# A Preliminary Study of the Bacterial Flora of a Revegetating Field and a Prairie

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## INTRODUCTION

This preliminary report is the result of an investigation that was carried out at the University of Oklahoma during the month of June, 1952. It is only one of a series of investigations that have been made on the University of Oklahoma Grassland Investigation Plots. Investigations have been made dealing with the following: vegetational composition, microclimate, plowing and mulching, and soil inhabiting organisms. The study of the fungi, and the effects of Krilium on the soil in these plots is still in progress.

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One of the difficulties in the study of soil microflora is the inability of investigators to get bacteria to grow upon ordinary media (1). Another difficulty which makes the numbers of bacteria found in certain samples of soil seem unreliable is the fluctuation in counts as determined by the plating technique (2). Taylor (6) demonstrated that these fluctuations occurred from hour to hour and Russel (5) stated that these variations occur even if soils are kept at a constant temperature and moisture content. These observations indicate that better techniques must be found in order to make more reliable studies of soil bacteria.

## Materials and Methods

Soil samples were taken from five stations in each area by means of a soil auger 1½ inches in diameter. These stations were spaced 10 paces apart over the areas. Four samples were taken from each station from depths of 0-6, 6-12, 12-18, and 18-24 inches. The A horizon was found to be from 0-12 inches, the B horizon from 12-18 inches, and the C horizon from 18-24 inches. The individual samples were analyzed to determine the bacterial numbers, types of bacteria found most abundantly, *p*H, and organic carbon content. A composite sample was made for each level from all stations and analyzed similarly.

Ten gram portions of soil of each sample were used to make 1-10,000, 1-100,000, and 1-1,000,000, dilutions. A one milliliter portion of each sample was then plated in Sabouraud's agar, nutrient agar, nutrient broth containing 12 per cent gelatin, and a tap water solution of 12 per cent gelatin. Each dilution was plated in triplicate on each type of medium. An average of the triplicate count was used as the count for that particular sample. The samples were incubated 5 days at a temperature of 25° C. The gelatin was cooled to a temperature of 18° C. after the incubation period in order to determine if liquefaction had taken place. The liquefaction of gelatin is one of the key characteristics used by Conn (1) to identify certain soil bacteria. The plate count was used to detect differences in numbers of bacteria found in the two areas studied.

The *p*H of each sample was determined by use of a Beckman model H2 *p*H meter. Organic carbon was determined by the Walkley-Black technique (4).

## Results and Discussion

The bacterial count was found to be higher in the 0-12 inch level in the prairie area than in the revegetating field (Tables I and II). A higher

TABLE I

*Total Bacterial Counts per Gram of Soil on Nutrient and Sabouraud's Agar*

| Depth | Sabouraud's Agar | Nutrient Agar |
|---|---|---|
| **Virgin Prairie** | | |
| 0- 6 in. | $15.6 \times 10^5$ to $19.5 \times 10^5$ | $26 \times 10^5$ to $4.5 \times 10^5$ |
| 6-12 in. | $11.3 \times 10^5$ to $14.5 \times 10^5$ | $12.4 \times 10^5$ to $28.8 \times 10^5$ |
| 12-18 in. | $7.4 \times 10^5$ to $9.9 \times 10^5$ | $7.7 \times 10^5$ to $9.9 \times 10^5$ |
| 18-24 in. | $4.3 \times 10^5$ to $5.5 \times 10^5$ | $3.7 \times 10^5$ to $5.6 \times 10^5$ |
| **Revegetating Field** | | |
| 0- 6 in. | $10.2 \times 10^5$ to $11.6 \times 10^5$ | $9.6 \times 10^5$ to $11.2 \times 10^5$ |
| 6-12 in. | $9.0 \times 10^5$ to $9.9 \times 10^5$ | $8.6 \times 10^5$ to $10.9 \times 10^5$ |
| 12-18 in. | $5.3 \times 10^5$ to $8.7 \times 10^5$ | $7.6 \times 10^5$ to $9.5 \times 10^5$ |
| 18-24 in. | $4.3 \times 10^5$ to $5.4 \times 10^5$ | $3.3 \times 10^5$ to $5.2 \times 10^5$ |

count was obtained in the 0-12 inch level in both areas. A similar observation was made by Harley (3) and Timonen (7) in samples of podsolic soils in which higher numbers of bacteria were found in the A horizon than in the C horizon. With an increase in depth, there is an increase in potentially anaerobic fungi and bacteria. Higher numbers of bacteria were obtained on both media used for samples of soil taken from the 0-12 inch level of the prairie plot than from the same level of the abandoned field. The bacteria in the lower levels of the two fields were nearly the same and no significant differences in numbers were obtained.

TABLE II

*Bacterial Counts of Composite Samples per Gram of Soil*

| Depth | Prairie | | Revegetating field | |
|---|---|---|---|---|
| | Sabouraud's Agar | Nutrient Agar | Sabouraud's Agar | Nutrient Agar |
| 0- 6 in. | 17.5 x $10^5$ | 42.2 x $10^5$ | 13.0 x $10^5$ | 10.9 x $10^5$ |
| 6-12 in. | 12.8 x $10^5$ | 25.3 x $10^5$ | 9.4 x $10^5$ | 8.7 x $10^5$ |
| 12-18 in. | 8.9 x $10^5$ | 9.4 x $10^5$ | 52.2 x $10^5$ | 5.5 x $10^5$ |
| 18-24 in. | 4.7 x $10^5$ | 5.4 x $10^5$ | 4.3 x $10^5$ | 3.2 x $10^5$ |

The colony characteristics, Gram's stain and the morphology and activity of the bacteria were observed on nutrient agar and nutrient gelatin. Differences in species composition of bacteria were observed in the two areas. *Bacillus mycoides*, and *Streptomyces spp.* were found to be present in essentially equal numbers in the two areas. In the virgin prairie area the following species were noted as being most abundant, *Arthrobacter spp.*, *Bacillus megatherium*, *Streptomyces spp.*, and *Bacillus mycoides* in that order. In the revegetating field *Agrobacterium spp.* were the most abundant bacteria. *Pseudomonas spp.*, *Micrococcus spp.*, *B. mycoides*, and *Streptomyces spp.* were also observed. Composite samples of the prairie and revegetating field soil showed a similar pattern.

The virgin prairie soil samples were generally alkaline, while the abandoned field samples were slightly acid (Table III). The quantitative and qualitative effects that the differences of *p*H on the bacterial populations should be investigated further.

TABLE III

*Variation in Range of pH of Soil Samples Collected on June 10, 1952.*

| Depth | Virgin Prairie | Revegetating Field | Virgin Prairie (composite) | Revegetating Field (composite) |
|---|---|---|---|---|
| 0- 6 in. | 6.7 to 7.3 | 6.6 to 6.8 | 6.9 | 6.5 |
| 6-12 in. | 6.8 to 7.4 | 6.7 to 6.8 | 7.1 | 6.6 |
| 12-18 in. | 6.7 to 8.3 | 6.4 to 6.7 | 7.2 | 6.4 |
| 18-24 in. | 7.1 to 8.7 | 6.6 to 6.9 | 7.5 | 6.5 |

Organic carbon was found not to be significantly different in the two plots. The prairie had an average organic carbon content of 2.34 per cent and the revegetating field 1.42 per cent (Walkley and Black values); the organic matter in the prairie was 2.89 per cent and that of the revegetating field 3.05 per cent.

This report was based on the analysis of one collection of soil samples, and therefore these results cannot be interpreted as those which would always be expected when a comparison is made between a prairie plot and a revegetating field. Further study should be made to determine the bacterial populations over a period of time.

SUMMARY

A preliminary investigation of a revegetating field and a virgin prairie revealed a significantly higher bacterial count in the prairie at the 0-12 inch level. Further investigation also revealed a difference in the bacteria species found in the two areas. Whether this difference is due to technique or to fluctuations in bacterial numbers, or to absolute differences in the two plots that enable more bacteria to live in the prairie soil was not ascertained.

## LITERATURE CITED

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