THE DETERMINATION OF MICROBIAL ACTIVITY IN SOILS
BY A TURBIDIMETRIC METHOD

by

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THE DETERMINATION OF MICROBIAL ACTIVITY IN SOILS

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INTRODUCTION

The soil microbiologist encounters considerable difficulty when attempting to evaluate numbers and activity of the soil flora. In the past some emphasis has been placed on the relationship between soil fertility and microbial activity. It has been found that high microbial numbers are often associated with soils relatively rich in organic matter, and a low population correlated with lower levels of the organic fraction.

Soil flora studies are usually carried out by microscopic and cultural methods. The principle of "enrichment" advocated by Beijerinck has enabled investigators to attain a degree of separation of specific cultural groups. Plate counts involving selective media are used widely. However, the latter procedures are costly, time consuming and often do not lend themselves well to experiments involving a large number of treated soils. For this reason, the present study was undertaken in order to investigate the possible use of a turbidimetric procedure in estimating the microbial activity of soil.
HISTORICAL

Various investigators have attempted to outline a method for a more comprehensive examination of the soil flora. In general the solution of this problem has met with little success. However, three basic methods are used to demonstrate the microbial activity of soil. These may be grouped as follows: microscopic methods, cultural methods, and procedures involving the decomposition of various organic materials. Since only the first two methods have been used for comparison purposes in the present study, the review of literature does not include the third approach.

In 1917 Conn (4, p. 257) proposed a method for the microscopic examination of the soil. The method depended upon staining dried films of soil on a microscopic slide with rose bengal. The technique assumed some importance because of its adoption, with few modifications, by Winogradsky (6, p. 259). However, the rather complex procedure proposed by Winogradsky has not proven acceptable (7, p. 259).

Cholodny (7, p. 622) advocated a modification of the microscopic technique proposed by Rossi (18, pp. 409-412). This method is now called the "Rossi and Cholodny's Contact Slide Method." The latter method consists of slitting the soil and inserting a microscopic slide. After a
short incubation period the slide is removed and the film of microorganisms which have become attached to the slide are stained. Difficulty, however, is encountered in expressing the results obtained in terms of microbial activity or numbers.

Other modifications of the direct microscopic method have been proposed. Thornton and Gray (25, p. 522) mixed a soil with a known amount of indigotin. He then established the relationship between the numbers of erythrosin-stained bacteria and indigotin particles and was able to measure the abundance of microorganisms in the soil. Thus Thornton and Gray (25, p. 522) believed that the application of his technique had far greater value than the original microscopic method. Apparently, this worker was able to solve the problem of interfering soil particles.

Jones and Mollison (15, p. 55) devised a method for counting soil microorganisms by an adaptation of the haemocytometer slide technique. A measured amount of soil in a melted agar gel was placed on a haemocytometer slide of known depth. The film was stained and differential counts made on a measured area of the film. In this respect, Jones and Mollison (15, p. 42) determined high counts of 1 to 4 billion organisms from soils that showed counts of 4 to 8 million bacteria using the standard plate procedure.

Hanks and Jones (10, p. 208) have severely criticized the direct microscopic method as a means of enumerating
bacteria. They pointed out that there are two important sources of error: losses of bacteria from the slide during staining and faulty selection of areas on the film for microscopic observations.

Even though the standard plate method is the one most commonly employed, various investigators have criticized its value (Wilson, 35, p. 407). A noted authority H. J. Conn (7, p. 257) aptly comments on the inherent disadvantages of the plate counting procedure: "A beginner in this field is apt to be assigned the task of plating one or two samples of soil; but after the colonies have developed about all he can do with them is to count the numbers—a matter of little significance in soil bacteriology. He may speculate as to what the various organisms are, and which kinds are of importance; but unless he is associated with someone well versed in the field, he has difficulty getting the information in fairly concise form." However, Jennison and Wadsworth (13, p. 390) reported that the standard plate count method for estimating bacterial populations is satisfactory if relative rather than absolute numbers of cells are wanted.

The plate method possesses several distinct advantages. Determinations can be made of the abundance in the soil of organisms that are capable of growing on selective media. Waksman and Fred (30, pp. 27-28) evaluated the use of differential media for the study and enumeration of the
microflora, and have shown the relative merits of various types.

Data were presented by James and Sutherland (11, pp. 72-86) to show the application of proven mathematical formulae in testing the accuracy of plate counts of field soils. These authors insisted that a soil sample must be plated on the day it is taken from the field before the counts can be accepted as satisfactory.

Skinner, Jones and Mollison (20, pp. 26-27) investigated the accuracy obtained with the standard plate count and the direct microscopic procedures. These workers found no correlation between the two methods, and attributed the discrepancies to; clumps of bacteria which remain aggregated, competition on plates, selectivity of plating media, and non-cultivation of obligate anaerobes. Skinner et al. concluded that the greatest value of the plate count procedure is qualitative in nature.

Jennison and Wadsworth (13, p. 342) experimentally proved that the dilution error and the distribution error are the chief sources of variation accounting for the total error involved in estimating bacterial numbers by the plating method. In this respect Waksman (26, p. 100) showed that the probable error involved in a quantitative determination of soil microorganisms can be worked out by combining a relatively large number of plates with a large number of soil samples.
The accuracy of the plate count with suspensions of *Pseudomonas fluorescens* and *Bacterium globiforme* was determined by Sutherland and James (22, p. 305). Two dilutions were made and four replicate plates. This was repeated 200 times. The investigators found the distribution values in the plating from each dilution used agreed quite well with the theoretical distribution.

For counting bacteria, the plating technique is generally preferred to the dilution technique because the same accuracy can be obtained with less effort and material. However, when some members of the population cannot grow as surface colonies, or when, for some other reason, the plating technique is inapplicable, the dilution technique must be used. Savage and Halvorson (19, p. 355) included data obtained by Phelps. The latter investigator used the dilution method for estimating *Escherichia coli* populations in drinking water. The interpretation of Phelps' results was not made until Ziegler and Halvorson (36, p. 609-639) calculated tables by which results of dilution studies could be converted into most probable population values. These workers showed the limitations of the dilution method, but believed that this technique offers a new method of studying the antagonistic effect of one organism upon another. Waksman (27, pp. 283-298) stated that the dilution method can be utilized not only for the determination of the total soil flora, but also for the study of specific physiological
groups. However, this method has proven too cumbersome for the routine analysis of soils.

For many years it has been the intent and hope of soil microbiologists to correlate or measure certain soil constituents and fertility by some microbiological method. A technique developed by Winogradsky (7, p. 259) used colonies of *Azotobacter* as an indicator of phosphorous deficiency in the soil. For several years, therefore, this approach was tested using various microorganisms and fungi as indicator organisms. The final outcome of these investigations showed that the use of indicator organisms did not produce favorable results.

The more fundamental question of relating any one type of organism to soil fertility was left open until Conn and Darrow (8, pp. 95-110) isolated *Bacterium globiforme* from fertile soils and found the organism absent in two less productive soils. Taylor and Lochhead (24, pp. 340-347) state: "If there does actually exist some easily isolated organism whose presence or absence in soil indicates, even to some degree, the crop producing power of the soil, or will aid in differentiating types of soil, the soil scientist will have at hand a new method for soil survey."

From 31 samples of Utah soils, ten of which were virgin, Greaves in 1914 (34, p. 164) found, with few exceptions, that almost twice the total count was obtained from virgin soil.
On analysis of many soil samples Waksman (28, pp. 321-346) concluded that the total number of microorganisms as determined by the plate method can serve as a function of the bacteriological condition of the soil and as an index of soil fertility. His conclusions were based on observations on which crop production ran nearly parallel with numbers of microorganisms in the soil.

The methods and approaches used in studying and in counting soil microorganisms are numerous. Apparently no one method provides a complete picture of the microbial complex in the soil.
EXPERIMENTAL METHODS

Soil Tested

The soil chosen for the principal part of the present investigation was classified as a Chehalis silty clay loam. In addition a forest nursery soil was obtained through the courtesy of the Soil Department, Oregon State College.

Soil Treatment

The type and number of soil microorganisms are influenced by the nature and availability of soil nutrients. For this reason, Chehalis silty clay loam was treated so as to provide significant differences in the soil flora. In one trial, Orzan (dehydrated waste sulfite liquor) was added so as to give a final concentration of 100 ppm nitrogen. Then the Orzan treated and the non-treated soils were allowed to incubate for approximately 60 days. A second experiment was designed to demonstrate the effect of readily available nutrients. In the latter case, dextrose was added to one lot of soil at a rate of 2000 ppm carbon; a second lot received dextrose and ammonium nitrate so as to give a C:N ratio of 20 to 1; a third portion received Bacto-peptone so as to provide 1000 ppm nitrogen. A fourth set was not treated and served as the control soil. Each soil was brought to 60 percent of the saturation capacity by the addition of either plain water (control) or water plus the above mentioned nutrients.
Culture Medium

Various media have been devised for the observation and isolation of soil microorganisms. In some instances, the medium has been prepared to favor a specific microbial group such as the Actinomycetes. In the present study, it was decided to use a medium favorable to a greater variety of microbial types. The medium decided upon was prepared as follows:

Glucose-------------10 gram
Gelatin-------------1 gram
Peptone-------------5 gram
Beef Extract-------3 gram
Tap Water-----------1000 ml
pH------------------7.0

When a solid medium was required 1.5% Bacto agar was added to the above medium.

Turbidimetric and Standard Plate Test Examination

The moisture content of each soil sample was determined and subsequent calculations made on a water-free basis. Ten gram aliquots of the desired soil sample were added to a 90 ml water blank. Then the diluted soil was placed on a reciprocating shaker for 15 minutes. The shaker revolved at 130 rpm through a distance of six inches. Ten ml of the soil suspension were pipetted from the center of the dilution bottle into another 90 ml sterile water blank, and this shaken about 40 times. Subsequent dilutions were carried out to include $10^5$, $10^6$, and $10^7$. In most instances these dilutions were used for inoculation of the test medium. One
ml of each soil dilution was pipetted into 9 ml of modified nutrient broth, respectively. The inoculated broth tubes were then placed on a rotary shaker and incubated at 30°C. Subsequently the tubes were checked at varying time intervals for the development of turbidity or increase in optical density. At the onset of the turbidity determinations the galvanometer was adjusted to a reading of G=100 (nutrient broth). A Beckman Model B spectrophotometer was used for the measurement of optical density. The most effective wave length was found to be 640 mu.

Standard plate counts were included for each test soil. These were carried out using the same dilution series described above for the turbidity determinations. However, Bacto agar was added to the modified nutrient broth medium to provide a solid plating medium.

All tests were designed so as to provide triplicate readings. Subsequent data are reported as the average of three separate determinations.

Microscopic Examination

The question of the nature of the major flora responsible for the rapid growth observed during the turbidity determinations was considered. After the onset of turbidity in the liquid medium, a loopful was removed to a glass slide and stained by the Gram's method. This step was repeated for all soil samples under study. In addition, slides were prepared from representative colonies which developed on
the solid plating medium. These slides were also stained using the Gram's method.
EXPERIMENTAL RESULTS

Orzan Treated Soils

The inclusion of Orzan in Chehalis silty clay loam has effected a significant increase in the numbers of soil microorganisms. The standard plate count used on the non-treated soil showed a total count of 2.9 million, whereas the Orzan treated soil tested 9.4 million microorganisms per gram of soil. The noted difference in total count was also indicated by the trends in turbidity shown in Figure 1. In this respect the observed variance in count was reflected by the lack of growth in tubes inoculated with the non-treated soil (Figure 1, lines 1, 2 and 3). On the other hand, liquid medium which received diluted inoculum from the Orzan treated soil, showed a slight development of turbidity at 12 hours and extensive growth density at the 24 hour period (Figure 1, lines 4, 5 and 6).

It became apparent early in the study that the soil dilution used represented the critical factor in the turbidimetric procedure. For example, when the one million dilution of the Orzan treated soil was used as the inoculum source, microbial growth resulted. However, when the ten million dilution was tested no growth was manifest in the nutrient broth. In this connection, the approximate total count may be estimated by using the principle of dilution to extinction. Failure of positive growth to develop with the
### Soil Sample Dilution Plate Count

<table>
<thead>
<tr>
<th>Sample Description</th>
<th>Dilution</th>
<th>Plate Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Orzan Added</td>
<td>100,000</td>
<td>2,900,000/gm. soil</td>
</tr>
<tr>
<td>No Orzan Added</td>
<td>500,000</td>
<td>2,900,000/gm. soil</td>
</tr>
<tr>
<td>No Orzan Added</td>
<td>1,000,000</td>
<td>2,900,000/gm. soil</td>
</tr>
<tr>
<td>Orzan Added</td>
<td>100,000</td>
<td>9,400,000/gm. soil</td>
</tr>
<tr>
<td>Orzan Added</td>
<td>500,000</td>
<td>9,400,000/gm. soil</td>
</tr>
<tr>
<td>Orzan Added</td>
<td>1,000,000</td>
<td>9,400,000/gm. soil</td>
</tr>
</tbody>
</table>

**Figure 1.** The Influence of Orzan on the Relative Microbial Activity of Chehalis Silty Clay Loam as Determined by the Standard Plate Count and the Turbidimetric Procedure.
<table>
<thead>
<tr>
<th>Soil Treatment—Sawdust Added at Ten Tons/Acre</th>
<th>Dilution</th>
<th>Plate Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. No Nitrogen Added</td>
<td>1,000,000</td>
<td>12,800,000/gm. soil</td>
</tr>
<tr>
<td>2. No Nitrogen Added</td>
<td>10,000,000</td>
<td>12,800,000/gm. soil</td>
</tr>
<tr>
<td>3. Nitrogen Added</td>
<td>1,000,000</td>
<td>8,500,000/gm. soil</td>
</tr>
<tr>
<td>4. Nitrogen Added</td>
<td>10,000,000</td>
<td>8,500,000/gm. soil</td>
</tr>
</tbody>
</table>

Figure 2. The Influence of Douglas Fir Sawdust on the Relative Microbial Activity of Chehalis Silty Clay Loam as Determined by the Standard Plate Count and the Turbidimetric Procedure.
ten million dilution and positive growth with the one million dilution set the count between one and ten million microorganisms. This observation was verified by the 9.4 million already derived from the standard plate count.

Sawdust Treated Soils

The soil samples obtained from the greenhouse conveniently lent themselves to a more critical evaluation of the turbidimetric procedure. In the case of the soil treated with sawdust only, the standard plate count was 12.8 million. However, when the sawdust and nitrogen were included together, the count was 8.5 million organisms per gram of soil. The noted difference in the plate counts can hardly be termed significant, yet these same counts are better expressed when considered in the light of the turbidimetric data (Figure 2, lines 2 and 4). The one million dilution of both soils showed little or no variation in turbidity trends (Figure 2, lines 1 and 3). However, the differences between these two soils were clearly demonstrated by the use of the higher ten million dilution (Figure 2, lines 2 and 4). Extensive growth density developed after a 24 hour shaking period when using the higher dilution of the soil not receiving nitrogen as the inoculum source. On the other hand the liquid medium receiving the ten million dilution from the sawdust treated soil plus nitrogen did not exhibit any turbidity at the end of a 36 hour period.
A second lot of soil obtained from the greenhouse had been treated with Douglas fir sawdust at the rate of 100 tons per acre. It was found that the three treated soils showed relatively little differences in bacterial numbers. The standard plate count on the non-treated soil was 7.5 million. The soil with sawdust added showed a microbial count of 7.6 million. In the case of the greenhouse soil that was treated with sawdust and nitrogen the count was found to be 8.45 million. The turbidity trends of the soils using the 100,000 dilution are depicted in Figure 3. As in the case of the standard plate count little difference in microbial activity was demonstrated by the turbidimetric procedure. It was significant that the soil treated with sawdust and nitrogen showed a higher plate count and correspondingly a more rapid increase in growth as depicted by the trends in turbidity (Figure 3, lines 1, 2 and 3).

The use of the one million dilution of the above soil samples demonstrated identical differences in growth density as that of the 100,000 soil dilution. The only significant variation was in the length of time required for initial turbidities to become visible (Figure 4, lines 1, 2 and 3). In the case of the higher dilution, the nutrient broth tubes showed little or no development of turbidity for 16 hours. At the end of a 24 hour period, optical density readings were almost equal for the same soils irrespective of the dilution.
Soil Treatment
1. Non-Treated
2. Sawdust Added at 100 Tons/Acre
3. Sawdust Added at 100 Tons/Acre plus Nitrogen

<table>
<thead>
<tr>
<th>Soil Treatment</th>
<th>Dilution</th>
<th>Plate Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Non-Treated</td>
<td>100,000</td>
<td>7,500,000/gm. soil</td>
</tr>
<tr>
<td>2. Sawdust</td>
<td>100,000</td>
<td>7,600,000/gm. soil</td>
</tr>
<tr>
<td>3. Sawdust</td>
<td>100,000</td>
<td>8,450,000/gm. soil</td>
</tr>
</tbody>
</table>

Figure 3. The Influence of Douglas Fir Sawdust on the Relative Microbial Activity of Chehalis Silty Clay Loam as Determined by the Standard Plate Count and the Turbidimetric Procedure.
Soil Treatment | Dilution | Plate Count
---|---|---
1. Non-Treated | 1,000,000 | 7,500,000/gm. soil
2. Sawdust Added at 100 Tons/Acre | 1,000,000 | 7,600,000/gm. soil
3. Sawdust Added at 100 Tons/Acre plus Nitrogen | 1,000,000 | 8,450,000/gm. soil

Figure 4. The Influence of Douglas Fir Sawdust on the Relative Microbial Activity of Chehalis Silty Clay Loam as Determined by the Standard Plate Count and the Turbidimetric Procedure.
Soil Treated with Glucose, Peptone and Ammonium Nitrate

A study of Figure 5 discloses that the addition of readily available nutrients to Chehalis silty clay loam brought about marked changes in the microbial flora of the test soil. The observed increase in numbers of microorganisms over the 72 hour period was most pronounced with the soil that was treated with dextrose. At 12 hours the standard plate count was 23.7 million. This figure became 239 million at the end of 24 hours, and 540 million at the end of 72 hours (Figure 5, line 2). The trends depicted by lines 3 and 4 in Figure 5 attest to the increases in microbial numbers of the soil treated with dextrose and nitrogen, and the soil treated with peptone. The control soil, which received no nutrients, demonstrated little change in microbial numbers over the course of the 72 hour period as shown by line 1, Figure 5.

Short Incubation Period

The turbidimetric procedure under investigation was devised to determine relative microbial activity of various soils. The artificially treated soils were at large enough variance at different time intervals to enable their being rated as to activity or numbers. Along with the standard plate counts, the turbidity method also demonstrated this variation.

Reference to Figure 6 discloses that the Chehalis silty clay loam treated with peptone showed the greatest
1. No Treatment.
3. Dextrose and NH₄NO₃ Added. C:N Equals 20/1.
4. Peptone Added at 1000 ppm Nitrogen.

Figure 5. The Influence of Various Treatments on the Microbial Activity of Chehalis Silty Clay Loam as Determined by the Standard Plate Count.
microbial activity as measured by the density of growth and the standard plate count. Approximately equal microbial stimulation was obtained with dextrose and nitrogen. The dextrose treated soil at the end of 12 hours showed a slightly higher plate count than the control soil. However, turbidimetrically there appears to be larger differences between the control soil and the dextrose treated soil. With the standard plate count, the soils at the end of 12 hours could be rated in order of decreasing activity at: four, three, two and one (Figure 6).

The plate counts computed after the 24 hour incubation period revealed the change in the relative position of the four soils in respect to the total count (Figure 7). Turbidimetrically, however, few differences were noted. This was due to the sudden rise in numbers and dilutions were not made to compensate for this increase.

As mentioned previously, at the 72 hour interval, plate counts of 540 million (dextrose), 280 million (dextrose and nitrogen), 48 million (peptone), and 18.9 million (control) were obtained. Using the treatment numbers shown in Figure 8, the above soils may be rated two, three, four and one in decreasing order of activity.

All previously mentioned data was obtained on wet samples. In order to check the influence of drying on the studied flora a portion of each sample was removed and air-dried for approximately three days. A comparison of Figures
Chehalis Silty Clay Loam
1. No Treatment.
3. Dextrose and \( \text{NH}_4\text{NO}_3 \) Added.
   C:N Equals 20/1.
4. Peptone Added at 1000 ppm Nitrogen.

sheet

Figure 6. Relative Microbial Index as Determined by the Standard Plate Method and the Turbidimetric Procedure on Test Soils Incubated for 12 Hours.
Chehalis Silty Clay Loam
1. No Treatment.
3. Dextrose and NH₄NO₃ Added.
   C:N Equals 20/1.
4. Peptone Added at 1000 ppm Nitrogen.

Figure 7. Relative Microbial Index as Determined by the Standard Plate Method and the Turbidimetric Procedure on Test Soils Incubated for 24 Hours.
Chehalis Silty Clay Loam
1. No Treatment.
3. Dextrose and NH₄NO₃ Added.
   C:N Equals 20/1.
   Peptone Added at 1000 ppm Nitrogen.

Figure 8. Relative Microbial Index as Determined by the Standard Plate Method and the Turbidimetric Procedure on Test Soils Incubated for 72 Hours.
5, 9 and 10 indicates that the relative numbers of microorganisms originally present in the most soils had decreased as a result of air-drying.

The greatest single decrease was noted with the dextrose treated soil in which case a drop from 540 million to 34 million was recorded. A decline in microbial count from 280 million to 79 million was found in the case of the soil to which dextrose and ammonium nitrate had been added. However, it is significant that the peptone treated soil showed a net increase in count of 39 million organisms per gram of soil after prolonged drying.

Depicted in Figure 9, lines 1, 2, 3 and 4 are the turbidity trends of the treated soils after being air-dried. The dilution used in this case was one million and differences noted among the soils can not be considered significant. However, reference to Figure 10 again illustrates the importance of the soil dilution used. In this case the critical dilution was ten million. The turbidity curves showed that slight development of optical density occurred at the end of 12 hours for the dextrose treated soil (Figure 10, line 2). The soil receiving the peptone treatment demonstrated extensive growth density at the end of the 12 hour period (Figure 10, line 4). It is also important to note the differences reflected by the turbidity curves of lines 3 and 4 in Figure 10. The peptone treated soil, with its plate count of 88 million organisms showed a rapid increase
Chehalis Silty Clay Loam
1,000,000 Dilution
1. No Treatment.
3. Dextrose and NH₄NO₃ Added.
   C:N Equals 20/1.
4. Peptone Added at 1000 ppm Nitrogen.

Plate Count

10,000,000/gm. soil
34,000,000/gm. soil
79,000,000/gm. soil
88,000,000/gm. soil

Figure 9. Relative Microbial Activity as Determined by the Standard Plate Count and the Turbidimetric Procedure on Air-Dried Samples of Test Soils Incubated for 72 Hours.
Chehalis Silty Clay Loam
10,000,000 Dilution

1. No Treatment.
3. Dextrose and NH₄NO₃ Added. C:N Equals 20/1.
4. Peptone Added at 1000 ppm Nitrogen.

Plate Count

- 10,000,000/gm. soil
- 34,000,000/gm. soil
- 79,000,000/gm. soil
- 88,000,000/gm. soil

Figure 10. Relative Microbial Activity as Determined by the Standard Plate Count and the Turbidimetric Procedure on Air-Dried Samples of Test Soils Incubated for 72 Hours.
in growth density, whereas the dextrose and nitrogen treated soil, with a plate count of 79 million organisms displayed a growth density curve that closely resembled the peptone density curve. Interestingly enough, a soil rating based on the plate count (four, three, two and one) corresponds to the turbidimetric rating of four, three, two and one.

Prolonged Incubation Period

The moist soils were incubated for prolonged periods of time: the control soil and the soil treated with dextrose for 53 days, and dextrose plus nitrogen treated soil and the peptone treated soil for 42 days. These were air-dried and subsequent dilutions were carried out for the standard plate count and the turbidimetric procedure.

The dilution finally used in this study was one million. The highest plate count of 68 million microorganisms per gram of soil was obtained with the air-dried peptone treated soil. This result is also reflected in the more rapid development of growth in broth shown in Figure 11, line 4. Soils treated with dextrose and with dextrose plus nitrogen gave microbial counts of 38 and 36 million, respectively. In this instance, the turbidity trends of these two soils are almost identical. The use of a ten million dilution probably would have brought out the differences in these four soils. However, from the turbidity trends, one can rate the soils as four, three, two and one in order
Chehalis Silty Clay Loam
1,000,000 Dilution
1. No Treatment.
3. Dextrose and NH₄NO₃ Added. C:N Equals 20/1.
4. Peptone Added at 1000 ppm Nitrogen.

<table>
<thead>
<tr>
<th>No Treatment.</th>
<th>Dextrose Added</th>
<th>Dextrose and NH₄NO₃ Added</th>
<th>Peptone Added</th>
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<td>8,300,000/gm. soil</td>
<td>38,000,000/gm. soil</td>
<td>36,000,000/gm. soil</td>
<td>68,000,000/gm. soil</td>
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Figure 11. Relative Microbial Activity as Determined by the Standard Plate Count and the Turbidimetric Procedure on Air-Dried Samples of Test Soils after Prolonged Incubation.
of decreasing microbial activity. From the plate count the order of three and two would be reversed.

Forest Soils

Turbidimetric determinations and standard plate counts were made on six forest soils obtained from the Soils Department. No data or information as to the previous history was supplied with these soils. Therefore numbers from one to six were designated as a means for identification.

The standard plate counts showed soil five had the highest microbial count of 64 million. Soil six showed the lowest plate count of 400,000. The bacterial numbers of the soils one, two, three and four were 2.47, 2.43, 1.97 and 2.66 million, respectively. According to the bacterial counts derived from the standard plate method, these six soils could be rated five, four, one, two, three and six in order of decreasing activity. Reference to Figure 12, demonstrated that turbidimetrically the major differences were in accordance with the plate counts. However, the ratings of soils one, two and three are not significant with either the plate counts or the turbidimetric determinations.

Figure 13, lines 5 and 6 showed that the large significant differences were demonstrated by using a higher dilution of one million. However, forest soils one, two, three and four were not significantly different. This was shown by the use of either dilution (Figures 12 and 13).
Figure 12. Relative Microbial Activity as Measured by the Standard Plate Count and the Turbidimetric Procedure on Air-Dried Samples of Forest Soils.
<table>
<thead>
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<th>Soil Number</th>
<th>Plate Count (10,000 Dilution)</th>
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<tbody>
<tr>
<td>1.</td>
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<td>2.</td>
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<td>3.</td>
<td>1,970,000/gm. soil</td>
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<td>4.</td>
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<td>5.</td>
<td>64,000,000/gm. soil</td>
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<td>6.</td>
<td>400,000/gm. soil</td>
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</tbody>
</table>

**Figure 13.** Relative Microbial Activity as Measured by the Standard Plate Count and the Turbidimetric Procedure on Air-Dried Samples of Forest Soils.
Microscopic Examinations

Microscopic examination of the microflora present in representative tubes was made when maximum turbidity was attained by the bacterial cultures. A loopful taken from these tubes and stained by the Gram's method showed that turbidity could be attributed to the growth of gram positive rods. In a few instances the organisms were found to be gram negative rods. Gram positive cocci were never found to be the cause of turbidity.

Standard plates were chosen from the 12, 24 and 72 hour intervals. These plates depicted on Figure 14, pages 37 and 38 represent a picture of the microscopic flora capable of growing on the media used. The best plates were picked irrespective of dilution, and therefore do not demonstrate any change of bacterial numbers. Ten colonies were picked from each plate, and gram stains were made. The types or kinds of microorganisms that represented the colonial growth are recorded in Table 1. A study of Table 1 indicated that the overall highest percentage of microorganisms on the plates were gram positive rods. However, the percentage of gram positive cocci was considerable.
TABLE 1

The Relative Percentages of Representative Microorganisms Found Present on the Plates Depicted in Figures 15 and 16.

<table>
<thead>
<tr>
<th>PLATE NO.</th>
<th>LARGE GRAM POSITIVE RODS</th>
<th>SMALL GRAM POSITIVE RODS</th>
<th>MIXTURE OF LARGE AND SMALL GRAM POSITIVE CELLS</th>
<th>GRAM POSITIVE COCCI</th>
<th>GRAM NEGATIVE RODS</th>
<th>YEAST CELLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>50%</td>
<td>10%</td>
<td>40%</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>2.</td>
<td>60%</td>
<td>20%</td>
<td>10%</td>
<td>--</td>
<td>10%</td>
<td>--</td>
</tr>
<tr>
<td>3.</td>
<td>60%</td>
<td>10%</td>
<td>30%</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>4.</td>
<td>10%</td>
<td>40%</td>
<td>30%</td>
<td>20%</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>5.</td>
<td>--</td>
<td>20%</td>
<td>60%</td>
<td>20%</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>6.</td>
<td>--</td>
<td>60%</td>
<td>40%</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>7.</td>
<td>20%</td>
<td>10%</td>
<td>--</td>
<td>30%</td>
<td>30%</td>
<td>--</td>
</tr>
<tr>
<td>8.</td>
<td>20%</td>
<td>10%</td>
<td>50%</td>
<td>20%</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>9.</td>
<td>20%</td>
<td>--</td>
<td>20%</td>
<td>30%</td>
<td>10%</td>
<td>20%</td>
</tr>
<tr>
<td>10.</td>
<td>40%</td>
<td>10%</td>
<td>30%</td>
<td>10%</td>
<td>10%</td>
<td>--</td>
</tr>
<tr>
<td>11.</td>
<td>--</td>
<td>--</td>
<td>80%</td>
<td>10%</td>
<td>10%</td>
<td>--</td>
</tr>
<tr>
<td>12.</td>
<td>10%</td>
<td>10%</td>
<td>30%</td>
<td>20%</td>
<td>20%</td>
<td>--</td>
</tr>
</tbody>
</table>
Legend for Plates Depicted on Figure 14, pages 37 and 38.

<table>
<thead>
<tr>
<th>Plate Number</th>
<th>Soil Treatment</th>
<th>Dilution</th>
<th>Sample Time</th>
<th>Plate Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>No Treatment</td>
<td>100,000</td>
<td>12 hours</td>
<td>10 million</td>
</tr>
<tr>
<td>2.</td>
<td>No Treatment</td>
<td>100,000</td>
<td>24 hours</td>
<td>17.3 million</td>
</tr>
<tr>
<td>3.</td>
<td>No Treatment</td>
<td>100,000</td>
<td>72 hours</td>
<td>16.9 million</td>
</tr>
<tr>
<td>4.</td>
<td>Dextrose Added</td>
<td>100,000</td>
<td>12 hours</td>
<td>23.7 million</td>
</tr>
<tr>
<td>5.</td>
<td>Dextrose Added</td>
<td>1,000,000</td>
<td>24 hours</td>
<td>289 million</td>
</tr>
<tr>
<td>6.</td>
<td>Dextrose Added</td>
<td>10,000,000</td>
<td>72 hours</td>
<td>540 million</td>
</tr>
<tr>
<td>7.</td>
<td>Dextrose plus Nitrogen</td>
<td>500,000</td>
<td>12 hours</td>
<td>67 million</td>
</tr>
<tr>
<td>8.</td>
<td>Dextrose plus Nitrogen</td>
<td>1,000,000</td>
<td>24 hours</td>
<td>200 million</td>
</tr>
<tr>
<td>9.</td>
<td>Dextrose plus Nitrogen</td>
<td>10,000,000</td>
<td>72 hours</td>
<td>280 million</td>
</tr>
<tr>
<td>10.</td>
<td>Peptone Added</td>
<td>500,000</td>
<td>12 hours</td>
<td>82 million</td>
</tr>
<tr>
<td>11.</td>
<td>Peptone Added</td>
<td>500,000</td>
<td>24 hours</td>
<td>184 million</td>
</tr>
<tr>
<td>12.</td>
<td>Peptone Added</td>
<td>1,000,000</td>
<td>72 hours</td>
<td>48 million</td>
</tr>
</tbody>
</table>
Figure 14. The Influence of Various Treatments on the Microbial Flora of Chehalis Silty Clay Loam as Shown by the Standard Plate Method.
Figure 14-continued. The Influence of Various Treatments on the Microbial Flora of Chehalis Silty Clay Loam as Shown by the Standard Plate Method.
DISCUSSION

The problem of estimating microbial numbers or activity in soil calls to ones attention various fundamental questions. In the present study an attempt was made to use a small segment of the total population as an indicator group. Naturally the question arises as to the validity of this approach. The studies dealing with Orzan and sawdust treated soil aptly demonstrated that massive changes in the soil population can be measured turbidimetrically by the increase in cell density of predominately gram positive types. Certainly the soil ratings obtained with the turbidimetric procedure compared well with those obtained by the usual standard plate method. In some instances the former method appeared more selective. Thus, the rapid rise in number of the gram positive cells appears to offer a valuable tool in a study of the soil flora.

In some instances the disadvantages of the turbidimetric procedure are of the same nature as one finds with the standard plate method. Since the medium used is somewhat selective, growth will be indicated by only those microorganisms that can develop on or in the medium used. This factor limits the range of organisms that can be studied.

As with the standard plate count, care and precision are prime prerequisites for the preparation of soil dilutions.
Standardization of dilution procedure is mandatory for reproduction of favorable results.

It is evident that soils which exhibit marked differences in total plate count are not difficult to rate using the turbidimetric approach. On the other hand, four forest soils which showed similar microbial counts were more difficult to rate. Thus, at least three different dilutions must be used in order to catch the critical dilution. In the case of widely divergent counts a lesser number of dilutions may be used. With proper choice of time interval, a single dilution could serve.

An inherent advantage of this procedure is that results can be obtained in a relatively short period of time. Standard plate procedures require a minimum time of four days before a count can be made. However, with the turbidimetric method, results can be obtained within 12-36 hours. The latter procedure is comparatively simple and requires only a little knowledge of dilution and the use of a colorimeter or spectrophotometer.

It should be emphasized that the method under study does not supply all the answers to problems encountered in the microbiological analysis of soil. This was hardly intended. However, it is to be hoped that the area under study has received worthy stimulation for future investigations of this type.
SUMMARY

Differences in microbial activity of variously treated soils were demonstrated by the turbidimetric procedure. These differences observed with the turbidimetric method correlated well with the standard plate count procedure. Soils which were treated gave a wide range of microbial activity. Whenever analysis of these soils was carried out, the turbidimetric method readily indicated the noted differences. Therefore, these soils could be rated in order of microbial activity.

The many advantages offered by the turbidimetric procedure are as follows:

1. Results are obtained within 12-36 hours.
2. It is more economical, since smaller amounts of media are needed.
3. The method is simple to perform, and the time involved for completion is less than for the standard plate procedure.
4. It is probably more accurate; less opportunity for error.
5. It is reliable in that it measures the relative abundance of organisms predominating at any given time.


11. James, Norman and Marjorie Sutherland. The accuracy of the plating method for estimating the numbers of soil bacteria, actinomyces, and fungi in the dilution plates. Canada journal of research, section C 17:72-86. 1939.


27. ______ Microbiological analysis of soil as an index of soil fertility. II. Methods of the study of the numbers of micro-organisms in the soil. Soil science 14:283-298. 1922.


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