0.2 to 1 per cent.

8. Miqual's medium for diatoms (modified).

Solution A 10.1 grams $KNO_3$ in 50 cc. of distilled water.

Solution B 2. grams $Na_2 HPO_4$ in 20 cc. water.

(1) c.c $HC_1$ concentrated 1 cc. $Fec_{12}$.

(2) grams $CaC_{12}$ dissolved in 20 cc water.

40 drops of A and 16 drops of B are added to 1,000 cc. of distilled water.

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