about 1 cm. apart, but the thickness of the slide should not exceed 2 mm. (Since the cover slips are not uniform in thickness, it is necessary to select two of the same thickness, about 0·1 mm. or slightly less.) Once the balsam is dried the depth of the chamber is determined by a micrometer and verified with a suspension of blood cells or yeasts against a hemacytometer. It is necessary to know the depth of the counting chamber so the volume can be calculated.

Preparation of Soil Suspension.

Winogradsky[27] used fractional centrifugation for his direct microscopy. As the counting chamber has a depth of only 0·1 mm., it is better to avoid sand grains in the suspension. Twenty gr. of soil is washed with 50 c.c. lots of water. After each washing, the sand deposit is examined under the fluorescence microscope. Three or four washings are necessary to obtain a sand practically free of algal cells. The water from different washings is collected in a measuring cylinder. The volume is made up to 200 c.c. After 20 minutes of shaking the cylinder is allowed to stand for five minutes. From different levels of the cylinder samples are taken and examined. Most of the algal cells are sedimented with the soil particles. Only a few still remain in suspension. Hand centrifugation for one minute brings all algal cells to the bottom. The algae can be concentrated in a soil suspension in this way. This is quite useful for soils of low algal population and for water samples. The suitable suspension for most soils examined by us is one part of soil for 5 to 10 parts of water. At higher concentrations the soil particles are too dense.

Method.

Focus the condenser of the lamp to produce a parallel beam and place the blue filter between the light and the microscope. Adjust the mirror of the substage condenser, then introduce a drop of soil suspension into the counting chamber. A drop of liquid paraffin is used to connect the slide with the condenser of the microscope so that the maximum aperture of the optical system is fully used. The light intensity is cut down by a neutral filter or by reducing the lamp voltage. Focus the microscope on the soil particles. Remove the eyepiece and the neutral filters. Increase the lamp voltage to produce the maximum light intensity. Adjust the mirror of the microscope carefully until a maximum intensity of light can be seen through the objective lens. Replace the eyepiece with the yellow filter. Focus the microscope on the red fluorescent spot and carefully adjust the mirror until the maximum fluorescent light is obtained. The microscope is then ready for use. Algal cells appear as red spots or lines.

Using a 10× objective lens and a 5× eyepiece count algal cells in 50 fields. If the soil suspension has a very low number of algae, count the total surface of the counting chamber, which has a definite area determined by direct measurement.

As we know the depth of the counting chamber we can calculate the number of algae per gr. of soil.

Effect of Fixatives.

The aim in studying fixatives is to preserve samples for future studies. The fixative also kills the algal cells and motile forms can be more easily counted. The classical fixatives with or without Cu_2SO_4 are not suitable as the fluorescence disappears quickly. Zinc fixatives preserve the fluorescence to some degree but it still fades significantly.[26] The fluorescence is preserved after heating.[16] Satisfactory results can be obtained by subjecting the samples to low temperature, which allows the cells to remain apparently unchanged but prevents them dividing. The value of hot and cold treatments in preventing changes in the number of algae is shown by the following experiments.

A suspension of soil containing algae was divided into three portions. Direct counting gave 180 cells per 20 fields. The first portion was kept at room temperature; the second in the refrigerator, and the third heated in boiling water. Counting at regular intervals showed that the number in the first portion had diminished slightly,

the second had a constant number of algae, and the third showed a significant loss of fluorescent cells, as seen in the table below:

	Room Temperature.	Refrigerator.	Heated at 100°.
0 hours	180	180	180
1 hour	182	178	179
3 hours	169	184	—
24 hours	152	172	87
48 hours	140	176	50
7 days	172	180	10

After seven days the first sample nearly regained the initial number. This could be due to the possible multiplication of algal cells at room temperature, especially since it was not kept in the dark. For routine work, we can use the refrigerator for storing samples.

Interference due to Other Plants and Resting Forms of Algae.

Few other types of plant are likely to be confused with algae. Moss protonemata are the main difficulty; these cannot be distinguished from filamentous algae under the fluorescence microscope. However, it can be converted into a light microscope in a few seconds, when careful examination can then distinguish the two types of filament. Confusion from the gemmae of liverworts can be similarly excluded.

The resting stages of algae introduce some difficulties. Certain cells do not fluoresce in the resting form (Hematococcus)* while others do. Fluorescence microscopy can only detect cells still containing chlorophyll.

Most works on soil algae deal with the morphology, cytology, taxonomy, and physiology. Very few papers are concerned with algal population in soils. A convenient historical review is given by Petersen.[12] Real progress was made when the culture technique was introduced, but we still know little about the soil algal populations. Demolon in 1944[5] stated: "Leur étude encore au stade floristique a permis d'en caractériser plusieurs centaines d'espèces; quant à leur nombre, il est régi par des circonstances inconnues." The culture technique was not the real answer for the study of soil algal population. Smith,[19] when discussing the isolation of single cells by micromanipulation, referred to the difficulties and wrote: "The problem is not so much a matter of picking out the individual organisms as it is one of finding a suitable nutrient medium once they have been picked out."

The biphasic or soil-water culture described by Pringsheim[14] seems to be the best for the culture of soil algae. Still, according to Pringsheim, some algae refuse to grow on these semi-natural media. Even using a great number of different media, we cannot assume that all species will grow. The data obtained with the artificial media may not represent the real population of soil algae for the following reasons: firstly, the cells living heterotrophically in the soil may develop chlorophyll in the artificial media; secondly, the artificial media may be selective for certain species.

A further disadvantage of the culture technique is the slow growth of algal colonies which involves delays of up to two or three months. Van Overeem[23] has reported that some aero-plankton algae took six months to give a visible colony. Filamentous and colonial algae introduce further difficulties. A single filament or colony can give one or many colonies according to the number of viable pieces formed from it during dispersion. This is particularly true with Cyanophyceae.[12] The tough mucous sheaths of these algae withstand shaking, so that the cells do not separate readily during dispersion. The time of shaking the sample of soil in the water is important in culture techniques; according to Petersen[12] shaking of twenty minutes gives a maximum number of colonies, prolonged shaking even reduced the number.

Furthermore, in a mixed culture the fast-growing species may crowd out slow-growing species. This can be avoided by high dilution when the number of slow-growing species is higher than or at least equal in number to the fast-growing algae.

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* Tisher, J., 1937 : Happe-Seyler's Zeitsch. Physiol. Chemie, 250 : 147.—According to Tisher a small amount of chlorophyll may still be present in the red form of Hematococcus.

On solid media (agar or silicagel) the Chlorophyceae may form zoospores which may spread and form new colonies. It may then be difficult to decide whether a colony is of primary or secondary origin. In addition, the growth of fungi may destroy the culture on solid media, especially when organic matter is added.

It therefore follows that the numbers of soil algae estimated by the dilution technique cannot be regarded as absolutely reliable. If the culture technique has given useful information they may not have an absolute ecological significance. The same problem has been discussed for soil bacteria[13] and soil fungi.[3] Only direct microscopy can resolve these difficulties.

Recent direct microscopic techniques introduced by Struger,[20] Jones and Mollison,[9][13] Manninger and Vamos,[11] Vamos,[25] and Tchan[21] deal with bacteria, fungi, and protozoa. No special attention is paid to the soil algae.

Counting algal cells under the microscope does not involve the use of an artificial medium. Therefore, all selection is avoided. If some filamentous or colonial algae are not broken down to single elements during the dispersion of soil in water, direct microscopy would count all the associated cells. Direct microscopy seems very attractive, but has some practical difficulties. The principle of direct microscopy consists in rendering visible the soil microorganisms suspended in water or in agar[13][9] by staining on a slide. Since some soils have only a few thousand algae per gramme, many microscopic fields under high power must be counted. For example, using a modified Chalodny's technique Verplancke[24] reported 3,700 to 10,000 algal cells per gramme of soil. On the other hand, Petersen[12] used direct microscopy only for soils showing a macroscopic growth of algae. Their techniques were quite complicated and not suitable for routine work.

Quispel[16] used a plastic film technique, but this is limited to the investigation of the vegetation on the surface of soil, rock, or water. In all existing counting techniques, the low power lens cannot be used because some unicellular algae are too small to be easily detected, especially when intimately associated with soil particles. These difficulties are overcome by the new fluorescence technique proposed here.

The use of fluorescence microscopy for the examination of algal populations in soils has, to the best of my knowledge, not been reported before. The technique proposed is simple, rapid, and easy to use. Although the technique, like the dilution method, does not discriminate between active and certain resting forms of algae, it has the advantage of being rapid and of excluding the non-photosynthetic forms. The long waiting period for the growth of algae is eliminated. It has been established that an inexperienced person can count 8 to 10 samples per day. It is also possible to study the daily variation of algal numbers in the soil. No expensive apparatus is involved because most of the accessories can be made in the laboratory.

The interference by moss protonomena and liverworts can be avoided. Even if we do count them by mistake, functionally they resemble algae. Especially when we compare the soil to a living organism[13][21] the anabolic activities are caused by autotrophic organisms.

As chlorophyll alone is visible under the fluorescence microscope, the morphology and taxonomy of the algae cannot be studied. As the fluorescence can be converted into an ordinary light microscope, the morphology and taxonomy can be studied separately.*

Summary.

A fluorescence microscopy technique for the estimation of algal populations in soil is rapid. All soil algae are counted without selection. No large quantity of glassware is needed. It excludes heterotrophic forms without chlorophyll. It can be adopted for the study of water algae. The method cannot be used for taxonomic work, but the microscope is easily convertible to a light microscope for this purpose.

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* Certain species of algae cannot be classified by simple morphology. The study of the life cycle is needed. By the introduction of a suitable fluorochrom, the technique may be adopted for the estimation of flagellata and microfauna of the soil. Further research is in progress.

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NOTES ON THE MORPHOLOGY AND BIOLOGY OF *ECTENOPSIS VULPECULA* WIED. VAR. *ANGUSTA* MACQ. (DIPTERA, TABANIDAE, PANGONIINAE).

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(Eleven Text-figures.)

[Read 29th October, 1952.]

Synopsis.

The genus *Ectenopsis* was erected by Macquart for *Chrysops vulpecula* Wied. Specimens described under other names by Macquart and Bigot were recorded as synonyms by Ricardo. Ferguson doubted whether they were really synonymous with *E. vulpecula* Wied. The relevant literature is quoted or listed.

Larvae, pupae and imagos of *E. vulpecula* Wied. var. *angusta* Macq. were found at Woolwich, Sydney, N.S.W. Larva and pupa are described and figured.

Introduction.

The genus *Ectenopsis* was erected by Macquart (1838) for *Chrysops vulpecula* Wied. (1828), a Tabanid, whose country was unknown, in the Berlin Museum. Walker (1848) in his List of the Specimens of Dipterous Insects in the Collection of the British Museum, lists "Ectenopsis vulpecula Macq., Chrysops vulpecula Wied.", with references, and a question mark for the country of origin. Macquart (1847) described *Pangonia angusta*, from Nouvelle-Hollande, in the collection of M. Bigot. Bigot (1892) described *Corizoneura angusta* and *C. rubiginosa*, from Australia, in his own collection. Ricardo (1915) recorded the synonymy of the above three species with *E. vulpecula* Wied. the type of the genus. Ferguson (1921) agreed that the three species were the same but doubted whether they were really synonymous with *E. vulpecula* Wied. He says: "I have not seen Wiedemann's original description, but apparently the name was applied to a species with black legs. I have taken a species at Sydney which has the legs, except the coxae, deep black, the wings are also smoky, almost deep black in fresh specimens, but fading somewhat with age, the palpi variable in colour, black to testaceous. Compared with this . . . are specimens in which the legs are yellowish (testaceous) and the wings clear, the stigma being inconspicuous in marked contrast to the black stigma of the other form.

"While I recognise that the species may prove sufficiently variable to include the two forms, I think that at any rate varietal names should be given to each. *E. vulpecula* Wied. evidently from all evidence, should be applied to the black legged form. . . . *E. angusta*, Macq. (= *E. angusta*, Bigot and *E. rubiginosa*, Big.) would apply to the paler legged form."

From this it can be seen that, over the years, there has been some confusion about the naming of the species and some doubt whether the specimens described belonged to one variable species or possibly to two distinct species.

The material which forms the subject of this paper includes thirteen imagos collected during one summer in a very small area at Woolwich, Sydney, N.S.W. The adults are the pale-legged form, though even among these few there is some variation in colour, none have wholly black legs, and the stigma of the wing is inconspicuous.

Adults were sent to Dr. I. M. Mackerras, who said: "The Pangonines are undoubtedly *Ectenopsis vulpecula* Wied. var. *angusta* Macq. as identified in the Ferguson collection."

Some were sent also to Mr. H. Oldroyd for comparison with Bigot's types of *rubiginosa* ♂ and *angusta* ♀ which are in the British Museum, where they are all under the one name *Ectenopsis vulpecula* Wied. "as placed by Miss Ricardo".

However, as it appears possible that two species may exist, the varietal name is used for these specimens.