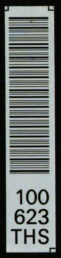
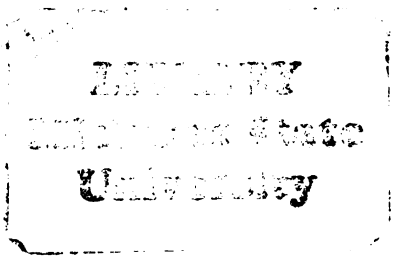




This is to certify that the

dissertation entitled

THE EFFECT OF SOIL MOISTURE
ON AMOEBA POPULATION

presented by

NAKISAH MAT AMIN

has been accepted towards fulfillment
of the requirements for

Master's degree in Zoology

Major professor

Date 12/22/83

THE EFFECT OF SOIL MOISTURE ON AMOEBA POPULATION

by

Nakisah Mat Amin

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements for
the degree of

MASTER OF SCIENCE

Department of Zoology

1984

ABSTRACT

THE EFFECT OF SOIL MOISTURE ON AMOEBA POPULATION

by

Nakisah Mat Amin

A pressure membrane extractor was used to measure the relationship between soil moisture and soil suction for a sandy loam soil. The lower and upper limit of available water for this soil was 25.46% and 3.79%. Diameters of soil pores which were emptied at corresponding suction levels were calculated. Soil pores larger than 7.5 μ m effectively maintained the most water which was required for amoeba growth.

A peak population density of amoebae in soil was achieved at soil moisture of 30%. Soil moisture levels higher or lower than 30% negatively affected the growth of amoebae. A comparison of the species composition of amoeba populations at soil mositures of 10% and 40% revealed no difference at these two moisture levels; however, the total number of amoebae was higher at a soil moisture of 40% (p 0.01). Acanthamoeba polyphaga was the most abundant of the various species identified.

E. coli was used to feed soil amoebae and the survival of this bacterium in soil showed that its numbers decreased over time. The factors which lead to this decrease, including predation by amoebae in soil, are discussed.

DEDICATION

To my parents

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation and thanks to Dr. Neal R. Band, my major advisor, for his patience and valued guidance during the course of this study. My thanks are also due to Dr. Richard J. Snider and Dr. Michael J. Klug for their constructive comments and helpful suggestions in preparing and writing this thesis.

I also wish to express my appreciation to Mr. Allen K. Sample, Dr. Sterling Thompson, and Nouri for giving me assistance in doing my research. I would like to thank Kay for editing this thesis and Barbara for her typing. To my close friends, I also acknowledge their help and support while finishing my study at this university.

Finally, a special thank you is extended to Abdullah for his encouragement and help which sustained me throughout my preparing this thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Naked amoebae are the most abundant among soil protozoa (Cutler, 1923). The genera Hartmannella Alexeieff, Naegleria Alexeieff, Vahlkampfia Chatton and Lalung-Bonnaire and Acanthamoeba Volkonsky are common naked soil amoeba (Crump, 1950; Geltzer, 1963). The cells of these amoebae are highly flexible, making it easy for them to move around in the soil microenvironment (Sleigh, 1973). Tropic forms consume other microorganisms such as bacteria and yeasts, demonstrating that their presence in soil is important as micropredators (Nikoljuk, 1969; Sleigh, 1973; Clarholm, 1981). To grow soil amoebae and other protozoa in a culture medium (not axenic culture), edible bacteria such as Escherichia coli (Migula) are used as food (Singh, 1946).

There is a close relationship between soil properties and amoeba life. Soil moisture is the most important factor for the growth of amoebae in certain soils (Stout & Heal, 1967). Water is held by adhesive and cohesive forces to soil particles and between water molecules, and it completely occupies soil pores when saturated (Foth, 1978). External forces are required to drain water from soil pores (Childs, 1940). Although measurement of soil moisture does not indicate potential energy of soil water, it gives an estimate of the air-filled pores (Griffin, 1963). A soil moisture characteristic curve is used to estimate soil pore sizes (Childs, 1940).

Objectives of the present study were to (a) evaluate the relationship between habitable soil pores and the amoeba population at various soil moisture levels; (b) determine relative species composition of amoebae at soil moistures

of 10% and 40%; and (c) since no previous studies have reported E.coli as food for amoebae in soil, another objective was to measure the survival of E.coli in soil.

LITERATURE REVIEW

Occurrence of Amoebae in Soil

The protozoan fauna are similar in all soils (Dixon, 1936), and their numbers are roughly related to bacterial numbers (Sandon, 1928). Of these soil protozoa, naked amoebae are the most abundant (Cutler, 1923). Their numbers increase in cultivated soil and the amoebae cause a 60% decrease in bacteria added to soil (Clarholm, 1981). The accumulation of protozoa in a rhizosphere can be traced to greater activity and multiplication of bacteria, which can serve as food for amoebae in that region (Linford, 1942).

In soil, most of the protozoa present are heterotrophic. The trophic forms of amoebae ingest other soil microorganisms such as bacteria or yeasts. Acanthamoeba castellanii (Douglas, 1930) consumes both unicellular and filamentous forms of cyanobacteria (Wright et al., 1981), and it has surface binding sites for flagella of seveal bacteria (Preston & King, 1982). Various authors have reported on the selectivity of soil protozoa in consuming different bacteria (Singh, 1946; Nikoljuk, 1969). Although naked amoebae present in soil are free-living and feed on other soil microorganisms, a few of them are potentially pathogenic to laboratory mice (Culbertson, 1971).

Naked amoebae commonly found in soil include the genera Hartmannella Alexeieff, Naegleria Alexeieff, Vahlkampfia Chatton and Lalung-Bonnaire and Acanthamoeba Volkonsky (Crump, 1950; Geltzer, 1963). Fellers and Allison (1920) conducted a quantitative survey of protozoa in New Jersey soil and found Naegleria gruberi (Schardinger, 1899) to be the most abundant soil amoeba. The

latter was subsequently re-identified by Sandon (1928) as Hartmannella hyalina (Dangeard). In New Brunswick soil, Sandon (1928) found that the predominant species of amoebae was very similar to Hartmannella hyalina. Dixon (1936) discovered 25 species of Rhizopoda in Russian soil, of which the three most common were Hartmannella hyalina, Limax sp x (Sandon, 1928), and Amoeba diplodea Hartmann and Nagler. Rhizopoda testacea were poorly represented. Although numbers and species of protozoa did not show a significant relationship with soil depth, more species and numbers of individuals were found in the top layer (Dixon, 1936). One factor found important to the occurrence of varied species of amoebae in soil was the number of bacteria present as food supply (Sandon, 1927).

Trophic forms of amoebae are frequently present in soil (Fellers & Allison, 1920); in its microenvironment, such organisms live on soil particles (Russel & Appleyard, 1915) and move about to graze on bacteria (Sleigh, 1973). When the soil microenvironment is inhospitable, amoebae will be encysting (Stout, 1973). Encystation can be induced by moistening the cysts or stimulation with compounds excreted by living bacteria (Crump, 1950; Drozanski, 1963).

Soil Water

Soil texture refers to the relative size of soil particles and aggregation of these particles into groups is called soil structure (Buckman & Brady, 1969). The United States Department of Agriculture categorizes soil particles as very coarse sand, coarse sand, medium sand, fine sand, very fine sand, silt, and clay. The proportion of all these particles defines a textural soil class by using "The Soil Textural Triangle" (Foth, 1978).

Since soil particles are not spheres, their arrangement or aggregation forms pore spaces of complex interconnected shapes (Collis-George, 1959). The

number and nature of pore spaces vary in an individual soil and are determined by size, shape, and arrangement of soil particles (Darbyshire, 1975). These pore spaces are either filled with gasses or various concentrations of gasses and water (Foth, 1978). To remove water from these pore spaces, the water-air interface has to be able to pass through the pore necks; this can be done only if the force applied is greater than the equivalent surface tension force (Collis-George, 1959).

In unsaturated soil, water is held to soil particles by adhesive forces and between water molecules by cohesive forces, which leaves some space for air-filled pores (Foth, 1978). At saturation, all of the pores are filled with water and there is a continuity or conductivity of water flow in soil. If it is not saturated, some of the pores become air-filled and water conductivity will decrease. When pressure (or suction) is applied to soil, the largest pores, which are most conductive according to Poiseuille's law (the rate of water flow is proportional to the fourth power of the pore radius), will be emptied first so the water only flows in smaller pores (Hillel, 1980).

Soil moisture content (or soil moisture) is a term used to express the ratio of water to soil, per mass, based on dry soil. Generally, this ratio is multiplied by 100 to express it in terms of percentage (Buckman & Brady, 1969). Soil water has two forms of energy, kinetic and potential. The movement of water in soil is very slow so that its kinetic energy is not significant compared with its potential energy, which becomes the major factor in determining the state and movement of water in soil. In the field, soil water is subjected to many forces. These forces may be combined to derive the total potential of soil water as is explained below.

$$\phi_t = \phi_g + \phi_\rho + \phi_o$$

where ϕ_t = total potential

ϕ_g = gravitational potential

ϕ_p = pressure or matric potential

ϕ_o = osmotic potential

The ellipses indicate the possible additional terms which can be included in this equation.

Among various potentials, pressure or matric potential describes the tenacity of soil water to soil particles (Hillel, 1980). Measurements of soil moisture do not express the potential energy of soil water, but give estimates of air-filled pores (Griffin, 1963). The relationship between soil moisture and corresponding soil suction is graphically expressed by a soil moisture characteristic curve (Childs, 1940) which is strongly affected by soil texture and structure (Hillel, 1980).

Soil water is available for plants in the range between field capacity (FC) and permanent wilting point (PWP). FC is the upper and PWP the lower limit of available water in soil (Buckman & Brady, 1969). Available-water capacity (AWC) depends on texture and structure of soil; it correlates negatively with the percentage of coarse sand and positively with the percentage of fine sand (Salter & Williams, 1965; Salter et al., 1966). Field capacity is commonly defined as moisture retained in soil two days after the soil was partially wet and represents the maximum storage capacity (Sykes & Loomis, 1967). In most soils, FC is equivalent to 1/3 bar of soil moisture suction (Baver et al., 1972). PWP is the percentage of moisture left in a soil that can cause permanent wilting in plants (Sykes & Loomis, 1967) and normally taken to be at 15 atm (bars) (Salter & Williams, 1965). As the water capacity of soil is affected by its texture and structure, these properties and AWC at varied levels, affect the numbers and species of amoebae.

Effects of Soil Properties on Amoebae

Soil structure and texture influence the distribution of soil microorganisms in soil (Heal, 1962). The number of protozoa retained in fine or coarse sand is much larger than those retained in silt or clay (Cutler, 1919). The diameter of soil spaces is also found to greatly influence the distribution of various testate amoebae (Heal, 1962). Amoebae with larger shells (or tests) are found to inhabit larger soil spaces within the organic soil horizon; amoebae with smaller tests inhabit smaller soil spaces in mineral layers. Mahler and Wollum (1981) reported that populations of Rhizobium sp were lowest in sandy and clay loam soils. The low occurrence of this bacterium in these soils limits the number of amoebae present. Generally, pore space sizes decrease with increasing depth with a corresponding decrease in the vertical direction of small cavities available for soil inhabitants (Wallwork, 1970).

The amount of water present in soil plays an important role in influencing soil atmosphere, soil temperature, and freedom of animal movement (Collis-George, 1959). Water volume is not constant, but varies with time. Water conduction is determined by soil particle size (Donahue et al., 1977). Sandy soil is more permeable than clay and water capillary action increases as particle size decreases (Collis-George, 1959). The last remnants of water in capillaries is held at the corner of the soil spaces due to surface tension and serves as habitat for the smallest soil inhabitants like protozoa and bacteria in dry soil. Alexander and Jackson (1954) observed soil bacteria in the film of colloidal material covering soil particles. To get water, soil microorganisms have to perform work equal to the energy expended in retaining water in soil (Collis-George, 1959). Soil animals have to maintain the osmotic pressure of their body fluids equal to the energy of water in soil. If not, water will diffuse out from their bodies, especially in dry soil. Normally these orgnisms form some impermeable outer

coverings (cysts) to prevent water loss; others tolerate extreme environmental conditions or shift to a new environment (Collis-George, 1959).

Moisture is the most significant property for the growth of amoebae in certain soils (Stout & Heal, 1967). This includes the alternative processes of wetting and drying of soil, movement of water to narrow pores, association of water film with mineral and organic colloids and variations in osmotic tension. Cutler and Dixon (1927) showed that reproduction of protozoa falls when the moisture content of soil drops below half of field capacity. The growing period of the ciliate Colpoda steini Maupas, 1883 decreased in a soil sample subjeted to increased suction (Darbyshire, 1976). The large amount of water lost from soil pores under this suction affects the growth of this species (Darbyshire, 1976). While no direct relationship has been found between bacterial number and soil moisture content (Waksman, 1916), Clark (1967) reported that at a moisture tension of three bars or higher, bacterial activities are reduced, especially at 15 bars of mositure tension (at PWP).

Although previous studies have not focused on the effect of soil aeration on amoebae, its effects on other aerobic soil microorganisms show that the presence of air (i.e., oxygen) in soil pores promotes their growth. The occurrence of aerobic and anaerobic bacteria in soil depends on pore space air. Filling up these pores with water reduces the availability of oxygen and affects aerobic bacterial growth (Clark, 1967). A study done by Winogradsky (1924), and cited by Skinner (1975), shows that the presence of Azotobacter (aerobic bacteria) and Clostridium (anaerobic bacteria) at various soil depths depends on water content which directly affects soil aeration. Azotobacter grow throughout the soil column at low soil water content (e.g., 15% or lower). Soil water content higher than 20% favors the growth of Clostridium. In general, lack of aeration slows down biological activities in soil. Decomposition and mineralization rates

of organic materials in soil will also be reduced (Baver et al., 1972). Aeration is the most effective factor influencing fungal activity in soil compared with moisture content, soil texture, and structure (Griffin, 1963). Williams et al. (1972) found that Streptomycetes grow best in pore spaces which are humid and air-filled, rather than water-logged.

Soil atmosphere is not static, but changes over time because of several on-going processes (Burges, 1967). Living organisms use oxygen from air-filled pores and produce carbon dioxide as a byproduct of respiration. The latter gas diffuses to the external environment and, likewise, fresh oxygen will diffuse down from the above ground atmosphere. The partial pressure of both gasses in the soil environment is controlled by the diffusion process. The CO_2/O_2 ratio depends on soil pore sizes and pathways (Collis-George, 1959). If pathways are straight and pore sizes large, this ratio will decrease. The diffusion coefficient for various water-air ratios is a linear function of air porosity (Penman, 1940). Carbon dioxide formed in soil pores is increased with depth (Kuhnelt, 1961). The response of soil organisms to this gas is variable (Burges & Fenton, 1953). Saprophytic soil protozoa show a positive chemotaxis to carbon dioxide at lower concentrations and show a reverse reaction at higher concentrations (Kuhnelt, 1961).

Such other soil properties as pH, salinity, and temperature also influence the life of soil amoebae. Soil water (with dissolved nutrients) tends to move from warmer to cooler areas, indirectly affecting microorganisms distribution (Cary & Maryland, 1972). A significant change in soil pH will affect its ionic condition (Buckman & Brady, 1969). Microorganisms are affected directly by the change in H-ion concentration which indirectly influences nutrient elements. Fluctuations in the reaction of soil solution also affect soil organisms. Bacteria and actinomycetes grow in mineral soils at relatively high pH (Warcup, 1951, cited by Warcup, 1967), but the tolerance of individual species of soil protozoa to

soil pH is largely undetermined (Heal, 1967). The presence of protozoa in various soils at different pH levels, however, gives an impression that they can grow within a relatively wide range of pH (Stout, 1956). Barnes and Ali (1917) showed that an increase of soil salinity will decrease the number of microorganisms, as well as inhibit some of their activities. Increasing salinities may trigger the encystment of soil protozoa (Band, 1963). The transformation of soil amoebo-flagellates, Naegleria gruberi is determined by the concentration of cations or anions in soil solution (Willmer in Stout & Heal, 1967). Fluctuations in soil aeration, pH, and temperature affect the growth and survival of amoebae and E. coli. It is useful to be aware, at least, of all these effects in examining the treatment effcts that are the focus of this study.

Escherichia coli

Escherichia coli is a gram-negative bacillus, indigenous to the gastrointestinal tract. E. coli is not a soil inhabitant, so its presence in soil is used as an indicator of faecal contamination and its density is proportional to the degree of faecal contamination (Geldreich et al., 1962).

Survival of E. coli in soil is very low due to the low availability of its specific required nutrients in soil (Waksman & Starkey, 1923; Waksman & Woodruff, 1940). Additional factors antagonistic to the success of E. coli in soil are the type of bacteria added, treatment, and temperature incubation of the soil. Waksman and Woodruff (1940) suggested bacteriostatic and bactericidal substances might be produced by soil actinomycetes.

MATERIALS AND METHODS

Soil Sampling and Treatments

Soil samples were taken in September 1981 and 1982 at the Rose Lake Wildlife Research Area (see Appendix A for sampling sites). These two soil samples were then used as sources for soil subsamples throughout the experiments. The area of each sample taken was approximately 18 x 18 square cm, using a shovel. To obtain homogeneous soil samples, grass Agropygon repens (Linneus) was discarded and the soil was passed through a 2.0 mm sieve and mixed thoroughly by hand before it was stored. Samples were kept in covered buckets and stored at 4C. Generally accepted numbers of amoeba species present in soil were not affected by storage (Sandon, 1927), especially when soil samples were taken in a dry, hot season with comparatively low moisture (Dixon, 1936). Soil texture and pH were determined by the Soil Testing Laboratory, Michigan State University. The soil used in this study was a sandy loam with pH 7.3 for the 1981 sample and pH 6.6 for the 1982 sample. Soil moisture was 3.90% and 8.63% (g water/g dry soil x 100) respectively, for the two soil samples. Experiments with soil suction and the effect of soil moisture on amoeba population were done using the first (1981) soil sample. The second (1982) soil sample was used for determination of amoeba species' composition and the survival of E. coli. The first and second soil samples are in the same soil textural class (sandy loam) so results obtained from using the two samples were correlated.

Determination of Soil Moisture at Various Suction Levels

A pressure membrane extractor (Soilmoisture Equipment Corporation) was used to determine the soil moisture (for moisture content) at various suction levels. After subsamples were subjected to each pressure, they were put into an oven at 105C for about 24 hours until their constant weight were attained to determine their moisture content (gravimetric method), which was expressed as g water/g dry soil x 100. The suction level used ranged from 0.3 bar to 15 bars, which is the range of available water for plants in soil. The results, soil moisutre and soil suction (expressed in bars), were used to plot a standard soil moisture characteristic curve for the soil under study. A one-way analysis of variance to find the variation of soil moisture under various soil suctions was done.

Enumeration of Amoebae in Soil

Enumeration of amoebae at various soil moisture levels in this study was done by Singh's (1946) method. The soil samples were moistened with distilled water to get variations in soil moisture levels and incubated before their numbers were counted. Details of these stepes added to Singh's method are explained below.

Variations in soil moisture levels were artifically made by adding known amounts of water to the soil subsamples. Knowing the soil moisture, the desired moisture level was achieved by adding an appropriate amount of water to the soil. Additional water was also used to make a suspension of E. coli as food for amoebae. A spatula was used to mix the water and soil before the soil was incubated.

The purpose of soil incubation was to measure the effect of additional water on the amoeba population within the incubation time. Ten g of moist soil was put in a 50ml sterile culture flask for enumeration of amoebae. A

suspension of E. coli (0.03g packed cell volume with 69% viable cells) was added to the soil in aqueous suspension. Soil with added bacteria was incubated at 23C for 48 hours before counting.

The following steps were carried out as described by Singh (1946). Deviations from his method are specified.

Soil Dilution Series

In the amoeba counting, incubated soil was diluted with 50ml Low Salt Solution (LSS) (Band & Mohrlok, 1969) instead of the normal salt solution used by Singh containing 2.92g of NaCl, 1.33g of $MgSO_4.7H_2O$ and 0.04g of $CaCl_2$ in 1000ml of distilled water to give the initial dilutin of 1:5. A set of 15 two-fold dilution series, ranging from 1/5 to 1/81, 902, was prepared. From each of such dilutions of a series, eight 0.5ml aliquots of the suspension were distributed to 8 wells of a 24-multiwell plate containing sterile Dilute Stock Agar Glucose (DSAG) containing 2.13g $McCl_2.6H_2O$, 0.136g KH_2PO_4, 0.568g Na_2HPO_4, 1.0g Trypticase, 1.0g Yeast Extract, 1.0g Glucose, 15g Bacto-Agar and 1000ml distilled water (Band, personal communication). In this experiment, DSAG was used to replace 1% agar containing only NaCl. Multiwell plates were used instead of glass rings and petri dishes to facilitate replication of the experiment. To support the growth of amoebae in multiwell plates, a drop of E. coli suspension was added to each well. The plates were incubated at 23C for four days before observation. Both vegetative forms and cysts were found healthy at that time, making species identification and clonal isolation relatively easy.

Amoeba Counting

The examination of each series of plates was done by direct observation under an inverted microscope. The density of the population of amoeba in various moisture-induced soil samples was calculated from the number of

negative wells in a given series. Using the table in the appendix presented by Singh (1946), the density of amoebae per g. dry soil was determined. Determination of positive wells was based on the occurrence of cysts and vegetative forms. Two replications of each treatment were performed. A one-way analysis of variance of log-transformed number of amoebae was done to determine soil moisture effects. A comparison of amoeba number at 10% and 40% of soil moisture levels was done using a student t-test which detailed species compositions examined in this study.

Species Identification

A method similar to the enumeration of amoebae in soil described above was used to identify amoebae at two soil moisture extremes. Soil moistures at 10% and 40% (g water/g dry weight soil x 100) were used for this purpose. The 1982 soil sample was used for species identification so the minimum soil moisture used was 10%. Species identification was based on morphology for both vegetative forms and cysts: locomotion for vegetative only, using descriptions by Page (1967a, 1967b, 1976). A phase contrast microscope was used to observe amoebae in wet-mount preparations and sizes were measured by using a calibrated ocular micrometer. A flagellation test was done to identify soil amoebo-flagellates by suspending amoeba cells in LSS, in wet amount preparations and sizes were measured by using a calibrated ocular micrometer. A flagellation test was done to identify soil amoeba-flagellates by suspending amoeba cells in LSS, in wet-mount preparation, without E. coli (Fulton & Dingle, 1967). The slides were left at room temperature at least for seven hours for transformation. Low Salt Agar (LSA) containing 1% Bacto-agar (Band & Mohrlok, 1969) was used as a medium in colonal isolation for further species identification.

Observations and identification were made based on two replicates of each treatment of soil moisture levels.

Experiments with Escherichia coli

Culture of Bacteria

Escherichia coli strain K12 was obtained from Dr. Robert Brubaker, Department of Microbiology and Public Health, Michigan State University. The cultures were transferred weekly and maintained in Tryptone-Agar Slants (0.5g NaCl, 1.0g Bacto-Tryptone and 1.0g Bacto-Agar in 100 ml of distilled water). Before it was used as food for amoebae, E. coli was grown in Tryptone Broth (0.5gm NaCl and 1.0g Bacto-peptone in 100 ml distilled water) for 24 hours at 35C. The cells were then centrifuged at 3020 x g, at 4C for 10 minutes and washed three times in LSS before they were weighed and diluted in distilled water. A similar amount of the E. coli in a method similar to enumeration of amoebae in soil (0.03g packed cells volume with 69% of viable cells) was used to examine the survival of this bacterium in soil (10g dry weight).

Cell Counting

For total plate counting with the surface plate technique, the initial dilution series was done by adding 50ml LSS to incubated soil. A decimal dilution series using 9ml of 0.1% Peptone Solution (1g Bacto-Peptone in 1000 ml of distilled water) was prepared as described by Deibel and Lindquist (1981). The diluent (0.1ml) at each dilution level that can give the colonies numbers between 30 and 300 was spread on the Levine Eosin Methylene Blue Agar plate (BBL) using a "hockey stick" glass rod. Triplicate sets of EMB plates were used for each dilution due to their success in producing E. coli colonies, green black, metallic sheen in color (Konenman et al., 1979). All plates were incubated in an

inverted position at 35C for 24 hours before counting. Experiments with E. coli were carried out at 25% soil moisture.

Biochemical Tests

A series of biochemical tests were conducted to identify colonies counted on EMB agar as E. coli. Colonies which were randomly taken from EMB plates were grown on Tryptone-Agar slants for 24 hours before identification. Tests for Indole, Nitrate, Triple Sugar Iron Agar (TSA), Citrate utilization, Methyl Red Voger Proskaur (MR-VP) and motility were utilized for this purpose. The tests and media followd standard procedures described by Koneman et al. (1979) and the DIFCO Manual (1977).

RESULTS

Soil Moisture Characteristic Curve

The soil moisture characteristic curve for the soil investigated (using the 1981 soil sample) is shown in Figure 1a. The curve shows a relationship between soil moisture and soil suction within the range 0.3 bar to 15 bars which is the range of available water for plants in soil. Results from a one-way analysis of variance showed that the amount of water loss varied at different soil suctions. F calculated was 192.730 which was highly significant compared with $F_{(8,27,0.01)}$ which was 3.26 (refer to Appendix B_1 for detailed ANOVA table). Variations in each replicates were presented as standard deviation of each mean (see Table 1). According to this curve, a soil suction of 0.3 bar corresponds to 25.46% (g water/g dry soil x 100) of soil moisture and a suction of 15 bars corresponds to soil moisture of 3.79% (Table 1). The soil moisture shows an exponential decrease at soil suctions greater than 0.3 bar until a soil suction of 5 bars is reached (Figure 1a). Beyond this soil suction, the amount of water drained from soil pores is almost constant; this is demonstrated by the horizontal part of the curve. There is little water left (3.97% of soil moisture) in the system after 5 bars of soil suction. The soil moisture at 014 bar was estimated from this curve at ca 10%.

Figure 1b describes the distribution of various sizes of soil pores. The diameter of soil pores is calculated by using the formula of Baver et al. (1972).

$$h = \frac{2\gamma \cos\phi}{\rho_{gr}}$$

Table 1

Soil Moisture Characteristic Curve for Sandy Loam Soil

Soil Suction (bars)	Pore Sizes (μ m)	Soil Moisture (g water/g dry soil x 100) Replicates #1	#2	#3	#4	Mean ± S_D
0.3	10.0	28.09	24.97	21.65	27.13	25.46 = 2.86
0.4	7.5	-----	-----	-----	-----	10.00*
0.5	6.0	7.51	8.38	7.65	7.80	7.84 ± 0.38
1.4	2.0	5.87	5.53	5.59	4.50	5.37 ± 0.60
2.0	1.5	5.20	5.01	4.72	4.30	4.81 ± 0.39
3.0	1.0	4.60	4.44	4.51	4.15	4.43 ± 0.19
4.0	0.75	4.40	4.30	4.10	3.80	4.15 ± 0.26
5.0	0.60	4.25	4.05	3.97	3.60	3.97 ± 0.27
6.0	0.50	4.16	3.88	3.86	3.39	3.82 ± 0.32
15.0	0.20	4.10	3.85	3.80	3.40	3.79 ± 0.29

*This value was estimated from Figure 1a.

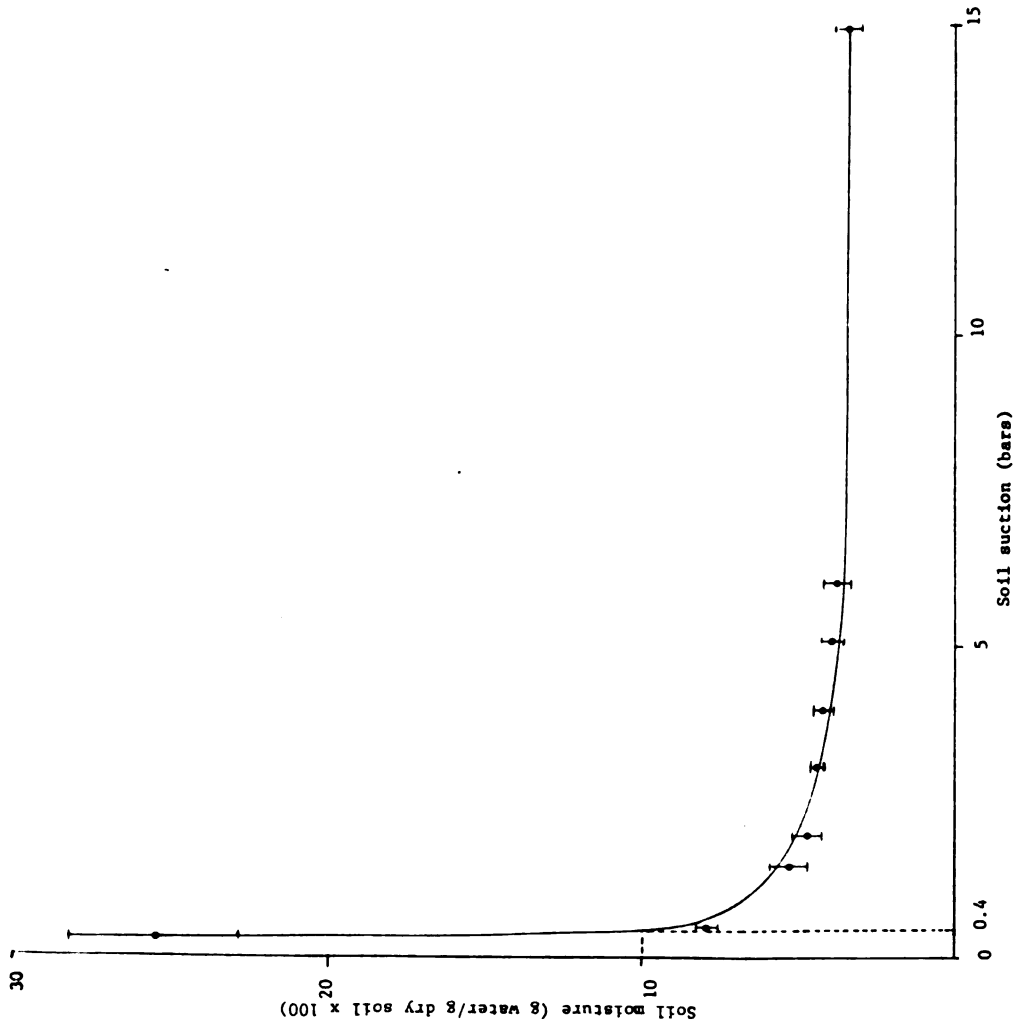


Fig. 1a. Soil moisture characteristic curve for sandy loam soil. Each point was taken from the average of 4 observations. Bar of each point represents the standard deviation (S_D) of each mean. Data given in Table 1.

where

h : height of water rise in a capillary tube with radius r

γ : water surface tension

ρ : water density

g : acceleration due to gravity

ϕ : the contact angle between water and soil pore (assumed to be zero)

which is then simplified as

$$d = 3/H$$

where

d: diameter of the soil pores in mm

H: the amount of tension which the water in the pore is in equilibrium. One bar is approximately 100cm of water suction (Griffin, 1963).

The relationship between soil moisture and the diameter of soil pores is shown in Figure 1b and Table 1. At saturation (ca. 48% of soil moisture), soil suction was equal to 0 bar, all soil pores were saturated. When pressure was applied to the soil, a certain size of pore was emptied. At 0.3 bar, soil pores with diameters of 10 μm or more were emptied, followed by other smaller pores as suction increased. Many pores were considered emptied when pressures higher than 5 bars were applied, as shown by a small amount of water loss beyond this suction (Figure 1a and Figure 1b). Pores emptying (a) and pores empty (b) in this system are shown in Figure 1b.

At 0.3 bar of soil suction, 25.46% of soil water remained in the system occupying soil pores with diameters of less than 10 μm. At a soil suction of 0.5 bar, remaining water in the soil (7.84% of soil moisture) was present in pores with diameters smaller than 6.0 μm. The amounts of water left in various sizes of pores for the rest of the data in Table 1 are explained in a similar manner. When soil suction exceeded 5 bars, all pores available in the soil were empty. In

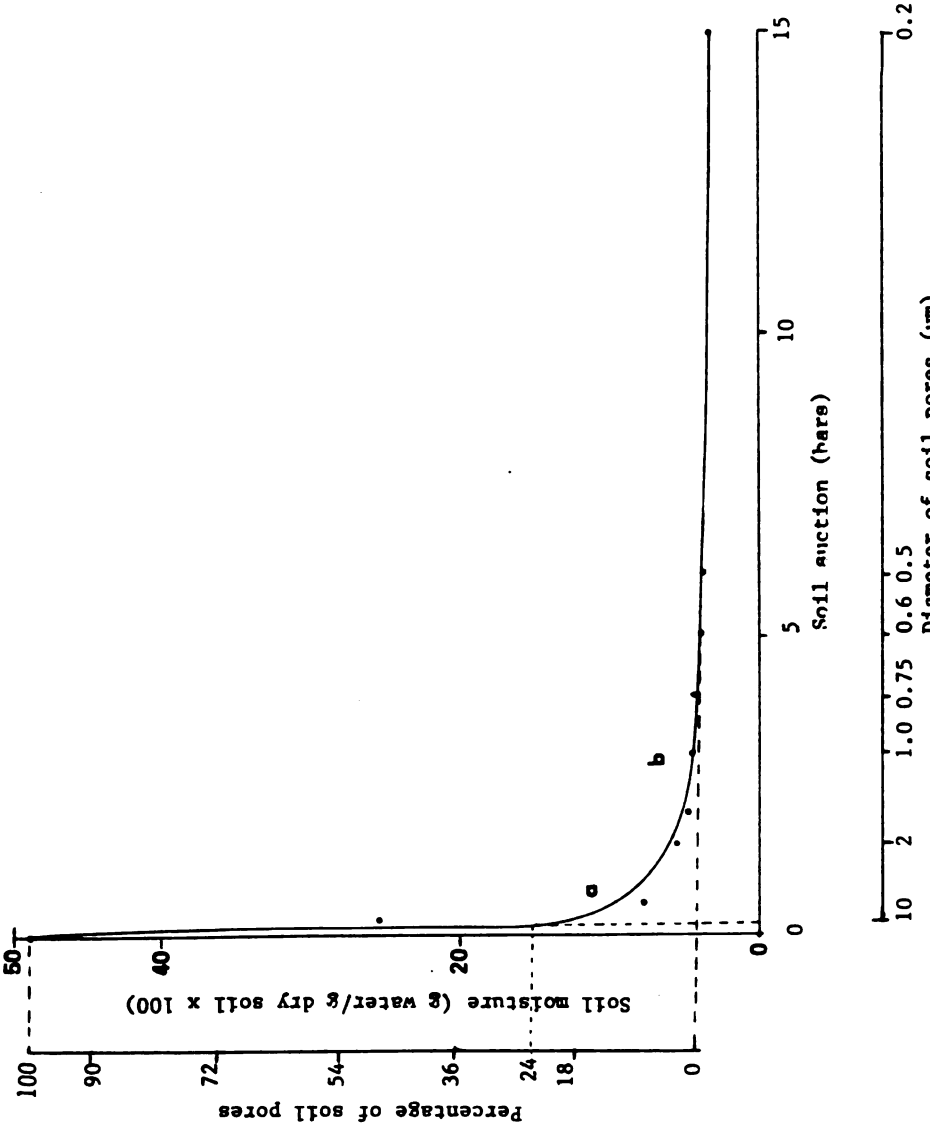


Fig. 1b. Soil moisture characteristic curve in relation to diameter of soil pores. Pores emptying are shown at a and pores empty at b.

this context, soil pores are said to be emptied when no water can be extracted as suction increases, even at 15 bars (see Figure 1b). Assuming the relationship between soil moisture and soil pore percentage is a straight line—that pores were empty at "b" and water-filled at zero bar—the percentage of soil pores with various diameters can be estimated (Wallace, 1958). So the percentage of soil pores with diameters of 0.6μm and 10μm for the soil investigated were about 24%. The rest of the pores in the system had diameters larger than 10μm. Since most water was lost between 0.3 bar and 5 bars of soil suctions, the soil pores which effectively contained water in the soil investigated (within the range of available water in soil) were the soil pores with diameters of 0.6μm - 10μm. At 15 bars soil suction, water retained in soil was 3.79% of soil moisture. This water occupies soil pores with diameters between 0.2μm and 0.6μm.

Effects of Soil Moisture on Amoeba Populations

Amoebae need a critical amount of water for growth as shown in Figure 2a. The maximum population of amoebae in the soil investigated was found at 30% soil moisture. Populations of these organisms decreased at soil moistures higher or lower than this critical point. A poor growth of amoebae was apparent between soil moistures of 5% and 20%, resulting in a low number of amoebae at these moisture levels. A dramatic increase in amoeba number was observed starting at a soil moisture of 25%. The amoeba numbers decrease again after a peak population is attained (i.e., at 35% and 40% of soil moistures). The effect of soil moisture on amoeba population was significant (p 0.01). This was shown by high value of F obtained which was 110.3442 from a one-way analysis of variance, as compared with $F(7,8,0.01)$ which was 6.18 (see Appendix B_2 for detailed ANOVA table). When the log-transformed number of amoebae was plotted against soil moisture, the population incrased in a straight line as soil moisture increased (Figure 2b). The linear relationship between amoeba

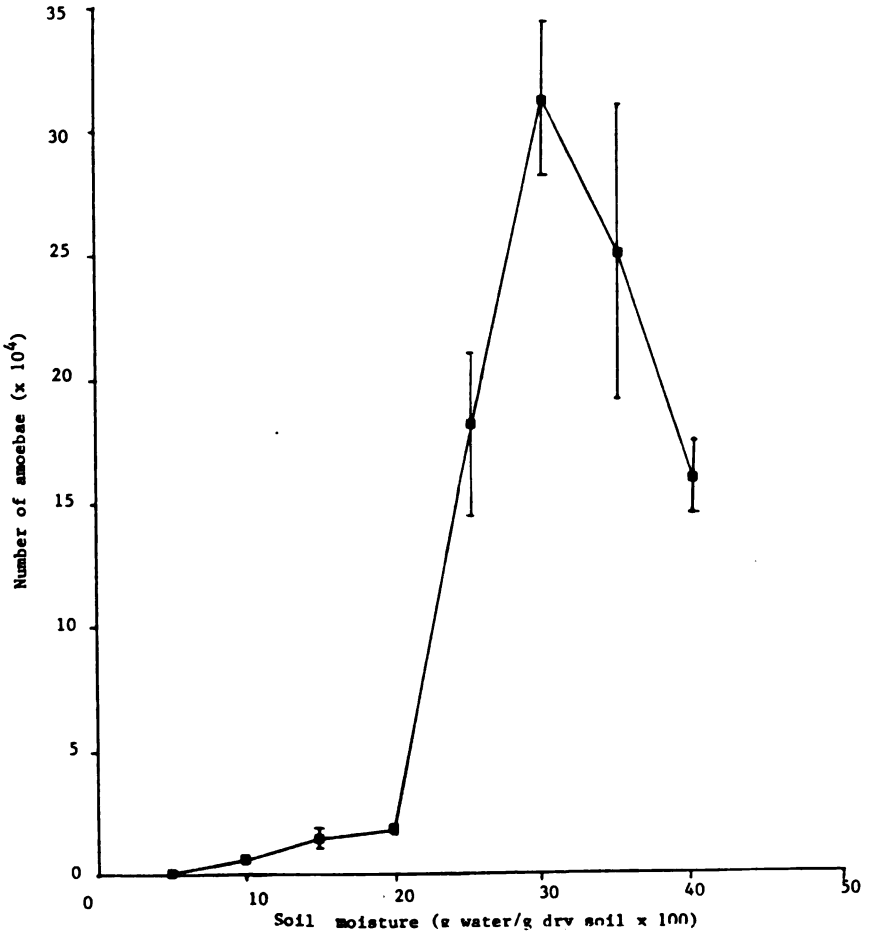


Fig. 2a. Effect of soil moisture on amoeba population. Each point is the average of two counts. Bar of each point represents the range of amoeba population counted. The amoeba number at 5% of soil moisture was 0.1 x 10^4 (see Table 2).

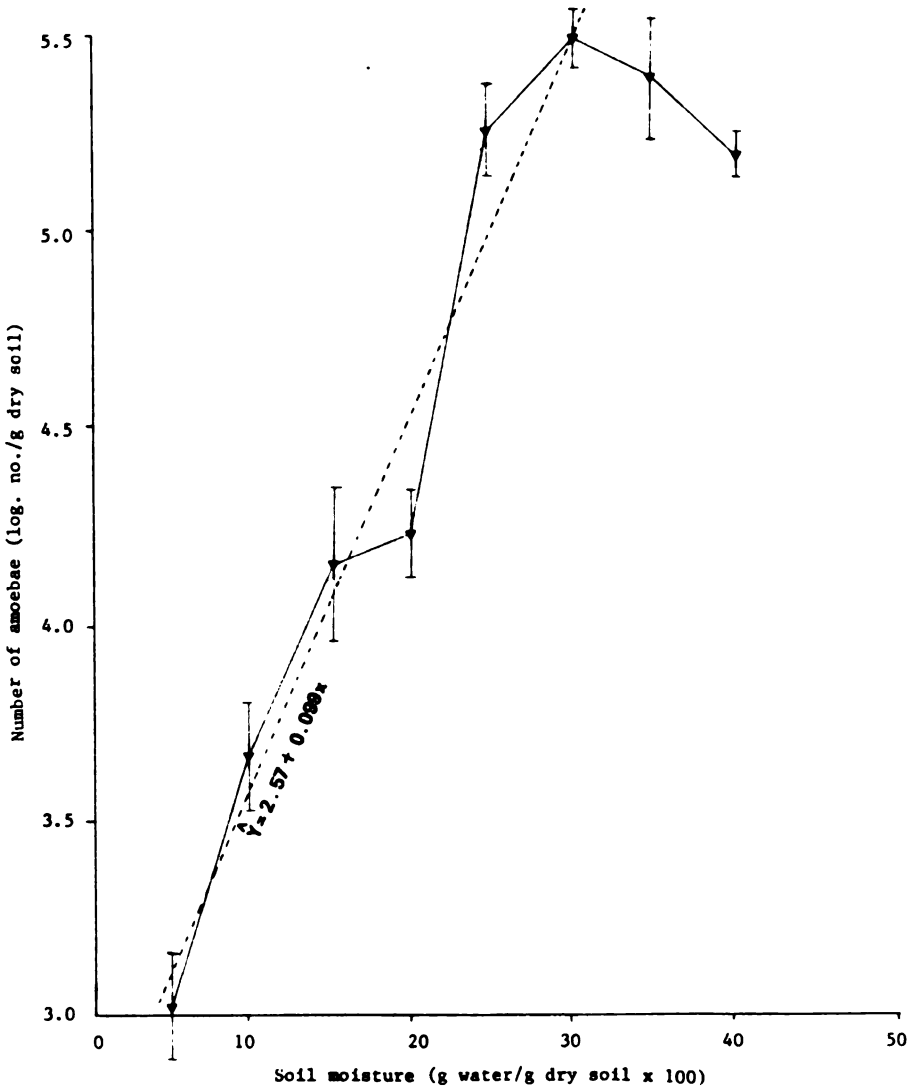


Fig. 2b. Log plot of amoeba population at various soil moisture levels Each point was taken from the average of two observations. Bar of each point indicates the standard deviation (S_D) of each mean.

population and soil moisture was obtained at soil moisture levels between 5% and 30%. A regression line of this relationship is $\hat{Y} = 2.57 + 0.099x$ (Figure 2b). The estimate value of β is 0.099 ± 0.01 log (no.)/ soil moisture (p 0.01). The value of t for testing $\beta = 0$ against $\beta > 0$ was 4.5249. The value of $t_{4,0.01}$ was 3.747. A regression test for the soil moisture levels of higher than 30% was not done, since only two data points were observed.

Amoeba Species at 10% and 40% of Soil Moistures

Amoeba species found at 10% and 40% soil moisture, with measurements of their cysts and vegetative forms are listed in Table 3. Occurrence of each amoeba species at these two moisture levels is roughly presented by frequency found, not absolute numbers. The total population of amoeba species at these two moisture levels was listed in Table 2. Data presented in Table 3 were for the second (1982) sample of the soil investigated based on two replications of the experiment.

There was no difference in species composition at 10% and 40% soil moisture, but generally the number of an individual species was increased at 40% of soil moisture (Table 3). The difference between the number of amoebae present at 40% and 10% of soil moisture levels was significant at p 0.01, using a student t-test (Table 2). The number of species of Vahlkampfia jugosa Page, 1967 was found to decrease at a soil moisture of 40%. Another species that was identified as a member of family Vahlkampfiidae was rarely observed at either soil moisture levels. Acanthamoeba polyphaga (Puschkarew, 1913) was commonly observed at soil moisture levels of 10% and 40%.

Survival of Escherichia coli in Soil

Escherichia coli counts, using Eosine Methylene Blue plates (EMB) and the spread technique, are shown in Table 4. The survival of this bacterium in natural

Table 2

Effect of Soil Moisture on Amoeba Population in Natural Soil

Soil Moisture (g water/g dry soil) x 100	Number of Amoebae/g Dry Soil replicates* #1	#2	Mean	Log No. of Amoebae /b Dry Soil replicates #1	#2	Mean ± S_D
40	175,000	145,000	160,000	5.2430	5.1614	5.2022 ± 0.058**
35	311,000	192,000	251,500	5.4928	5.2833	5.3881 ± 0.148
30	282,000	344,000	313,000	5.4502	5.5366	5.4934 ± 0.061
25	145,00	211,000	178,000	5.1614	5.3243	5.2429 ± 0.115
20	14,500	20,500	17,500	4.1614	4.3118	4.2366 ± 0.106
15	18,800	10,200	14,500	4.2742	4.0086	4.1414 ± 0.188
10	5,540	3,600	4,570	3.7435	3.5563	3.6499 ± 0.132**
5	832	1,270	1,047	2.9154	3.1038	3.0096 ± 0.133

*Variations in amoeba population at each level of soil moisture were not significant (p 0.05) (Singh, 1946).

**The mean of 40% at soil moisture differs from the mean at 10%, at a 1% level of significance, using a student t-test.

Table 3

Amoeba Species at 10% and 40% of Soil Moistures

	Soil Moisture (g water /g dry soil x 100) 10	40	Length in Locomotion (μm)	Cyst Diameter (μm)
1. Acanthamoeba polyphaga (Puschkarew, 1913)	++++	++++	12 - 17	9.9 - 15.2
2. Acanthamoeba castellanii (Douglas, 1930)	+	++	-	13.0 - 17.0
3. Vahlkampfia jugosa Page, 1967	++	+	12 - 17	7.5 - 9.6
4. Hartmannella vermiformis Page, 1967	++	+++	6 - 13	4.0 - 9.5
5. Hartmannella exudans Page, 1967	++	+++	7 - 21	3.8 - 5.8
6. Fam: Vahlkampfiidae Jollos, 1917	+	+	16 - 18	
7. Naegleria gruberi (Schardinger, 1899)	++	+++	5 - 10	4.3 = 6.0

++++ the most frequently found
+++ frequently found
++ less frequently found
+ rare

All data presented above were based on two replicates of observation.

Table 4

Survival of *E. coli* in Natural Soil

Incubation Time (Days)	Cell Counts /g Dry Soil (x 100) replicates #1	#2	#3	Mean	Log Cell Counts /g Dry Soil replicates #1	#2	#3	Mean ± S_D
0	4.79	4.49	3.77	4.35	8.6803	8.6522	8.5763	8.6363 ± 0.054
1	2.40	2.22	3.17	2.59	8.3802	8.3464	8.5011	8.4092 ± 0.081
2	1.44	1.61	1.05	1.37	8.1584	8.2068	8.0212	8.1288 ± 0.096
3	0.72	0.77	0.79	0.76	7.8573	7.8865	7.8976	7.8805 ± 0.021
4	0.69	0.25	0.30	0.41	7.8388	7.3979	7.4771	7.5713 ± 0.235
5	0.86	0.07	0.10	0.34	7.9345	6.8451	7.0000	7.2600 ± 0.589
6	0.07	0.11	0.02	0.07	6.8451	7.0414	6.3617	6.7494 ± 0.350
7	0.04	0.03	0.02	0.03	6.6021	6.4771	6.3010	6.4601 ± 0.151
8	0.01	0.002	0.003	0.005	6.000	5.3013	5.4771	5.5927 ± 0.364

soil in this study was examined at a soil moisture of 25% for a period of eight days only, using the 1982 soil sample. The number of E. coli decreased over time in a linear form (Figure 3). The best fitting of the straight line is given by the equation $\hat{Y} = 8.8 - 0.357x$. The estimated value of β is -0.357 ± 0.03 log (no.)/day ($p < 0.01$). The value of t for testing $\beta = 0$ against $\beta < 0$ was -13.2342. This value was significant at $p < 0.01$ ($t_{2,0.01}$ was -2.998). The colonies counted on EMB plates gave positive reactions for Idole, Methyl Red, Nitrate and Triple Sugars Iron Agar tests. Negative reactions were obtaiend for Voges-Proskauer, Simmon's Citrate and motility: this bacterium was non-motile (Table 5). The results confirmed that the black metallic-sheen colonies that formed and were counted on EMB plate were actually E. coli.

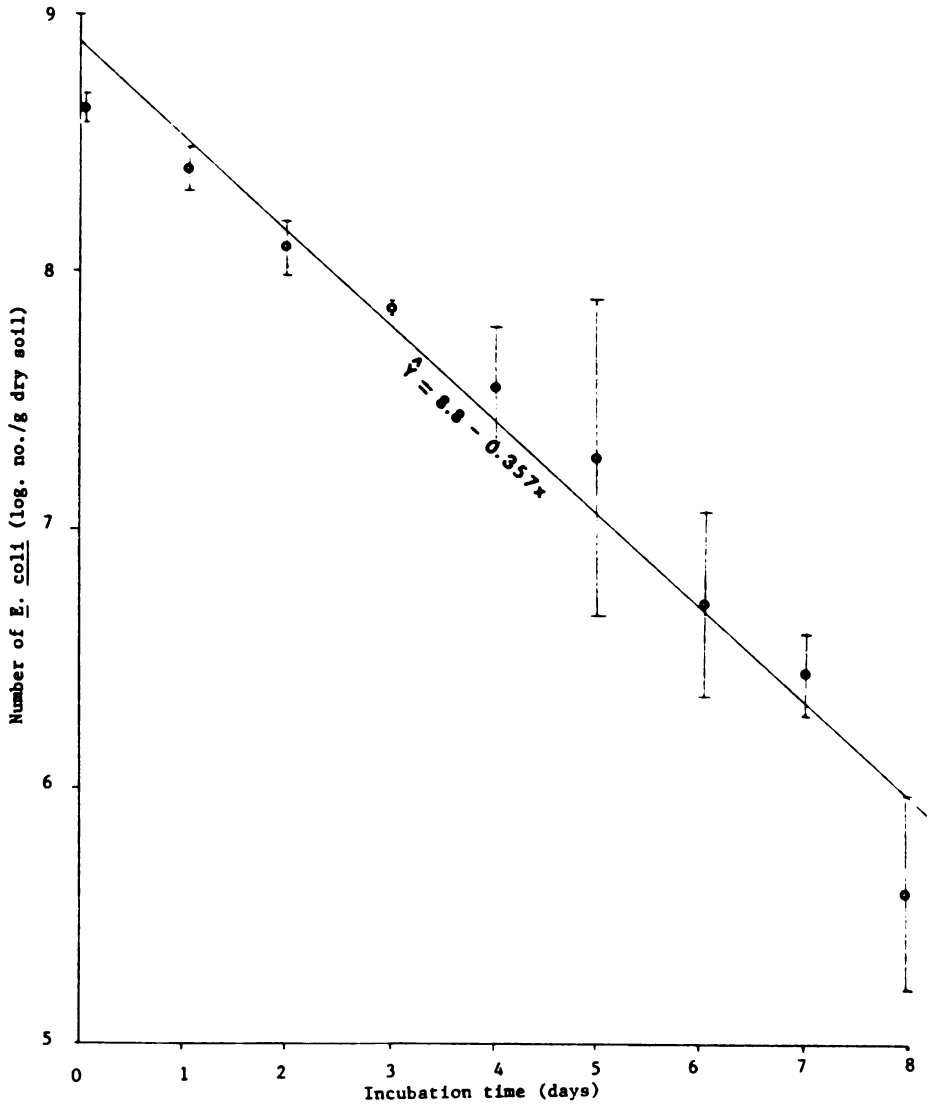


Fig. 3. Survival of E. coli in natural soil. Bar of each point indicates the standard deviation (S_D) of each mean which was taken from the average of 3 cell counts.

Table 5

Biochemical Tests of *Escherichia coli*

Tests or Substrates	Replicates of *E. coli* Colonies #1	#2	#3	#4
Indole	+	+	+	+
Methyl Red	+	+	+	+
Voges-Proskauer	-	-	-	-
Simon's Citrate	-	-	-	-
Nitrate	+	+	+	+
Triple Sugar Iron Agar	+	+	+	+
Motility	non-motile	non-motile	non-motile	non-motile

DISCUSSION

Soil Moisture Characteristic Curve

The soil moisture characteristic curve for a sandy loam soil (Figure 1a) graphically demonstrates the differing levels of water retained in soil at various soil suctions. The shape of this curve is determined by the soil texture and structure (Hillel, 1980) and may also be influenced by the history of soil usage of drying and wetting (Griffin, 1963). The curve shows that at 0.3 bar of soil suction, the corresponding soil moisture was 25.46% which was close to the estimated mean value for a sandy loam of 26% which was derived from the regression relating percentage of different particle size fractions and soil moisture content described by Salter and Williams (1969). The lower limit of available water in the soil investigated was 3.79% which corresponded to 15 bars of soil suction. The estimated value of soil moisture at this pressure was 9%. The slight difference between these two values might have been due to the nature of the soil used for this investigation. The value proposed by Salter and Williams (1969) was taken from an average of several soil samples in their study. The water retained in the soil in the present study at both FC and PWP was comparatively low (the corresponding soil moistures were 25.46% and 3.79%) and it increases when the soil texture becomes finer (Salter & Williams, 1965).

The maximum water that could be retained by this soil at saturation was ca 48% (g water/g dry soil x 100), and all pores in the system were considered to be saturated. When suction developed, some of this water was drawn out of the pores. The pores which were emptied at corresponding suction levels are shown

in Table 1 and Figure 1b. Bigger pores were emptied first because they were most conductive according to Poiseuille's law (Hillel, 1980). At field capacity of 0.3 bar, only 25.46% water was left in the soil, which was the upper limit of available water for the soil investigated. As soil suction increased, the amount of water dropped gradually until it reached a soil suction of 5bars (Figure 1a). The amount of water loss was almost constant beyond this suction, even at 15 bars (i.e., at PWP). The water left in the soil at PWP was bound to soil particles by adhesive and cohesive forces, cannot be used, causing permanent wilting in plants (Foth, 1978; Sykes & Loomis, 1967), and this may affect the availability of water for soil microorganisms too.

As seen in Figure 1b, about 24% of the pores in the soil investigated had diameters between 0.6 μm and 10 μm; the rest of soil pores were larger than 10 um. This means that this sandy loam soil contained a number of large pores which could retain a considerable amount of water when saturated or at field capacity. This would influence the nature of microflora present (Bhaumik & Clark, 1947). When the suction was higher than 0.3 bar, these large pores were emptied and less water retained in the small pores for which higher soil suction was required. The results obtained in this study agreed to previous findings (Hillel, 1980). The application of pressure to the soil caused the pores filled with the air that replaced the water in the system. In soil, air and water wre inversely related: when water fills the pores, only a little air is present in the soil; and when the water is drawn out of the pores, the pores fill with air (Buckman & Brady, 1969). At higher pressure, only a very little water retained in the soil and, tightly bound to soil particles, it could not be drained, even at 15 bars of soil suction. However, this water could be drawn out of the soil if it was put in an oven at 105C for 24 hours at which the equivalent pressure was 10,000 bars (Foth, 1978).

Effects of Soil Moisture on Amoeba Population

The peak population of amoebae in this study was achieved when the soil moisture was at 30% (Figure 2a). The amoeba populations decreased at soil moistures below or above this moisture level. The population decreased at soil moisture below 25% were also observed by Cutler and Dixon (1927) for protozoan populations in their study. When the numbers of amoebae were log-transformed and plotted against soil moistures, they showed a linear increase with increasing soil moisture until that moisture reached 30% (Figure 2b). The smaller amount of amoeba growth at various soil moisture levels below 25% was shown as short lines connected to each point within this range (Figure 2a).

This study showed that soil moistures ranging from 25% to 30% represented optimum water availability for the rapid growth of amoebae. Active amoebae need a moist environment for feeding, as well as oxygen for respiration (Sleigh, 1973). At these moisture levels, an equilibrium exists among the solids, liquids, and gasses of the soil system, providing optimum soil conditions for microorganisms. Above the optimum soil moisture, at 35%, there was a slight decrease in amoeba number and it dropped further, below the number of amoeba at field capacity, when the soil moisture was at 40%. Filling the large soil pores with water created an anaerobic environment, preventing amoebae from growing. Although the effects of anaerobic conditions on amoeba populations in soil were not addressed in this study, a study done on bacteia showed that the growth of aerobic bacteria was affected by filling the soil pores with water, due to the lack of oxygen in soil (Clark, 1967). The effect on amoebae would be similar, either directly because amoebae are aerobic organisms, or indirectly because amoebae are bacterial consumers in soil.

Another factor which can explain the decrease in amoeba numbers at higher levels of soil moisture is the production of organic substances in flooded

soil which can be toxic to microorganisms in soil. Osa-Afiana and Alexander (1979) found that sulfides and organic acids were produced in flooded soils and one of these substances, butyric acid, caused the death of root-nodule bacteria in their investigation. Wolin (1979) found the organic acids' toxicity for soil microorganisms incrased as soil pH decreased. The pH of the sandy loam soil in the present study was relatively high (pH 7.3 for the 1981 soil sample) so the toxicity of these acids was relatively low. If the acids existed in high concentration, however, they would cause injury to these organisms and have a significant effect on trophic interactions of soil microecology (Osa-Afiana & Alexander, 1979). If water is added to the soil, according to Rost and Fieger (1923), it will lower the soil pH. This phenomenon may contribute to many aspects of the relationship between soil moisture and other soil properties and merits future study.

At the lower ranges of soil moisture (e.g., at 20% or less), amoebae grow poorly (Figure 2a and Table 1), but their increase in number is almost linear with increasing soil moisture within this range. The small amount of water present in this low range favors the multiplication of amoebae although at very slow rates. Even though 20% or lower soil moisture will allow much air to be present in soil (too many pores are air-filled), this is not the only factor that is required for the growth of amoebae. A moist environment is important for amoebae to move, feed, and multiply. The size of amoeba population increased at soil moistures above 25% proved that water was important for their growth. Protozoa failed to multiply at the 10% moisture level due to the inability of these organisms to move to their prey (Osa-Afiana & Alexander, 1979) and Losina-Losinsky and Martinov (1930) noticed that amoebae moved very slowly in loam soil at 15% to 20% of soil moisture. Bryant et al. (1982) discovered that in dry soil, no bacteria were ingested by amoebae because the amoebae were in an inactive state.

When the environment is unfavorable, particularly when the soil becomes too dry, the movement of amoebae will be limited, the organisms will be inactive, and normally they will encyst. If sufficient water is added to the soil, it will induce excystation (Bryant et al., 1982). The additon of water to dry soil can cause an increase in protozoan numbers within two to four days (Osa-Afiana & Alexander, 1979), due to excystation when the soil is wet.

In this study, the additon of water to the soil produced favorable conditions for amoebae to excyst and reproduce in a relatively short time (within 48 hours of incubation). This phenomenon is similar to what happens in nature as unfavorable conditions for cell divison are relieved (Stout, 1973). The numbe of these organisms shown in Figure 2a and Table 2 is the result of this relatively swift multiplication in soil within 48 hours of incubation.

Each point in Figure 2a was calculated from the average of two total counts (each count represents each replication of each treatment), that is, the counts which included both vegetative forms and cysts. These two counts were not significantly different because they did not exceed eight negative wells in their original counts (p 0.05) (Singh, 1946). In nature both vegetative forms and cysts are commonly present in soil even though only the former state play an important role as micropredators. In this study, differentiation in the number of cysts from vegetative forms, by treatment with 2% HCl to kill the vegetative forms (Cutler, 1920), was not done. However, Alexander (1961) found that at low soil moisture, the cystic stage of amoebae were predominantly present in the soil. The occurrence of cysts and vegatative forms in the environment always fluctuates, so the total count of both of these forms, rather than only one, better represents the normal population.

Based on Figure 1b, the 0.3 bar suction, the pores that were effectively emptied were the pores which had diameters bigger than 10μm. So the ca. 25%

water retained in soil at this suction was found in soil pores with diameters less than 10 μm. Amoebae in soil use oxygen from air-filled pores and move into the moist environemnt between pore spaces with diameters of less than 10 μ m. Amoebae can move into soil pores which are smaller than themselves because they can change the shape of their bodies easily (Elliott et al., 1980). The water remaining in pores after draining, however, will restrict their movement in soil (Darbyshire, 1976). The results of the present study explained this phenomenon, and this is why the amoeba number varied at different soil moistures. When the suction increased, the smaller pores were emptied and the amoebae encysted because they were not able to move to a new environment. If the soil water in this study had not restricted the movement of these organisms, they might have shifted to a new environment, as was seen when amoebae entered smaller soil pores in the presence of nematodes, i.e., the phenomenon of escapism from predators (Elliott et al., 1980).

In this study amoebae were found to inhabit the soil pores which had diameters larger than 10 μ m. Amoebae show good growth at soil moistures between 25% and 40% (Figure 2a); and even at 40% soil moisture, when the soil environment is considered anaerobic for amoebae, the population was higher than the population at 20% or less. Therefore, a moisture level of 25% to 40% is needed for rapid growth; the oxygen loss at these levels is less important. Referring to Figure 1b, the range of soil moisture that promoted good growth of amoebae was when soil suction was at 0.3 bar or less, which corresponded to effectively drained pores of size 10 μm or larger. As the soil suction increased (e.g., at 3 bars), 4.43% (ca. 5%, Table 1) of soil water was left in the soil. This water occupied the pores with diameters of less than 1.0 μm, and amoeba number was low at this soil moisture level (Figure 2a and Table 2). This meant that the absence of water in larger pores (with a diameter bigger than 10 μ m) which

amoebae typically inhabit, affected their growth and movement. The soil organisms moved easily in coarse soil, compared with fine soil, because the former had a greater number of larger water-filled soil pores (Elliott et al., 1980).

The number of protozoa in soil is always related to the bacterial number present (Sandon, 1928; Chlarholm, 1981), so any unfavorable physical conditions which affect the bacterial population will also affect an amoeba population. Too much or too little water present in soil limits the activity of bacteria and may slow their rate of multiplication, thus limiting the supply of food for amoebae.

Amoeba Species at 10% and 40% of Soil Moisture

The amoeba species found in sandy loam soil with a pH of 6.6, in artifically induced conditions of 10% and 40% of soil moisture, are shown in Table 3. The ratio of amoeba species present at these soil moisture levels was similar, but the number of each species was increased at 40%. This explained why the total number of amoebae was higher at 40%, as compared with 10% (p 0.01) (see results in Table 2). Since the soil moisture levels used were 10% and 40%, the amoeba species identified in this study occupied the soil pores with diameters bigger than 7.5μm (Table 1).

The number of species found at these two levels of soil moisture failed to show any differences because the soil samples used in this experiment were taken from the same bucket which was stored at 4C. The addition of water induced the excystation of those species already present in the soil; thus it incrased the number in each species, but not the number of species. A difference in number of species present at 10% and 40% of soil moisture might be expected if the soil samples were taken from two sites in the field, with moisture content for each site was at 10% and 40%. Although the types of soil

protozoa present in soil vary according to soil moisture (Bamforth, 1969), other factors such as soil texture, fertility, pH, and temperature are also important in ifluencing these organisms to be varied in certain soil environment. In this study, Acanthamoeba polyphaga was found to predominate in soil at both soil moisture levels.

Even though the number of all species seemed to be higher at 40% than 10%, Vahlkampfia jugosa was rarely found at 40%. This can probably be explained by the sensitivity of this particular species to the presence of oxygen in soil. At 40% of soil moisture the soil pores are water-filled, less oxygen is present in soil, and this may have limited the ability of this amoeba to multiply. The number of this species was found to be higher at 25% of soil moisture (data are not shown) than at 10% and at 40%. This signified that at 25%, the soil microenviornment was optimum for this amoeba to grow and multiply. At 40% of soil moisture, not only were other amoebae numbers increased, but the number of ciliates such as Coloda sp also increased. A crowded environment may increase competition for soil spaces and available food (LeFevre et al., 1952, cited by Bamforth, 1963).

One species that was found to belong to family Vahlkampfiidae, based on its morphology, locomotion, and negative results with a flagellation test was very rare at both moisture levels in this study. The cyst of this species could not be investigated, as attempts to isolate its cyst were unsuccessful because of its sparceness in the soil. The identification of Acanthamoeba castellanii was based only on the morphology of its cyst, rather than its vegetative form.

In this experiment, DSAG was used as a medium for isolating amoebae from soil and LSA was used to make clonal isolation. This use of different media may explain why the sizes of V. jugosa and N. gruberi were smaller than Page's descriptions (19067a). Another reason may be that different strains of these

ameobae were present in the soil investigated. The morphology and size of amoebae are not always constant, so it was difficult to measure them accurately (Adam, 1964). Size and morphology vary depending on the specimen's growth conditions, culture age, and method of preparation (Culbertson, 1971), as well as structure, composition, and fertility in nature (Reinhard et al., 1969). The confirmation of these two species was completed after doing a flagellation test which took about seven hours for N. gruberi to give a positive result at room temperature. V. jugosa was recognized by observing at its locomotion which was erruptive, characteristic of family Vahlkampfiidae, and also by the morphology of its cysts and negative results on a flagellation test.

The identification of amoebae in this study was done based on their morphology and locomotion of trophic forms, cyst morphology as described by Page (1967a, 1967b, 1976), and flagellation tests (Fulton & Dingle, 1967). Due to time limitations, identification based on nuclear structure and patterns of mitosis and division were not done, although at present they are considered the most reliable techniques for identifying and classifying the amoebae (Pussard, 1973; Singh & Humaiah, 1976). However, the detailed explanations by Page were helpful for identification of these species.

Survival of Escherichia coli in Soil

The presence of Escherichia coli in soil was found to decrease linearly over an eight day incubation time (Figure 3). Klein and Casida (1967) made a similar observation after 24 days incubation although the decrease was not linear. In their study the decrease of the E. coli population was less abrupt when sugar was added to the soil or when one percent of autoclave-sterilized soil was added to normal soil. The decrease in E. coli numbers in the non-nutrient-amended soil in

the present study was due to a dying out of cells because of a lack of the food necessary to support the growth of a species nonindigeneous to soil.

McGrew and Mallette (1965) found that viable cells of E. coli decreased with time in normal soil but showed a stable growth (represented by a straight horizontal line) when fed with glucose. This phenomenon signified that glucose was used for cell maintenance, but not for growth. While starving due to the lack of food in the environment, the endogenous metabolism of E. coli was increased and cellular glycogen was used very rapidly (Dawes & Ribbons, 1965).

Cell "aging" may also cause a decrease in E. coli numbers with incubation time in soil. As the age of cells increases, their respiration rates will decrease, and the cells eventually will die out. During the aging process, the cellular content (i.e., ribonucleic acid) was found to be oxidizing, resulting in an accumulation of uracil in the suspension medium (Clifton, 1966).

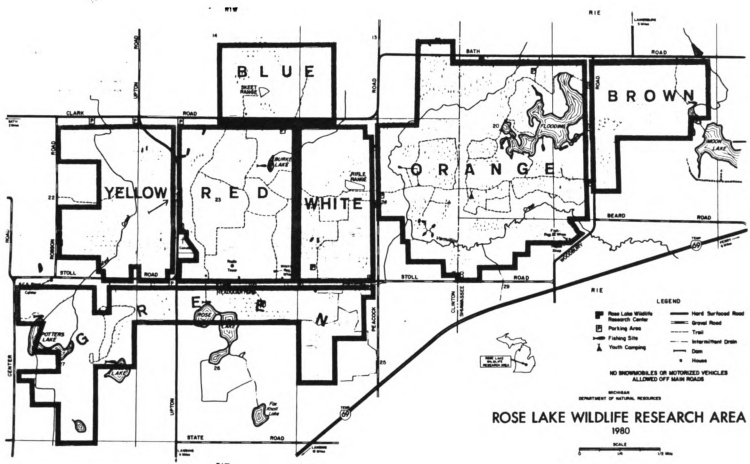
Waksman and Woodruff (1940) discovered that by adding E. coli to the soil, the total soil population was increased, due to the rapid multiplication of antagonistic bacteria, particularly spore-forming bacteria, to exclude this bacteria from the soil. This factor causes E. coli to die out when added to the soil. The addition of one percent of autoclave-sterilized soil, which killed some of the antagonists, probably helped the E. coli to live longer in normal soil. However, Klein and Casida (1967) claimed that the decrase of E. coli in soil is not caused by toxic compounds produced by indigeneous microorganisms, but by its inability to slow down its metabolic rate in keeping with the low availability of usable organic carbon in soil.

There is an inverse relationship between numbers of bacteria and numbers of active amoebae in soil (Cutler, 1923; Cutler & Crump, 1920) and the decrease in E. coli population in this study was also due to predation by amoebae. In fact, this was the main reason why E. coli were added to the soil; that is, to serve as

food for amoebae. Even though some indigenous bacteria present in soil can provide natural food for amoebae, the additional food was needed to support the normal growth of amoebae in moister soils. It is assumed that in this experiment, E. coli that were added to the soil could occupy the soil spaces or the water films that were accessible to amoebae. The decrease of the E. coli population because of predation (the term partial predation may be appropriate here as E. coli was not the only food availale in soil) will never be total because the bacteria will not be totally consumed by amoebae (Habte & Alexander, 1975). Bdellovibrio bacteriovorus Stopl and Starr, a parasite of gram-negative bacteria (Starr & Baigent, 1966) which is present in any American soil can also contribute to limiting of the establishment of E. coli in soil (Klein & Casida, 1967).

The decrease of the E. coli population in the soil in this study was less abrupt over time because the pH of the sandy loam soil used was relatively high (i.e., 6.6. for 1982 sample). E. coli can survive longer in alkaline soils than acid soils (Cuthbert et al., 1955); this may be comparable to the actual pH environment of E. coli in the gastrointestinal tract. Since E. coli can survive even after 48 hours of inoculation in soil, it can serve as food for amoebae as long as the amoebae only consume its vegetative forms, not its dormant form or dead cells as observed by Darbyshire (1976) on Colpoda steini with Azotobacter.

APPENDIX A



Appendix A : A map of Rose Lake Wildlife Research Area. An arrow indicates the site for soil samplings. The soil was described as Boyer sandy loam and Boyer complex by United States Department of Agriculture Soil Conservation Service (1978).

APPENDIX B

APPENDIX B

A one-way analysis table for fix effect of soil suction on soil moisture. Data given in Table 1.

Source	d.f.	SS	MS	F
Suction	8	1572.787	196.623	192.730
Error	27	27.5450	1.0202	
TOTALS:	35	1600.523		

$F_{8,27,0.01} = 8.21$

H_o = no effect of soil suction on soil moisture

H_1 = soil suction affects soil moisture

F calculated F table, therefore accept H_1.

A one-way analysis table for fix effect of soil moisture on amoeba population. Data given in Table 2.

Source	d.f.	SS	MS	F
Moisture	7	11.8954	1.6993	110.3442
Error	8	0.1230	0.0154	
TOTALS:	15	12.0184		

$F_{7,8,0.01} = 6.18$

H_o : no variation in amoeba population due to soil moisture

H_1 : there is variation in amoeba population due to soil moisture

F calculated F table, therefore reject H_o

LITERATURE CITED

LITERATURE CITED

Adam, K.M.G 1964 A comparative study of Hartmannellid amoebae. J. Protozool. 11:423-430.

Alexander, M. 1961. Introduction to soil microbiology. John Wiley and Sons, Inc. NY. page 105-113.

Alexander, F.E.S. and R.M. Jackson. 1954. Examination of soil microorganisms in their natural environment. Nature. 174:750-751.

Bamforth, S.S. 1963. Microhabitat and community structure as ecological factors for protozoa. In: Progress in Protozoology. Ed. Ludvik, J., J. Lom and J. Vavra. Academic Press, NY. page 301-302.

_____. 1969. The effect of moisture and vegetation on soil protozoa. Anon. Uspekhi. Protozoologii. (Progress in Protozoology). Nauka, Leningrad, USSR. page 196-197.

Band, R.N. 1963. Extrinsic requirements for encystation by the soil amoeba Hartmannella rhysodes. J. Protozool. 10:101-107.

_____ and S. Mohrlok. 1969. The respiratory metabolism of Acanthamoeba rhysodes during encystation. J. Gen. Microbiol. 59:351-358.

Barnes, J.H. and B. Ali. 1917. Alkali soils; some biochemical factors in their reclamation. Agr. J. India. 12:368-389.

Baver, L.D., W.H. Gardner and W.R. Gardner. 1972. Soil Physics. John Wiley and Sons, Inc., NY. page 187.

Bhaumik, H.D. and F.E. Clark. 1947. Soil moisture tension and microbiological activity. POroc. Soil. Sci. Soc. Am. 12:234-238.

Bryant, R.J., .L.E. Woods, D.C. Coleman, B.C. Fairbanks, J.F. McClellan and C.V. Cole. 1982. Interactions of bacterial and amoebal populations in soil microcosms with fluctuating moisture content. Appl. Environ. Microbiol. 43:747-752.

Buckman, H.O. and N.C. Brady. 1969. The Nature and Properties of Soils. The Macmillan Com., London. 653 pages.

Burges, A. 1967. The soil system. In: Soil Biology. Ed. Burges, A. and F. Raw. Academic Press, London. page 1-13.

_____ and E. Fenton. 1953. The effect of carbon dioxide on the growth of certain soil fungi. Trans. Brit. Mycol. Soc. 36:104-108.

Cary, J.W. and H.F. Maryland. 1972. Salt and water movement in unsaturated frozen soil. Soil Sci. Soc. Am. Proc. 36:549-555.

Childs, E.C. 1940. The use of soil moisture characteristics in soil studies. Soil Sci. 50:239=252/

Collis-George, N. 1959. The physical environment of soil animals. Ecology. 40:550-557.

Clarholm, M. 1981. Protozoan grazing of bacteria in soil--impact and importance. Microb. Ecol. 7:343-350.

Clark, F.E. 1967 Bacteria in soil. In soil Biology. Ed. Burges, A. and F. Raw. Academic Press, London. page 15-49.

Clifton, C.E. 1966. Aging of Escherichia coli. J. Bacteriol. 92:905=912.

Crump, L.M. 1950. The influence of bacterial environment on the excystment of amoebae from soil. J. Gen. Microbiol. 4:16.

Culbertson, C. G. 1971. The pathogenicity of soil amoebas. Ann. Rev. Microbiol. 25: 231-254.

Cuthbert, W.A., J.J. Panes and E.C. Hill. 1955. Survival of Bacterium coli, type I and Streptococcus faecalis in soil. Soc. Appl. Bacteriol. 18:408-414.

Cutler, D.W. 1919. Observations on soil protozoa. J. Agric. Sci. 9:430-444.

_____. 1920. A method for estimating the number of active protozoan in the soil. Ibid. 10:135-143.

_____. 1923. The action of protozoa when inoculated into sterile soil. Ann. Appl. Biol. 10:137-141.

_____ and L.M. Crump. 1920. Daily periodicity in the numbers of active soil flagellates: with a brief note on the relation of trophic amoebae and bacterial numbers. Ibid. 7:11-24.

_____ and A. Dixon. 1927. The effect of soil storage and water content of the protozoan population. Ibid. 17:247-254.

Darbyshire, J.F. 1975. Soil protozoa- Animalcules of the subterranean microenvironment. In Soil Microbiology, A Critical Review. Ed. N. Walker. John Wiley and Sobn. NY. page 147-163.

_____. 1976. Effect of water suctions on the growth in soil of the ciliate Colpoda steini, and the bacterium Azotobacter chroococum. J. Soil. Sci. 27:369-376.

Dawes, E.A. and D.W. Ribbons. 1965. Studies on the endogeneous metabolism of Escherichia coli. Biochem. J. 95:332-343.

DIFCO Manual. 1977. Ninth Edition. Difco Laboratories, Detroit, Michigan. USA. 341 pages.

Deibel, H.R. and J.A. Lindquist. 1981. General Food Microbiology Laboratory Manual. Burgess Publishing Company, Minneapolis, Minnesota. page 1-3.

Dixon, A. 1936. Soil protozoa: their growth on various media. Ann. Biol. 24:442-456.

Donahue, R. L., R.W. Miller and J.C. Shickluna. 1977. Soil: Introduction to soil and plant growth. Prentice-Hall, Inc., Englewood Cliffs, NJ. 363 pages.

Drozanski, W. 1963. The influence of bacteria on the excystment of soil amoebae (abstract). In: Progress in Protozoology. Ed. Ludvik, L., J. Lom and J. Javra. Academic Press. NY. page 330.

Elliott, E. T., D.C. Coleman, R.V. Anderson, C.V. Cole, H.W. Hunt, L.E. Woods, W.D. Gould and J.F. McClellan. 1980. Microbial trophic structure and habitable pore spaces in soil. In: Giesy, J.P., Jr. (Ed.) Doe symposium series. Vol:52. Microcosms in Ecological Research. (Microfiche) page 1050-1070.

Fellers, C.R. and F.E. Allison. 1920. The protozoan fauna of the soils of New Jersey. Soil Sci. 9:1-25.

Foth, D. 1978. Fundamentals of soil science. John Wiley and Sons, NY. 436 pages.

Fulton, C. and A.D. Dingle. 1967. Appearance of the flagellate phenotype in populations of Naegleria amoeba. Dev. Biol. 15:165-191

Geldreich, E.E., C.B. Huff, R.H. Bordner, P.W. Kabler and H.F. Clark. 1962. The faecal coli-aerogenes flora of soils from various geographical areas. J.Appl Bact. 25:87-93.

Geltzer, J.G. 1963. On the behaviour of soil amoeba in the rhizospheres of plants. Pedobiol. 2:249-251.

Griffin, D.M. 1963. Soil moisture and the ecology of soil fungi. Biol. Rev. 38:141-166.

Habte, M. and M. Alexander. 1975. Protozoa as agents responsible for the decline of Xantheomonas campestris in soil. Appl. Microbiol. 29:159-164.

Heal, O.W. 1962. The abundance and microdistribution of testate Amoebae (Rhizopoda, Testacea) in Sphagnum. Dikos. 13:35-47.

_____. 1967. Quantitative studies on soil amoebae. In Progress in Soil Biology. Ed. Graff, D. and J. E. Satchell. North Holland Publ. Co., Amsterdam. page 120-126.

Hillel, D. 1980. Fundamentals of Soil Physics. Academic Press, NY. page 123-156.

Klein, D. A. and L.E.J. Casida. 1967. Occurrence of Bdellovibrio bacteriovorus in soil capable of parasitizing e. coli and indigenous soil bacteria. Can. J. Microbiol. 13:1235-1241.

Koneman, E. W., S.D. Allen, V.R. Dowell and H.M. Sommers. 1979. Color Atlas and Textbook of Diagnostic Microbiology. J. B. Lippincott Company, Philadelphia. page 55-110.

Kuhnelt, W. 1961. Soil Biology. Rodale Books Inc. Emmaus, Penn. page 234-237.

Linford, M.B. 1942. Method of observing soil flora and fauna associated with roots. Soil Sci. 53:93-103.

Losina-Losinsky, L. and P. F. Martinov. 1930. A method of studying the activity and rate of diffusion of bacteria in the soil. Soil Sci. 29:349-362.

Mahler, R.L. and A.B. Wollum. 1981. The influence of soil water potential and soil texture on the survival Rhizobium leguminosarum and Rhizobium japonicum isolates in soil. Soil Sci. Soc. Am. J. 45:761-766.

McGrew, S.B. and M.F. Mallette. 1965. Maintenance of Escherichia coli and assimilation of glucose. Nature. 208:1096=1097.

Nikoljuk, V.P. 1969. The character of inter-relationships between soil protozoa on bacteria. In Progress on Protozoology, IIIrd. International Congress on Protozoology. Ed. Strelkov, A.A., K.M. Sukhanova and I.B. Raikov. Pub. House (Nauka), Leningrad Branch, Leningrad. page 180.

Osa-Afiana, L.O. and M. Alexander. 1979. Effect of moisture on the survival of rhizobium in soil. Soil Sci. Soc. Am. J. 43:925-930.

Page, F.C. 1967a Taxonomic criteria for limax amoebae, with descriptions of 3 new species of Hartmannella and 3 of Vahlkampfia. J. Protozool. 14:499-521.

_____. 1967b. Re-definition of the genus Acanthamoeba with descriptions of 3 species. Ibid. 14:709-724.

_____. 1976. An illustrated key to freshwater and soil amoeba. Freshwater Biological Association, Scientific Publicaiton No:34. U.K. 152 pages.

Penman, H. L. 1940. Gas and vapour movements in soils. J. Agric. Sci. 30:437-462.

Preston, T. M. and C.A. King. 1982. Interaction between the cell surface of Acanthamoeba castellanii and certain bacteria (abstract). J. Protozool. 20:639.

Pussard, M. 1973. Modalites de la division nucleaire et taxonomic chez les Amibes (Amoebida). Protistologica. 9:163-173.

Reinhard, L.V., A.P. Traveleyev and I.K. Bulik. 1969. Ecology of soil protozoa in forests of Ukrainian Steppe Zone. Anon. Uspekhi. Protozoologgi. (Progress in Protozoology.) Nauka, Leningrad, USSR.

Rost, C.D. and E.A. Fieger. 1923. Effect of drying and storage upon the hydrogen-ion concentration of soil samples. Soil. Sci. 16:121-126.

Russell, E. J., and A. Appleyard. 1915. The atmosphere of the soil: its composition and the causes of variation. J. Agric. Sci. 7:1-48.

Salter, P. J. and J.B. Williams. 1965. teh influence of texture on the moisture characteristics of soil. II. Available-water capacity and moisture release characteristics. J. Soil Sci. 16:310-317.

_____. 1969. The influence of textures on the moisture characteristics of soil: relationships between particle-size composition and moisture contents at the upper and lower limits of available-water. Ibid. 20:126-131.

Salter, P. J., G. Berry and J.B. Williams. 1966. The influence of texture on the moisture characteristics of soil. III. Quantitative relationships between particle-size composition and available-water capacity. Ibid. 17:93-98.

Sandon, H. 1927. The compositon and distribution of the protozoan fauna of the soil. Oliver and boyd, Edinburgh. 237 pages.

_____. 1928. A study of the protozoa of some American soils. Soil. Sci. 25:107-119.

Singh, B. N. 1946. A method of estimating the number of soil protozoa, especially amoebae, based on their differential feeding on bacteria. Ann. Appl. Biol. 33:112-119.

_____ and V. Hanumaiah. 1976. Studies on pathogenic and non-pathogenic amoebae and the bearing of nuclear division and locomotive form and behavior on the classificaiton of the order Amoebida. Association of Microbiologist of India. Monograph No:1. 80 pages.

Skinner, F.A. 1975. Anaerobic bacteria and their activities in soil. In: Soil Microbiology. A critical review. Ed. Walker, N. John Wiley and Sons, NY. page 1-19.

Sleigh, M. 1973. The biology of protozoa. Edward Arnold, London, page 248-253.

Starr, M. P. and N.L. Baigent. 1966. Parasitic interactions of Bdellovibrio bacteriovarus with other bacteria. J. Bacteriol. 91:2006-2017.

Stout, J.D. 1956. Reaction of ciliates to environmental factors. Ecology. 37:178-191.

Stout, J.D. 1973. The relationship between protozoan populations and biological activity in soils. Am. Zool. 13:193-201.

Sykes, D.J. and W.E. Loomis. 1967. Plant and soil factors in permanent wilting percentages and field capacity storage. Soil. Sci. 104:163-173.

United States Department of Agriculture Soil Conservation Service. 1978. Soil Survey fo Clinton County, Michigan. Map sheet no. 66.

Waksman, S.A. 1916. Bacterial numbers in soils, at different depths and in different seasons of the year. Soil Sci. 1:363=380.

_____ and Starkey. 1923. Partial sterilisation of soil, microbial activities and soil fertility. Soil Sci. 16:247-268.

_____ and H. B. Woodruff. 1940. Survival of bacteria added to soil and the resultant modificaitons of the soil population. Ibid. 50:421-427.

Wallace, H.R. 1958. Movement of eelworms. I. The influence of pore and moisture content of the soil on the migration of larvae of the beet eelworm, Heterodera schachtii, Schmidt. Ann. Appl. Biol. 46:74-85.

Wallwork, J.A. 1970. Ecology of soil animals. McGraw-Hill, London. page 81-108.

Warcup, J.H. 1967. Fungi in soil. In: Soil Biology. Ed. Burges, A. and F. Raw. Aca;demic Press. page 51-102.

Williams, S. T., M. Shameemullah, E. T. Watson and C. I. Mayfield. 1972. Studies on the ecology of Actinomycetes in soil. IV. The influence of moisture tension on growth and survival. Soil. Biol. Biochem. 4:215-225.

Wright, J.L., K. Redhead and H. Maudsley. 1981. Acanthamoeba castellanii, a predator of cyanobacteria. J. Gen. Microbiol. 125:293-300.

Wolin, M. J. 1969. Volatile fatty acids and the inhibition of E. coli growth by rumen fluid. Appl. Microbiol. 17:83-87.