BACTERIAL RESPONSE TO THE SOIL ENVIRONMENT

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J. W. Boyd, T. Yoshida, L. E. Vereen,
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ABSTRACT

Studies to elucidate some of the basic mechanisms by which surface pollution may gain entrance to subsurface water supplies were conducted.

Measurements of survival of selected bacteria in certain soil types found in Larimer County, in an area northeast of Fort Collins, Colorado, were made. Samples of two soil types were analyzed chemically and the effects of soil moisture, organic matter, chelation agents and soil sterility upon bacterial life were observed. The role of bacterial predators found in soil was determined, as were the roles of selected inorganic and organic compounds and soil extracts upon bacterial nutrition and/or survival. The collected data showed that while moisture and nutritive value of soils were important for bacterial survival, microbial overpopulation was a major cause of bacterial death. Microbial predators existing in these soils had little or no effect on bacterial survival.

Further research was initiated to determine the effects of particulate matter and bacterial surface charge on the mobility of bacteria. Data showed that the size of sand granules and the specific type of ion present in bacterial suspensions greatly affected the mobility of bacteria through sand columns. Bacterial surface charges (zeta potential) were determined in water and in the presence of certain ions. Specific ions markedly changed the zeta potential of the bacteria tested. An attempt was made to correlate the zeta potential data with the mobility values obtained with sand columns.

The value of a "Pollution Index" rating for soils was discussed.

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BACTERIAL RESPONSE TO THE SOIL ENVIRONMENT

J. W. Boyd[1], T. Yoshida[2], L. E. Vereen[3],
R. L. Cada[4] and S. M. Morrison[5]

CHAPTER I

INTRODUCTION

Although it is well known that direct contamination by man has rendered some rivers and lakes useless as domestic or industrial water supplies, little is known about less dramatic forms of pollution including bacterial contamination of many other surface water sources. Thus, attention must be directed toward underground water resources, especially since these have recently been shown to be in more intimate relationship with surface waters than originally believed. Contaminated surface waters may reach and contaminate our ground waters; it is possible that contaminated ground waters may move through aquifers and subsequently contaminate a surface water at a lower elevation. Therefore, any effort to reduce or eliminate the pollution problem in surface waters must be accomplished by a study of ground water pollution.

The geographic area under investigation, the area northeast of Fort Collins, Colorado (Larimer County), contains five major soil types, two of which were selected for tests in these experiments. Agriculture is a principal industry including irrigated and non-irrigated crop cultivation, dairying, and sheep and cattle feeding. Much of the lower Boxelder Valley is irrigated by diverted Cache la Poudre River water and Colorado Big Thompson Project water. These waters are supplemented by ground water sources. High salt contamination in some ground water contributes to local problems of stock water toxicity and salt accumulation in the soils. Water from oil-brine pits, run-off from cattle feedlots, ensilage pits, and domestic raw sewage outlets contribute to ground and surface water contamination.

Classically, the microbiologist thinks of pollution as contamination by human or animal pathogens. Those pathogenic organisms found in soil are shed from the intestinal tract or remains of infected animals into the environment and are usually in numbers too low to be detected by ordinary or routine bacteriological methods. Rather than a direct study of pathogenic microbes, the bacteriologist uses indicator organisms such as Escherichia coli, which are found abundantly in feces. This analysis, still used today, does not take into account the plant pathogens, the toxicity of the soil environment to the bacteria, and all or any of the factors affecting bacterial life and movement. Since the classical test for pollution cannot allow for these factors, other studies are required for characterization of the potential quality of our water resources.

Because soils may contribute a large number of bacteria to drainage water, which

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in turn can influence the bacterial contamination in ground water, studies must be conducted on (1) the persistency of bacteria in the upper layer of soil and (2) the movement of bacteria through the soil into underground water supplies.

In order to investigate these two factors, the principal objectives of this study were set up as follows:

1. To measure the persistency or longevity of enteric bacteria in two soils of Larimer County.

2. To determine what changes in bacterial survival rates may be attributed to the chemical and physical characteristics of these soils.

3. To evaluate the effect of specific ions on the mobility of bacteria through an artificial soil column.

4. To measure the difference of bacterial surface charge (zeta potential) observed in distilled water and in ionic solutions, and to correlate this change of charge with the change of bacterial mobility in sand columns containing like solutions.

5. To suggest criteria for a new index of soil pollution which would more effectively meet the needs of the water resource scientist.

CHAPTER II

SURVIVAL OF ENTERIC ORGANISMS IN SOIL

The rapid death of enteric organisms in soils has been well documented but few investigators have attempted a detailed study of the characteristics of the soil that contribute to the decline in microbial numbers. This study is an attempt to correlate some of the soil characteristics with their effect on the survival of Escherichia coli and Streptococcus faecalis.

A. Materials and Methods

1. Soils - The two soils chosen according to their variation in physical and chemical characteristics were Greeley fine sandy loam (G) and Weld fine sandy loam (W), the latter being part of the predominant soil around Fort Collins, Colorado. The upper crust of soil was scraped away and samples were taken from the top 6 inches. Stones and debris were removed by sieving through 2 mm size apertures, while soil moisture was expressed as a ratio of grams of water per gram dry weight of soil. Water holding capacity was expressed as a ratio of water held in saturated soil to the dry weight of soil.

Soil analysis was obtained from the Soils Testing Laboratory (Colorado State University, Fort Collins, Colorado) in order to see if chemical and physical variation affected the survival rate of micro organisms.

2. Cultural techniques - The following microbial cultural techniques were employed. Any variations in methodology are mentioned in the appropriate place in the text. Cultures of Escherichia coli (wild strain) and Streptococcus faecalis, obtained from the collection of the Department of Microbiology, Colorado State University, were grown on a rotary shaker (New Brunswick) for 12-16 hours at 35°C. Cells were harvested by centrifugation and washed three times with 0.002 N potassium dihydrogen phosphate prior to suspension in distilled water. The concentration of cells was determined by optical density measurements at 450 mμ (Klett-Summerson photoelectric colorimeter, Model 9008, Klett Mfg. Co., N.Y.).

Wide-necked screw-capped glass jars containing 10 gm (dry wt) soil were inoculated with cells and placed in a 20°C incubator during the experiments. All serial dilutions were made with 0.002 N KH_2PO_4 blanks while the standard tests (APHA, 1965) were used for the detection of coliform organisms. Plate count agar (Difco) was used in the counting of indigenous soil microflora, with incubation for 3-5 days at 28°C.

B. Experimental Results

1. Death rate in unadulterated soils - The data given in Table 1 indicate that Weld and Greeley soil types showed their greatest differences in ionic concentration. The Greeley soil had far greater amounts of sulfate, calcium, magnesium, sodium, and potassium ions than did the Weld soil. The survival rates for both E. coli and S. faecalis were similar in both soils. Figure 1 shows the rapid decrease of inoculated fecal streptococcus which, after a short lag period, reached the steady state at a level similar to that of the coliform. Weld soils may have had a slight advantage in supporting the life of coliforms, but differences were not clear for S. faecalis. The stationary level was the same whether large or small inocula of cells were used, although the logarithmic rate of decline differed.

Table 1. *Soil analysis of Weld (W) and Greeley (G) soil types*

	W	G		W	G
Organic Matter	1.4%	2.0%	pH	7.6	7.4
Texture (%)			Cations (ppm)		
Sand	52	58	Calcium	110.0	472.5
Silt	25	16	Magnesium	26.5	205.0
Clay	23	26	Sodium	25.5	77.5
Anions (ppm)			Potassium	23.5	80.5
Carbonate	0.0	0.0	Mn (ppm)	3.24	5.36
Bicarbonate	126.8	122.0	Iron (ppm)	4.1	4.4
Chloride	20.0	44.0	Copper (ppm)	0.66	0.64
Sulfate	85.5	1118.8	Zinc (ppm)	0.45	0.56
Nitrate	9.0	20.0			

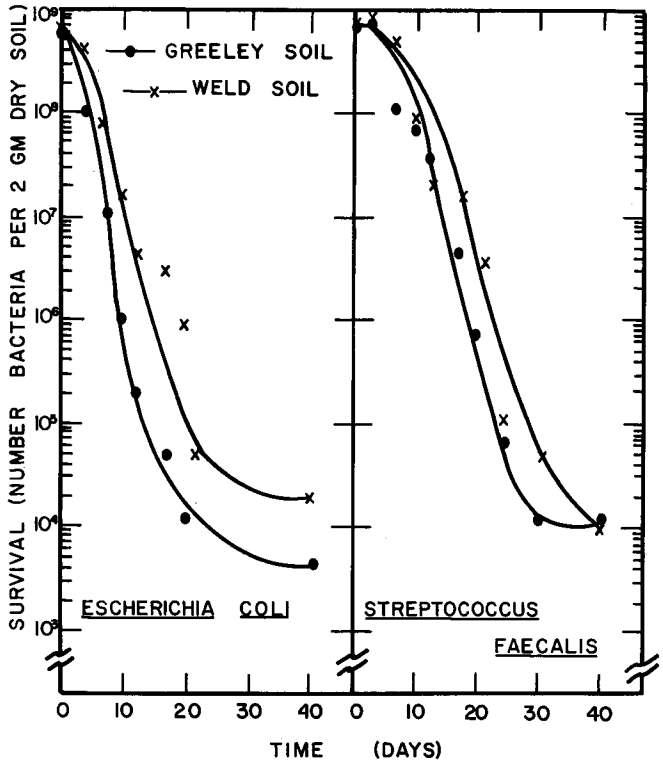


FIG. 1. SURVIVAL OF ENTERIC BACTERIA IN GREELEY FINE SANDY LOAM (G) AND WELD FINE SANDY LOAM (W). [THE MOISTURE CONTENT OF SOILS WAS KEPT AT THE MAXIMUM WATER HOLDING CAPACITY, 61.5% AND 48.1% (DRY SOIL BASIS), RESPECTIVELY.]

2. Effect of soil moisture - The data presented in Table 2 show that increased soil moisture enhanced the survival capacity of E. coli in soil W but was not significant at the 1 percent level (Stearman, 1955) in soil G after 14 days incubation.

3. Effect of organic matter (sewage) - An attempt was made to determine the effect of the addition of organic matter in the form of sewage effluent (Fort Collins Sewage Treatment Plant) to the soil substrates. After the effluent was analyzed to determine its organic solids content, it was added to the soil in its original aqueous form. The results shown in Figure 2 indicate that organic matter had little effect on E. coli in the initial period of study, but enhanced survival after a period of 14 days.

4. Effect of chelation - Analysis of the soils showed soil G to contain a greater concentration of ions, especially sulfate, magnesium, and calcium, than soil W. An

Table 2. Survival of Escherichia coli at selected soil moistures

Soil	Time (Days)	Viable Cell Counts at Selected Moistures 10%	20%	40%	50%
W	0	1400[1]	780	1500	870
	2	240	600	1100	740
	4	15	390	630	490
	7	8	96	200	190
	14	3	5	10	16
G	0	1300	800	1400	930
	2	850	430	1300	570
	4	490	210	420	330
	7	40	71	190	380
	14	2	7	8	18

[1] Multiply data by 10^5 to obtain counts.

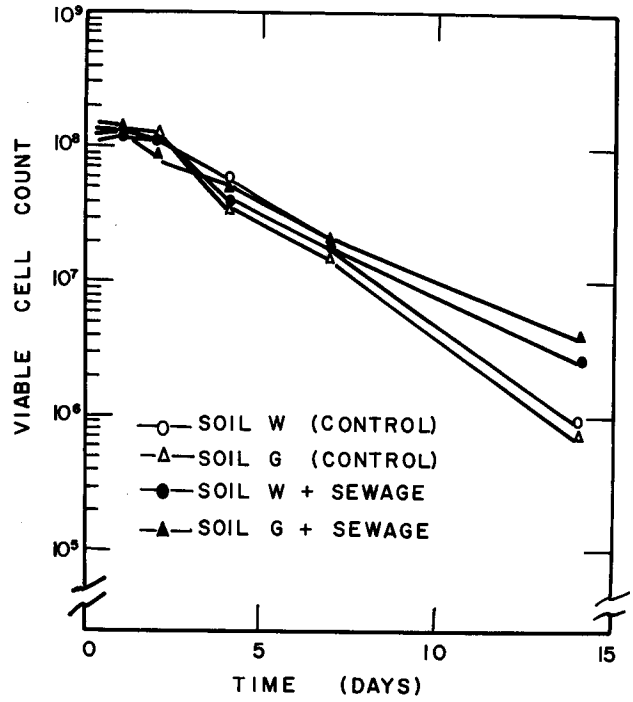


FIG. 2. THE EFFECT OF SEWAGE EFFLUENT ADDITION TO SOIL ON ESCHERICHIA COLI VIABILITY. (SEWAGE ADDITION MADE TO OBTAIN 150 PPM ADDED ORGANIC MATTER. FINAL SOIL MOISTURE = 20%. COUNTS BASED ON NUMBER OF BACTERIA PER GM DRY SOIL.)

attempt was made to tie up the cations by the addition of a chelating agent, ethylenediaminetetraacetic acid (EDTA, Matheson, Coleman and Bell). In soils not supplemented with nutrients, the presence of a chelating agent appears to be detrimental to the survival of E. coli (Figure 3). Similar results were obtained when E. coli and S.faecalis were suspended with anion exchange resins. In both cases, life rapidly fell off.

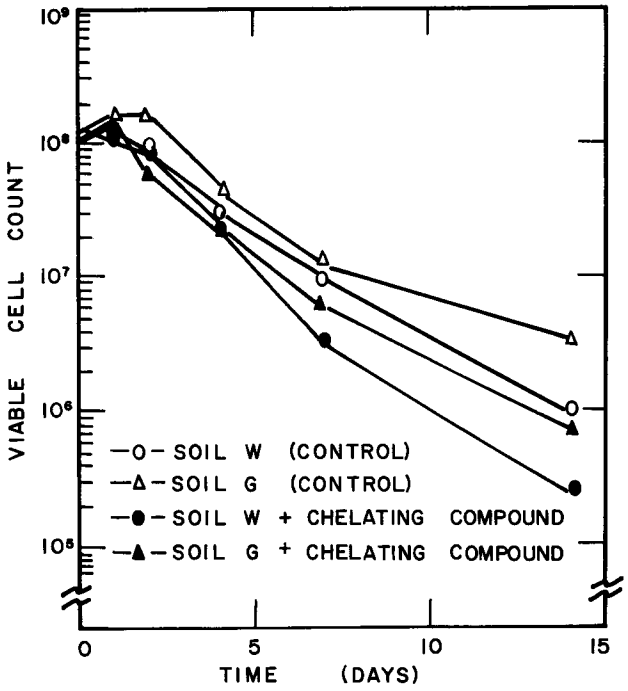


FIG. 3. THE EFFECT OF CHELATING AGENT (ETHYLENEDIAMINE TETRAACETIC ACID) ADDITION ON THE VIABLE COUNTS OF ESCHERICHIA COLI IN THE SOILS. (MOISTURE AND COUNTS AS IN FIG. 2. ONE-TENTH GM ETHYLENEDIAMINE TETRAACETIC ACID (EDTA), AS 10% SOLUTION, ADDED TO 10 GM SOIL.)

organisms. This observation agrees with that of Gray and Wilkinson (1965) who postulated that the chelating agent acts by pulling off metallic ions bound to the cell wall.

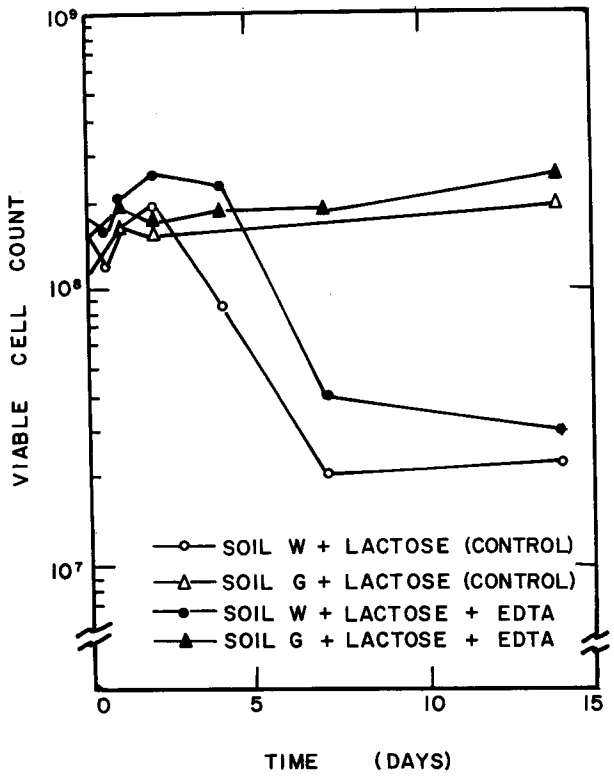


FIG. 4. THE EFFECT OF CHELATING AGENT (EDTA) ON THE VIABLE COUNTS OF ESCHERICHIA COLI IN SOILS (W AND G) WITH LACTOSE ADDITION. (METHODOLOGY AS GIVEN FOR FIG. 3 WITH 34.2 MG LACTOSE ADDED PER GM SOIL.)

When the chelating agent and a nutrient, lactose, were added to the two soils, an increase in survival was noted over the control soils to which only nutrient was added (Figure 4). When identical experiments were designed to take into account the total bacterial count, similar results were obtained. Apparently the nutrient material masked or covered the effect of the chelating agent or possibly changed the mechanism by which lactose was metabolized. The detrimental effect of the chelating agent upon microbial survival is probably either by direct inhibition or by immobilization of cations necessary for the maintenance of the

5. Effect of salt additions - Another attempt to recognize an ion effect was made by adding ammonium sulfate and calcium nitrate to soil W in amounts calculated to bring concentrations of these compounds to the level found in soil G. Although it appears (Figure 5) that there was a difference in the survival rate in soil W with the addition of ammonium sulfate as compared to control soil W, the slopes of the curves indicate that this difference was not too significant, and subsequent experiments altering the ammonium level indicated little appreciable effect by this ion. In soil W with calcium nitrate, results similar to those with ammonium sulfate were observed.

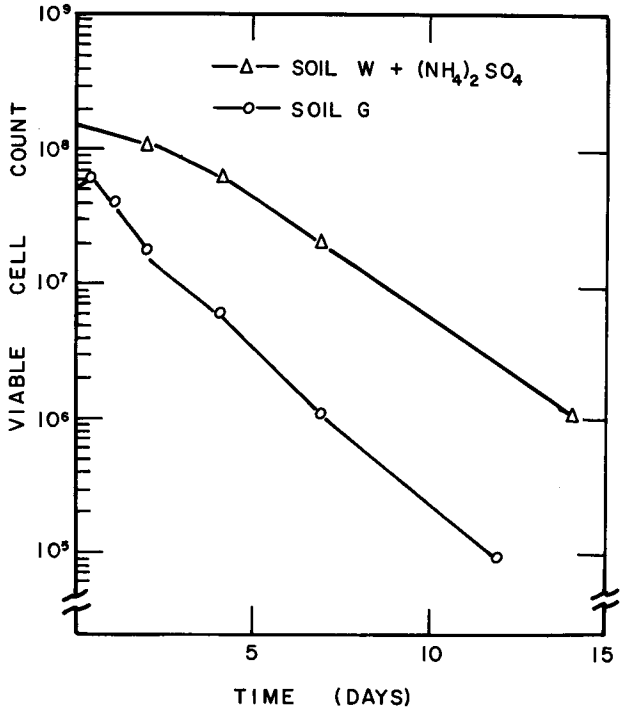


FIG. 5. THE EFFECT ON ESCHERICHIA COLI SURVIVAL IN SOIL W WHEN SULFATE LEVELS WERE RAISED TO THOSE OF SOIL G. (1.32 MG $(NH_4)_2SO_4$ ADDED TO ONE GM SOIL W. COUNTS BASED ON NUMBER OF BACTERIA PER GM DRY SOIL.)

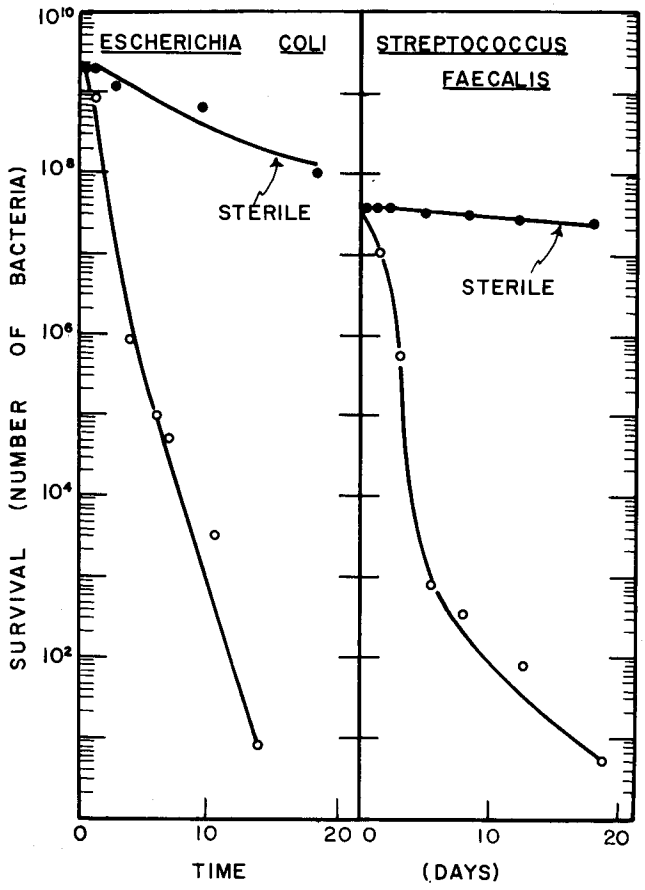


FIG. 6. THE EFFECT OF SOIL STERILIZATION ON THE SURVIVAL OF ENTERIC BACTERIA. (COUNTS BASED ON NUMBER OF BACTERIA PER GM DRY SOIL. STERILIZATION OBTAINED BY USING 120°C FOR 30 MIN.)

6. Effect of soil sterilization - It has been repeatedly suggested that the biological factors in soil would explain the rapid death of newly introduced microorganisms, for results indicate that microorganisms survived much longer in sterilized than unsterilized soil. This was confirmed with the Greeley loam as shown in Figure 6. The results indicate that the sterilized soil (sterilized by autoclaving at 120°C for 30 min) provided a preferential effect on the survival of both E. coli and S. faecalis. This could be due to the killing of some antagonistic microorganisms and/or providing hitherto unavailable nutritional component(s).

The "shock" of adding washed organisms in the logarithmic growth phase may have contributed to the initial decline in number, but most of the effect may be attributed to undetermined characteristics of the soil. The leveling off period is probably due to the multiplication of resistant forms.

7. Effect of nutrition - Nutrient materials (lactose and glucose) added to the soil substrates were conducive to the survival of E. coli (Table 3). These results support observations by Klein and Casida (1967) which state that nutrient compounds indigenous to the soil are not in a form available for coliform utilization, and support our findings with autoclaved soil. Phosphate and yeast extract added alone and in combination did not contribute significantly to survival of this microbe, although yeast extract favored an initial increase in numbers.

Table 3. *Survival of Escherichia coli in soil with the addition of selected nutrient substances*

Addition[1]	Amount Added to Soil (mg/gm soil)	Time (Days) 0	0.5	1	2	4	7	14
		Soil W						
Control	--	150[2]	--	--	110	63	20	10
Lactose	34.2	61	95	140	140	87	60	20
Glucose	18	17	22	66	83	83	140	59
Yeast extract	30	110	370	62	580	120	7	0.65
K_2HPO_4	30	110	130	130	110	37	6	0.91
Yeast extract + K_2HPO_4	30+30	100	180	300	230	22	9	0.82
		Soil G						
Control	--	140	--	--	130	42	2	0.78
Lactose	34.2	50	190	280	470	100	350	140
Glucose	18	19	100	200	430	870	540	130
Yeast extract	30	110	600	850	840	130	6	2
K_2HPO_4	30	120	160	180	150	34	8	2
Yeast extract + K_2HPO_4	30+30	100	290	590	350	24	9	0.67

[1] Soil moisture kept at 20%.
[2] Multiply data by 10^6 to obtain counts per gm dry soil.

8. Effect of indigenous soil microflora- For this portion of the work, E. coli or S. faecalis was grown in tryptone-soy broth (BBL division of BioQuest) on a rotary shaker at 30°C for 18-20 hours. After centrifugal harvesting and three distilled water washings, cells were diluted to 10^9 cells/ml. Eosin-methylene-blue agar (Difco) and Bacto-m-enterococcus agar (Difco) were used to determine survival of E. coli and S. faecalis respectively. Protozoa were observed via direct microscopic examination and by culture and after a one week incubation on a glucose-mineral agar[1] slant at room temperature. Myxamoebae and myxobacteria were examined by the method of Singh (1947a, 1947b). Lytic actinomycetes were examined on glucose-mineral agar plates embedded with E. coli or S. faecalis; if present, clear transparent zones appeared around the colony. The technique of Stolp and Starr (1963) was used for the examination of Bdellovibrio sp., with B. bacteriovorus (ATCC 15143), an organism showing bacteriolytic action against the E. coli used in this investigation, being used as the control organism.

The presence of other bacteriolytic microorganisms was determined by use of the following procedure: soil samples were inoculated into plates containing one of three media [yeast extract-peptone agar (0.5% and 1%, respectively), glucose-mineral agar, and plate-count agar (Difco)] which previously had been plated with a thick suspension of E. coli or S. faecalis (10^{10} cells/plate). The inoculated plates were incubated at 20°C, 25°C, and 35°C, respectively, prior to examination for plaque formation indicative of bacteriolysis. The soil extract coming through a 0.3 μ pore size filter (Millipore Corporation, Bedford, Massachusetts) was examined for bacteriophage using a similar procedure to that used for Bdellovibrio sp. except that nutrient agar (Difco) rather than yeast extract-peptone agar was used in phage detection.

The following procedure was used for the fractionation of a soil suspension. Fifty grams of Greeley soil were dispersed in 50 ml distilled water by shaking for one hour at room temperature. This soil suspension was filtered through a Millipore membrane of 8 μ pore size followed by a membrane of 1.2 μ pore size to obtain fractions containing particles in two size ranges:

Fraction I: 1.2 μ to 8 μ (contained bacteria, protozoa, etc.)

Fraction II: <1.2 μ (contained bacteria, Bdellovibrio sp., phage, etc.).

Soil was extracted for a few hours at room temperature with an equal amount of distilled water before filtration with the 0.3 μ pore size filter membrane. The filtrate, having a pH of 8 and considered bacteria-free, was termed soil infusion.

Both E. coli and S. faecalis showed marked decline in numbers after 10 days incubation with soil infusion containing either Fraction I (1.2 μ - 8 μ) or Fraction II (<1.2 μ) of soil suspension (Figure 7). No remarkable decline was observed in controls containing heat treated (100°C for 5 min) Fractions I or II. In the first few days of the experiment, the number of E. coli increased slightly in soil infusion medium regardless of the presence of soil suspension fractions; S. faecalis showed greater ability to survive than did the coliform.

[1] Glucose mineral agar: $(NH_4)_2SO_4$ - 2 gm; K_2HPO_4 - 6 gm; Na-citrate·$2H_2O$ - 1 gm; Mg SO_4·7 H_2O - 0.2 gm; glucose - 5 gm; agar - 15 gm; Dist. water - 1000 ml.

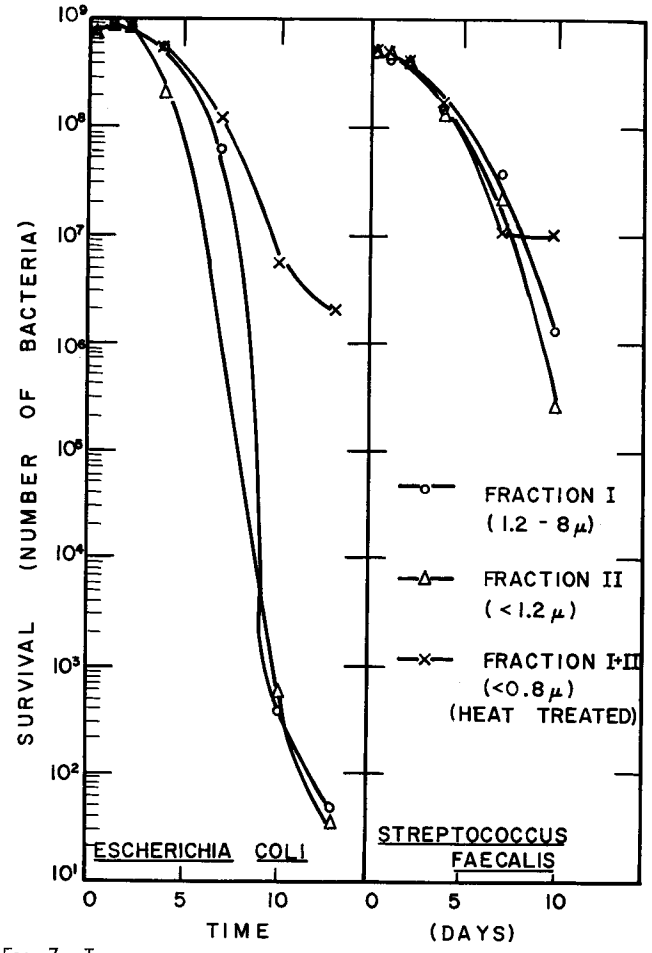


FIG. 7. THE EFFECT OF FRACTIONATED SOIL SUSPENSION ON THE SURVIVAL OF ENTERIC BACTERIA. (FRACTIONS OBTAINED BY MILLIPORE FILTRATION; HEAT TREATMENT = 100°C FOR 5 MIN; FILTRATE FRACTIONS ADDED TO STERILIZED SOIL INFUSION AT 1 TO 5 RATIO.)

the survival of the two test organisms. The results shown in Figure 8 indicate that the enriched Fraction II enhanced the death rate of E. coli and S. faecalis. Heat treated enriched Fraction II had no effect.

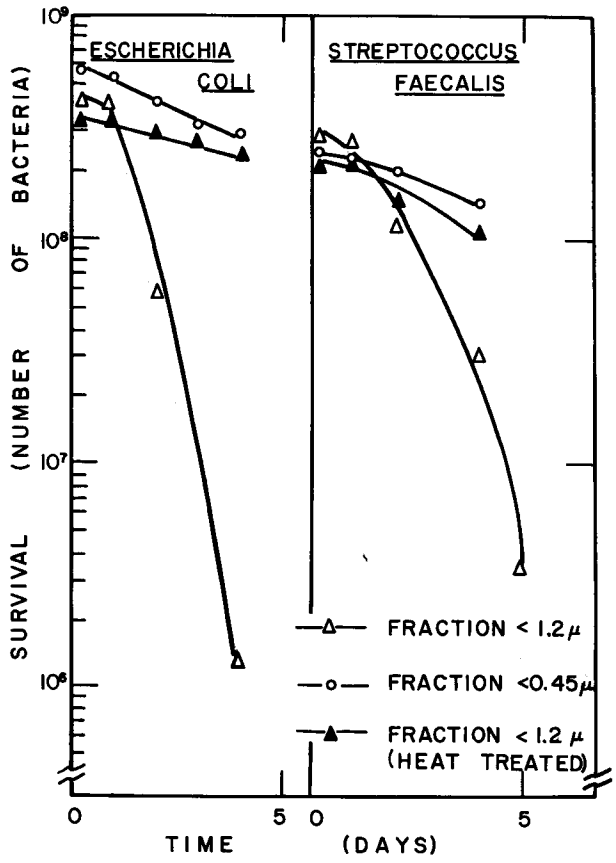


FIG. 8. THE EFFECT OF ENRICHED FRACTIONATED SOIL SUSPENSIONS ON THE SURVIVAL OF ENTERIC BACTERIA. (METHODOLOGY AS GIVEN FOR FIG. 7)

It was presumed that any microbes parasitic upon either E. coli or S. faecalis would have multiplied when incubated in the presence of the enteric organisms and could be detected readily. Therefore, after 10 days of incubation in the first experiment, enrichment tests were attempted. The reaction vessels were opened and refractionated by the Millipore technique. The vessels containing Fraction I (1.2 μ - 8 μ) were filtered with a 0.45 μ filter membrane and the filtrate was examined for Bdellovibrio sp. without success (larger bacteria should have been removed by the 0.45 μ membrane, leaving any vibrios and virus in the filtrate). Those vessels containing Fraction II (<1.2 μ) were refiltered with a 1.2 μ membrane, and the filtrate was again tested for its effect on

Efforts to isolate protozoa, myxamoebae, myxobacteria, lytic actinomycetes, bacteriophage or other predatory organisms from the enriched fractions were unsuccessful.

It was observed that both soil suspension Fractions I and II contained a large and predominant bacterial flora, suggesting that the very number as well as type of bacteria existing in soil may inhibit the growth of the two test organisms, probably by means of nutritional competition. Various workers have reported that small amounts of glucose promoted the survival of E. coli (McGrew and Mallette, 1965 and Mallette, 1963). Others (Kononova, 1966) have found that certain soil extracts (aqueous, alcoholic, or salt) and a number of amino acids, free sugars, and low molecular weight organic acids isolated from

soils have a beneficial effect on E. coli survival. These materials are found in low concentration in soils, and it is not surprising that there is keen competition for this limited food supply.

For the soil tested in these experiments, it is likely that nutritional deficiencies brought about the microbial competition were an important cause of death for both E. coli and S. faecalis. It is not probable that any parasitic and/or lytic microbe played an important role in the survival of these two bacterial strains.

C. Discussion

A number of factors affecting the survival of enteric bacteria in soil have been studied. It should be apparent that other variables exist which could relate to microbial survival.

We have shown that for two Larimer County soils, Greeley and Weld fine sandy loams, the soil moisture content and the available amount of organic matter and nutrients are capable of affecting microbial life. There appears to be a wide range of salt tolerance for Escherichia coli, as exemplified by a comparison of this organism's survival in soil G and soil W. Soil G, quite high in its concentration of ions, had about the same effect on bacterial survival as did the soil W. Microbial predators were of no significance in our tests with soil G.

From the controlled experiments performed with pure cultures, it could be postulated that a non-optimum amount of any one of the variables could bring about microbial death. Of course, soil is a complex ecosystem, and each living and non-living entity in the soil relates to the other entities about it. Microorganisms are competing with each other for available nutrients and moisture in soil. It is not difficult to suppose that certain components have a preferential effect upon a particular species of bacteria and that, with other variables at optimum, the type and quantity of nutrition available determines a population character and size. Further, many microbes release or manufacture a material toxic to others and/or themselves. Only if the toxicity is removed, perhaps by rain's leaching action, by aging, or by metabolism of the toxic substance by other organisms, can certain of the microbes survive.

Thus, we have a complex system governed by many variables. When conditions are suitable for its survival, an enteric microflora may exist for an indefinite period of time. Because these types of bacteria may reach and pollute ground and surface water sources, it is important to know when and if these conditions can exist for a particular soil. Although progress has been made, more work is needed before a model to make such assumptions can have accuracy.

CHAPTER III

BACTERIAL MOVEMENT THROUGH PARTICULATE MATTER

The actual mechanisms of the adsorption of viruses or bacterial cells to a soil (or other) particle is poorly understood. Various researchers have claimed that one or more factors affect adsorption, including pH, type of ions present, or the electrokinetic properties of either or both the bacteria and the adsorbing materials.

Tschapeck and Garbosky (1950) stated that the adsorption of Azotobacter depends mainly on the electrokinetic potential of the bacterium, which in turn depends on the pH of the suspension medium.

According to the findings of Curry and Beasley (1960) mechanical filtration was the main process by which bentonite particles were removed from suspension by the carborundum particles, 65 microns (μ) in diameter; adsorption of the bentonite onto the carborundum also occurred.

Zvyagintsev (1962) reported on the adsorption of the microorganisms by soil particles. He found that the microbial count of a soil-water mixture was far higher if the microbes were desorbed by a material such as sodium pyrophosphate than if they were dispersed by 10 minutes of shaking. This worker also studied the adsorption of bacterial cells to soil particles by direct microscopy; he found that the number of organisms adsorbed to a particle increased with particle size. The cells were arranged around the particles either laterally, in concentric circles, or radially, with one end of the cells pointed toward the particle.

In order to study the relationship between ground water and surface water pollution, a basic understanding of bacterial movement through and adsorption by porous material is essential. This section is devoted largely to the elucidation of some of the factors affecting movement and/or adsorption.

A. Materials and Methods

1. Column design - Multiple plexiglass columns (Hyde Corp., Grenloch, New Jersey) 123 cm long and 7.0 cm wide (I.D.) were fitted with one-hole rubber stoppers, glass and rubber tubing, and nylon stopcocks prior to placing in vertical position by rod-framing (Figure 9). Several layers of clean gauze were placed over the stopper hole to keep the exit free of particles. After each use, the columns were emptied, detergent washed, distilled water rinsed and air dried. The stopper-stopcock setup was washed, rinsed, and boiled in distilled water in order to remove any clumps of cells that might have lodged in crevices. The column, thus prepared, was filled to a height of 1 meter with porous material (glass beads or various sized sand particles) which had been acid washed (5 per cent HCl followed by thorough tap and distilled water rinses) and dried in enamel pans 20-48 hours at 150°C.

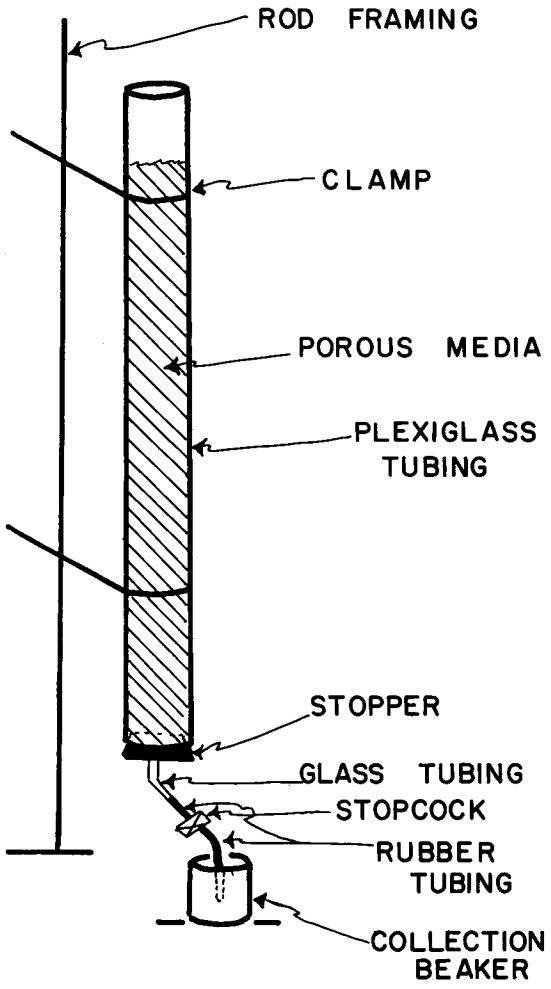


FIG. 9. COLUMN FOR BACTERIAL MOBILITY STUDIES IN POROUS MEDIUM.

2. Porous material - To simulate a porous soil in the columns, three materials were used. The first material was glass beads of 0.17 - 0.18 mm diameter (Matheson Scientific). A "coarse" and a "fine" sand were also used (Ottawa Silica Co., Ottawa, Ill.). The coarse one was a quartz sand with a uniformity coefficient (Briggs and Fiedler, 1966) of 1.33 and a representative particle

size of 0.46 mm. In these instances, representative particle size is taken to be the sieve size that retained 50 per cent of the weight of the sample. The fine material was a silica sand (Ottawa) having a uniformity coefficient of 2.04 and a representative particle size of 0.26 mm.

3. Organisms and cultural techniques - Organisms were from the culture collection of the Department of Microbiology, Colorado State University, except the Staphylococcus sp.. The latter was a fresh isolate from the skin of a laboratory worker.

Bacterial cells were grown in one-half strength plate-count broth (Difco) for 18-20 hours at 35°C and then harvested by centrifugation. They were washed two times with distilled water before dilution to about 10^6 cells per ml in the desired metal ion solution. A Klett-Summerson photoelectric colorimeter (blue filter, 450 mμ) was used for estimation of cell concentration. In preliminary experiments, cells were washed with the specific ion solution to be tested. Results were comparable to those using a distilled water wash; therefore, the ion solution wash was omitted in subsequent trials. The cell-ion suspensions were made at 10^{-3} N concentration unless otherwise noted and after a 20 minute adjustment period, were diluted in sterile distilled water for plating on plate-count agar (Difco) to obtain an exact initial count of the cells.

The cell-ion suspensions were poured through filled plexiglass columns such that a hydraulic head of 1 meter ± 5 cm was kept on the stopcock at the base of the column. After 800 ml had passed through the stopcock, a 50 ml sample was collected in a sterile container.

Dilutions of this sample, as well as dilutions of a saved portion of the original suspension, were made and again plated on plate-count agar. Plating of the original suspension at the end of each experiment served as a control to measure the toxicity of the ions and to account for other factors that might have brought death to the test organisms during the course of the experiment

All plates were incubated at 35°C for 20 hours prior to counting with the exception of those plates containing Staphylococcus. These were incubated 40-48 hours for ease in counting. Samples of bacteria-ion suspensions were checked for pH, turbidity and/or color before and after filtration. The latter tests were made with a Klett-Summerson photoelectric colorimeter at 450 mμ (blue filter).

B. Experimental Results

1. Porous media - Preliminary data showed that both size and character of the particulate filtration media were important. Tests were made using three types of particles with both wet and dry columns. Serratia marcescens was the test organism. Since similar results were obtained with predampened and dry columns, dry columns were used in further experiments. The results from a test of particles are given in Table 4. None of the media had appreciable ability to filter S. marcescens from distilled water suspensions. However, when an electrolyte (0.01 N NaCl) was added to the bacterial suspension, marked differences became apparent. Coarse sand and glass beads gave similar results while fine sand effectively removed all bacteria from the suspension.

Table 4. *Effect of particle size and NaCl on percolation of Serratia marcescens*

Substrate Material	Suspending Solution	Bacteria per ml: Initial & Control[1] Counts (IC)	Bacteria per ml: Final (Percolated) Count (PC)	Log Difference (log IC - log PC) or $\log \left[\frac{IC}{PC}\right]$
Coarse Sand (0.45 mm)	H_2O	15×10^5	14×10^5	0.03
	0.01 N NaCl	19×10^5	1.2×10^5	1.20
Fine Sand (0.26 mm)	H_2O	19×10^5	21×10^5	--
	0.01 N NaCl	23×10^5	$<1 \times 10^0$	>6.4
Glass Beads (0.17--.18 mm)	H_2O	15×10^5	17×10^5	--
	0.01 N NaCl	19×10^5	0.38×10^5	1.70

[1] The initial cell counts (IC) and nonpercolated control counts (CC) were identical.

2. Bacterial filterability - Since our research was concerned with the pollution of water resources by bacterial movement through soils, it was important to determine what effect ions and fertilizer compounds common to agricultural soils might have on the filterability or mobility of various bacteria. The concentration of these materials is set low enough to be found in soil leachates or as a result of man's contamination.

Because the "activity" or filterability of a bacterial species will vary somewhat from day to day, it was advisable to make specific comparisons on the effect of ions or compounds only if all tests were percolated through the columns simultaneously. Therefore, racks were set up so that as many as nine percolation columns could be used at one time. When all of the columns were in use, three hours elapsed between plating the initial count (IC) and the control count (CC).

The first series of experiments were designed to test the effect of common salt (NaCl) on various organisms, and the results are given in Table 5. The data given in this and subsequent tables have been calculated on a logarithmic basis, so that results are given as the log of the number of bacteria removed by column filtration. This decrease was calculated via two methods:

a) Log (No. viable bacteria at experiment end) (CC)
minus
Log (No. bacteria percolated through column) (PC)

or

$$\text{Log } [\frac{CC}{PC}]$$

b) Log (No. viable bacteria at experiment beginning) (IC)
minus
Log (No. bacteria percolated through column) (PC)

or

$$\text{Log } [\frac{IC}{PC}]$$

The first method of calculation gave the lowest value and presumed that the rate of death was the same in the column as in the original flask of solution. The second method assumed that no death of bacteria took place. The actual value probably lay between these two calculations, probably weighted toward results obtained with method (a).

$$\text{Log } \{[\frac{IC}{PC}]_{salt} \times [\frac{PC}{IC}]_{H_2O}\} = SFV_u$$

The 0.01 N NaCl used in the first experiment (Table 5) had little or no toxic effect on the test bacteria. Apparently, the Staphylococcus sp. used was somewhat fragile in distilled water for it lost viability more readily than in salt solution. All organisms tested showed a marked decrease in numbers when filtered with this concentration of saline, with S. marcescens showing the greatest change in numbers. The data also show that sand filtration without added ions could, at times, remove a sizable number of bacteria.

Tables 6 and 7 give the results of typical experiments using the 4 test organisms with many different solutions. By comparing the SFV_c and SFV_u values, the toxic effect

Table 5. *Effect of 0.01 N NaCl on the mobility of various bacteria in sand columns*

Organism	Suspending Solution	Bacteria per ml IC[1]	CC	PC	log [CC/PC]	SFV_c	log [IC/PC]	SFV_u
S. marcescens	NaCl	24×10^5	28×10^5	$<1\times10^0$	>6.5		>6.4	
	Water	20×10^5	16×10^5	53×10^4	0.48	>6.0	0.58	>5.8
E. coli	NaCl	80×10^4	70×10^4	24×10^2	2.46		2.52	
	Water	63×10^4	95×10^4	57×10^4	0.22	2.24	0.04	2.48
E. coli B	NaCl	16×10^4	19×10^4	99×10^1	2.28		2.21	
	Water	15×10^4	19×10^4	47×10^3	0.61	1.67	0.50	1.71
Staphylococcus sp.	NaCl	49×10^4	30×10^4	5×10^1	3.78		3.99	
	Water	48×10^4	13×10^4	49×10^2	1.42	2.36	1.99	2.00

[1] IC = initial count; CC = control count; PC = percolated count; SFV_c = salt filtration value controlled; SFV_u = salt filtration value uncontrolled.

To obtain a logarithmic value which allowed consideration for distilled water controls, the logarithms of the filtration values were subtracted.

Thus: $\text{Log}_{salt} [\frac{CC}{PC}] - \text{Log}_{H_2O} [\frac{CC}{PC}] =$

salt filtration value controlled (SFV_c) (full allowance for death during experiment)

or

$$\text{Log } \{[\frac{CC}{PC}]_{salt} \times [\frac{PC}{CC}]_{H_2O}\} = SFV_c$$

and

$$\text{Log}_{salt} [\frac{IC}{PC}] - \text{Log}_{H_2O} [\frac{IC}{PC}] =$$

salt filtration value uncontrolled (SFV_u) (no allowance for bacterial death during experiment)

or

of some of the ions may be measured. E. coli B and the Staphylococcus sp. were little affected by any of the solutions tested. Cu^{++}, Ca^{++} and Zn^{++} were toxic for S. marcescens, while Cu^{++}, Fe^{+++} and Ca^{++} showed toxicity toward E. coli. By comparing the Log $[\frac{CC}{PC}]$ and Log $[\frac{IC}{PC}]$ values obtained with water, one may note the effect of distilled water on the test organisms. E. coli B and the Staphylococcus sp. lost viability more readily in water than in salt solutions, while E. coli and S. marcescens lost little or no viability in water during the course of the experiments.

In trials with some common soil fertilizers (Table 6), the monovalent ammonium ion showed definite activity toward Serratia. Urea was ineffective as a filter aid in the concentration used (0.001 M).

Table 6. *Effect of different ions and compounds on the mobility of Serratia marcescens in sand columns*

Organism	Suspending Solution[1]	Bacteria per ml IC[2]	CC	PC	log [CC/PC]	SFV_c	log [IC/PC]	SFV_u
S. marcescens	Water	$64x10^4$	$67x10^4$	$17x10^2$	2.60		2.58	
	Ca^{++}	$62x10^4$	$52x10^3$	$<1x10^0$	>4.7	>2.1	>5.8	>3.2
	Cu^{++}	$46x10^4$	$68x10^2$	$<1x10^0$	>3.8	>1.2	>5.7	>3.1
	Fe^{+++}	$32x10^4$	$22x10^4$	$2x10^0$	5.04	2.44	5.20	2.62
(A)	Mg^{++}	$74x10^4$	$53x10^4$	$<1x10^0$	>5.7	>3.1	>5.9	>3.3
	$Borate^{=}$	$66x10^4$	$54x10^4$	$14x10^4$	0.59	--	0.47	--
	Na^{+}	$58x10^4$	$47x10^4$	$36x10^0$	4.11	1.51	4.21	1.63
	Zn^{++}	$70x10^4$	$36x10^4$	$1x10^0$	5.56	2.96	5.84	3.26
(B)	Water	$20x10^5$	$19x10^5$	$23x10^4$	0.92		0.94	
	Ca^{++}	$18x10^5$	$72x10^4$	$<1x10^0$	>5.9	>5.0	>6.3	>5.4
	Cu^{++}	$13x10^5$	$50x10^2$	$<1x10^0$	>3.7	>2.8	>6.1	>5.2
	Mg^{++}	$21x10^5$	$16x10^5$	$5x10^0$	5.50	4.58	5.62	4.68
	Zn^{++}	$20x10^5$	$17x10^5$	$<1x10^0$	>6.2	>5.3	>6.3	>5.4
(C)	Water	$66x10^4$	$88x10^4$	$90x10^3$	0.99		0.87	
	K^{+}	$69x10^4$	$68x10^4$	$16x10^4$	0.63	--	0.64	--
	NH_4^{+}	$62x10^4$	$68x10^4$	$22x10^2$	2.49	1.50	2.45	1.58
	Urea	$78x10^4$	$70x10^4$	$99x10^3$	0.85	--	0.90	--
(D)	Water	$61x10^4$	$49x10^4$	$94x10^3$	0.72		0.81	
	NH_4NO_3	$47x10^4$	$45x10^4$	$44x10^2$	2.00	1.28	2.03	1.22
	$(NH_4)_2SO_4$	$54x10^4$	$52x10^4$	$50x10^3$	2.00	1.28	2.03	1.22
	K_2HPO_4	$62x10^4$	$47x10^4$	$14x10^4$	0.53	--	0.64	--

[1] Unless otherwise noted, all cations were used as chlorides except Mg^{++} and NH_4^{+} which were used as sulfates. $Borate^{=}$ was used as the sodium salt. Concentrations were at 0.001 N except Mg^{++} and $borate^{=}$ which were at 0.002 N and urea which was at 0.001 N.
[2] As used for Table 5.

Table 7. *Effect of different ions and compounds on the mobility of coliforms and a Staphylococcus sp. in sand columns*

Organism	Suspending Solution[1]	Bacteria per ml IC[2]	CC	PC	log [CC/PC]	SFV_c	log [IC/PC]	SFV_u
E. coli B	Water	$56x10^4$	$38x10^4$	$31x10^4$	0.09		0.26	
	Ca^{++}	$48x10^4$	$28x10^4$	$11x10^4$	0.41	0.32	0.65	0.39
	Cu^{++}	$6x10^4$	$30x10^3$	$42x10^0$	2.85	2.76	3.15	2.89
	Fe^{+++}	$56x10^4$	$34x10^4$	$1x10^0$	5.53	5.44	5.75	5.49
(A)	Mg^{++}	$56x10^4$	$54x10^4$	$35x10^3$	1.19	1.10	1.20	0.94
	$Borate^{=}$	$48x10^4$	$39x10^4$	$48x10^4$	--	--	0	--
	Na^{+}	$47x10^4$	$42x10^4$	$19x10^4$	0.34	0.25	0.39	0.13
	Zn^{++}	$44x10^4$	$16x10^4$	$70x10^3$	0.36	0.37	0.80	0.54
(B)	Water	$10x10^4$	$70x10^3$	$25x10^3$	0.45		0.62	
	Ca^{++}	$10x10^4$	$25x10^3$	$26x10^1$	1.98	1.53	2.59	1.97
	Cu^{++}	$26x10^3$	$19x10^3$	$<1x10^0$	>4.3	>3.8	>4.4	>3.8
	Mg^{++}	$13x10^4$	$10x10^4$	$5x10^0$	4.30	3.85	4.41	3.79
	Zn^{++}	$10x10^4$	$36x10^3$	$12x10^0$	3.48	3.03	3.92	3.30
E. coli	Water	$47x10^4$	$58x10^4$	$59x10^4$	0		0	
	Ca^{++}	$45x10^4$	$22x10^4$	$40x10^2$	1.74	1.74	2.05	2.05
	Cu^{++}	$30x10^4$	$96x10^2$	$<1x10^0$	>3.0	>3.0	>5.5	>5.5
	Fe^{+++}	$40x10^4$	$56x10^3$	$80x10^2$	0.84	0.84	1.70	1.70
	Mg^{++}	$42x10^4$	$40x10^4$	$15x10^4$	0.43	0.43	0.45	0.45
	$Borate^{=}$	$49x10^4$	$36x10^4$	$62x10^4$	--	--	--	--
	Na^{+}	$40x10^4$	$36x10^4$	$37x10^4$	0	0	0.03	0.03
	Zn^{++}	$34x10^4$	$26x10^4$	$28x10^2$	1.97	1.97	2.08	2.08
Staphylococcus sp.	Water	$48x10^5$	$21x10^5$	$10x10^5$	0.32		0.68	
	Ca^{++}	$68x10^5$	$30x10^5$	$18x10^1$	4.22	3.90	4.58	3.90
	Fe^{+++}	$36x10^5$	$18x10^5$	$22x10^1$	3.91	3.59	4.21	3.53
	Mg^{++}	$49x10^5$	$37x10^5$	$20x10^2$	3.27	2.95	3.40	2.72
	$Borate^{=}$	$40x10^5$	$27x10^5$	$10x10^3$	2.43	2.11	2.60	1.92
	Zn^{++}	$36x10^5$	$24x10^5$	$90x10^0$	4.43	4.11	4.60	3.92

[1] As used for Table 6
[2] As used for Table 5.

The results of the experiments (Tables 6 and 7) may be summarized as follows. These values are based on SFV_c. Only slight changes in the placement of any ion or compound were noted when values were based on the SFV_u value.

Organism	Rank of "filter aids"
E. coli	$Cu^{++}>Zn^{++}$, $Ca^{++}>Fe^{+++}$, $Mg^{++}>Na^{+}>Borate^{=}$
E. coli B	$Fe^{+++}>Cu^{++}>Mg^{++}>Zn^{++}>Ca^{++}$, Na^{+}, $Borate^{=}$
Staphylococcus sp.	Zn^{++}, Ca^{++}, $Fe^{+++}>Mg^{++}>Borate^{=}$
S. marcescens	Zn^{++}, Mg^{++}, Ca^{++}, Cu^{++}, $Fe^{+++}>NH_4^{+}$, $Na^{+}>K^{+}$, $Borate^{=}$, Urea

Borate seemed to actually stimulate the passage of bacteria through the columns, acting in a manner similar to the desorbing compounds mentioned by Zvyagintsev (1962).

3. pH and turbidity measurements - The data (Table 8) show the variations in turbidity and pH that were measured on bacterial suspensions before and after column filtration. The cloudiness and/or color of a solution of ferric chloride is completely removed by passage through sand. Even though solutions of $FeCl_3$ became darker and turbid on standing, filtration still removed all color and cloudiness from a 0.001 N solution. On the other hand, sodium borate, di-potassium hydrogen phosphate and di-ammonium hydrogen phosphate increased in turbidity when filtered through sand.

Distilled water was used as it came from the still (Barnstead) without further treatment. This led to variations in pH depending on the amount of carbon dioxide dissolved, and the water was always slightly acid. When various compounds were dissolved in water, some pH changes occurred, the most noticeable being the marked acidity of $FeCl_3$ solutions and the elevated pH of sodium borate solutions. Since acid pH is supposed to favor removal of bacteria by filtration (Tschapek and Garbosky, 1950), this could explain, at least in part, the mode of action of $FeCl_3$ in sand columns. Similarly, the elevated pH of sodium borate solutions might be related to its desorption action (Tables 6 and 7).

With the exception of borate solutions, all bacteria-ion suspensions dropped in pH as they passed through the columns. It seems a reasonable conclusion that these columns were exchanging ions - i.e. exchanging a metallic cation for a hydrogen ion.

Table 8. *Turbidity and pH changes occurring as solutions pass through sand columns*

Organism	Turbidity[1] Before	Turbidity[1] After	pH[2] Before	pH[2] After	Suspending[3] Solution
E. coli B	2	3	5.4	5.4	Water
	2	1	6.3	5.3	Ca^{++}
	2	0	5.2	4.2	Cu^{++}
	140	0	3.6	3.5	Fe^{+++}
	3	0	6.4	4.4	Mg^{++}
	3	39	8.5	8.7	$Borate^{=}$
	5	5	5.9	5.3	Na^{+}
	2	14	5.7	5.2	Zn^{++}
E. coli	0	10	5.7	5.5	Water
	0	2	5.4	4.2	Ca^{++}
	0	1	5.1	4.1	Cu^{++}
	250	5	3.3	3.2	Fe^{+++}
	1	0	5.2	4.3	Mg^{++}
	1	61	8.0	8.7	$Borate^{=}$
	0	3	5.6	5.0	Na^{+}
	2	0	5.5	4.1	Zn^{++}
Staphylococcus sp.	1	6	6.4	5.9	Water
	1	2	6.0	5.4	Ca^{++}
	0	0	5.1	4.2	Cu^{++}
	133	1	3.4	3.4	Fe^{+++}
	2	2	5.5	4.9	Mg^{++}
	2	34	8.7	8.9	$Borate^{=}$
	2	9	5.8	5.4	Na^{+}
	2	2	5.8	4.7	Zn^{++}
S. marcescens			5.9	5.0	Water
			5.7	4.8	Ca^{++}
			5.1	3.8	Cu^{++}
(A)	167	0	3.3	3.1	Fe^{+++}
			5.4	4.3	Mg^{++}
			8.4	8.6	$Borate^{=}$
			6.1	5.6	Na^{+}
			5.8	4.2	Zn^{++}
(B)	0	3	5.6	5.1	Water
	0	0	5.5	4.5	KCl
	0	27	7.05	6.8	K_2HPO_4
	0	30	6.95	6.6	$(NH_4)_2HPO_4$
	0	2	5.75	5.3	$(NH_4)_2SO_4$
	0	4	5.8	5.0	Urea
(C)	2	5	6.5	5.65	Water
	0	5	5.4	4.5	NH_4NO_3
	4	11	5.6	5.4	$(NH_4)_2SO_4$
	3	32	6.95	6.9	K_2HPO_4

[1] Turbidity measured with Klett-Summerson photoelectric colorimeter, 450 mμ.
[2] Distilled water used as prepared and contained variable amounts of CO_2.
[3] As used for Table 6.

4. Ferric chloride experiments - In order to more fully understand the mechanisms of bacterial filtration, simple experiments were designed using ferric chloride. Columns were impregnated with $FeCl_3$ solutions at concentrations ranging from 0.002 N to 0.01 N. Color was measured before and after percolation (Table 9). At greater than 0.002 N concentration, the columns were overloaded and colored fluid passed through the sand. It might be supposed that at the higher concentrations, there were more Fe^{+++} ions than reactive sites (sand surfaces) at a specified time.

5. Column elution experiment - Because organisms were omitted in the ferric chloride experiment, and because the accumulated evidence seemed to indicate that an ion and/or bacterial exchange was taking place in the sand column, certain tests were made to elucidate this premise.

Table 9. *Removal of ferric chloride by sand filtration*

Concentration of $FeCl_3$	Percent Color and/or Turbidity Removed[1]
0.01 N	75[2]
0.005 N	90[2]
0.002 N	100[3]

[1] Measured after 24 hours standing with Klett-Summerson photoelectric colorimeter at 450 mμ.
[2] Measured after 800 ml passed through column.
[3] Measured after 800 and 3000 ml passed through column.

A column which had been used to remove S. marcescens from an ammonium nitrate suspending solution was allowed to drain overnight, a cover being put on its open end to prevent drying. Damp sand was dug out of the top and base of the column, weighed, and eluted with an aliquot of sterile distilled water. The data (Table 10) show that more bacteria per gram damp sand were trapped at the top of the column than at its base. Since the ammonium ion enhanced the removal of bacteria from the filtrate (Table 6), it would appear that NH_4^+ aided in the entrapment of bacteria - especially at the first surfaces (column top) contacted as they passed through the column. The data also show the ability of S. marcescens to maintain viability after 24 hours contact with sand and 0.001 N NH_4NO_3 at room temperature

Table 10. *Elution of bacteria from a column containing ammonium nitrate*[1]

Location	Bacteria per gm Damp Sand
Top of column	54×10^4
Base of column	3.4×10^4

[1] Approximately 350 gm damp sand eluted with 200 ml sterile distilled water.

C. Discussion

The character, quality and surface area of particulate matter, the type and concentration of ions in the bacterial suspending solution, and the strain of bacteria, itself, were factors influencing the removal of bacteria by filtration through particulate matter.

To effect bacterial removal, the particulate filtration medium must have a large surface area per unit of volume. Fine sand (0.26 mm diameter) fitted this criterion. Certain metallic cations in concentrations not uncommon to soils enhanced the removal of bacteria by filtration, as did the common fertilizer ion, NH_4^+. The anions Cl^-, $SO_4^=$ and NO_3 were without effect, while borate$^=$ and urea actually dispersed bacteria. Sodium ion markedly affected the removal of bacteria when used at a concentration of 0.01 N but was relatively ineffective at 0.001 N. Further, the viability of certain bacterial strains was limited in the presence of some common cations, while other microbes survived exposure to the same concentration of cation without ill effect.

In many ways, the system of column filtration resembled a model emulating surface or rain water passing through soil. For example, the high quality water of rain leaches ions and picks up organisms as it passes over soils. At the same time, the type and quantity of ions present may facilitate the removal of bacteria by soil particles. Thus, we are again presented with a complex natural system that contains a delicate system of checks and balances. However, from the data collected, it seems a reasonable conclusion that a weak bond exists between particle surface-cation-microbe and that this bond is easily destroyed or removed.

CHAPTER IV

ELECTROPHORETIC MEASUREMENTS

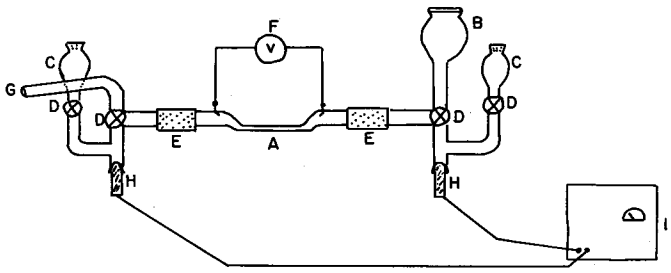
Measures of bacterial surface charge (zeta potential) in water and in the presence of ions were determined in order to elucidate the premise that a microbe-ion-sand surface complex exists. Zvyagintsev (1962) has shown that bacteria can be attracted to a soil particle, and it is conceivable that an ion may enter into a complex with the bacterial surface so that the microbe is more readily attracted to a particle - or even that an ion may form a bridge between the living cell and the sand particle.

A. Materials and Methods

1. Organisms and cultural techniques - Cultures of Escherichia coli, E. coli strain B, Serratia marcescens, and Staphylococcus aureus were obtained from the stock cultures of the Department of Microbiology, Colorado State University. Cells were grown in trypticase soy broth (BBL) for 18-24 hours at 35°C.

2. Preparation of cell suspensions - The ions of individual salts, the salt, its pH and concentrations used with cell suspensions are given in Table 11. The cells were harvested by centrifugation, washed twice with, and resuspended in the ion solution to be tested. For the electrophoretic studies, the prepared cells were suspended by visual turbidity comparison in the test solution at 10^6 and 10^7 cells per ml.

3. Electrophoretic equipment - Microelectrophoresis was conducted using a horizontal cataphoresis chamber and accessory glassware (Northrup-Kunitz apparatus from Arthur H. Thomas Co., Philadelphia) of which a diagrammatic sketch is given in Figure 10. The assembly was mounted such that, with the microscope in place for observation, the chamber rested firmly across the microscope stage. Current was applied across the chamber at 20-30 volts, depending on the relative velocity of the cells being observed.



A- CATAPHORESIS CHAMBER
B- BULB FOR CELL SUSPENSION
C- BULBS FOR ELECTROLYTE
D- STOPCOCKS
E- CONNECTING RUBBER TUBING
F- VOLTMETER
G- CHAMBER OUTFLOW
H- ZINC ELECTRODES
I- DC POWER SUPPLY

FIG. 10. DIAGRAMMATIC SKETCH OF THE CATAPHORESIS CHAMBER AND ACCESSORY EQUIPMENT.

Table 11. *Salt ions, compound, concentration and pH used in electrophoretic studies*

Ion	Compound	Concentration (Normality)	pH
--	Water	--	6.5
Na^+	NaCl	0.01	6.5
Cu^{++}	$CuCl_2$	0.001	5.4
Zn^{++}	$ZnSO_4$	0.001	5.8
Fe^{+++}	$FeCl_3$	0.001	3.3
$B_4O_7^=$	$Na_2B_4O_7$	0.001	8.9
Ca^{++}	$CaCl_2$	0.001	6.8
Mg^{++}	$MgSO_4$	0.001	6.8

Following the suggestions of Black and Smith (1962), the stationary plane for observations (that level at which movement of charged particles is not influenced by liquid flow due to the electric field) was used for all measurements.

The rate of movement of the various bacterial cells in the electrophoretic field was determined in the presence of the ions given in Table 11 using a binocular microscope for observations. The ocular grid of the microscope was calibrated using the 44X objective and 10X ocular for a 440X magnification. Timing of all movement was by stop watch.

NaCl studies were made over a wide pH range while other ion solutions were used at their "natural" (unmodified) solution pH, for pH adjustment with NaOH or similar material might have caused complexes capable of removing the test ion from solution; further, added NaOH would change the cation concentration of the test solution.

Response of each test organism, as mediated by its surface charge, to the selected substrates within the charged field was expressed as its zeta potential. The zeta potential of the cells was determined from the average velocities of the observed cells using distilled water as the control.

By definition, zeta potential (ζ) in millivolts (mv) is the linear movement in (cm/sec)/(volt/cm) multiplied by the Helmholtz-Smoluchowski factor. This factor equals 129,700 at 25°C, the temperature at which mobilities were determined (McBain, 1950).

B. Experimental Results

The results of the electrophoretic studies using 0.01 N NaCl as the suspending solution are shown in Figure 11. The zeta potentials of all the bacteria studied dropped sharply as the pH of the fluid environment was reduced below pH 5; the potentials of the organisms suspended in distilled water are shown as controls. In each case, the zeta potentials of the cells were considerably higher in distilled water than in 0.01 N NaCl.

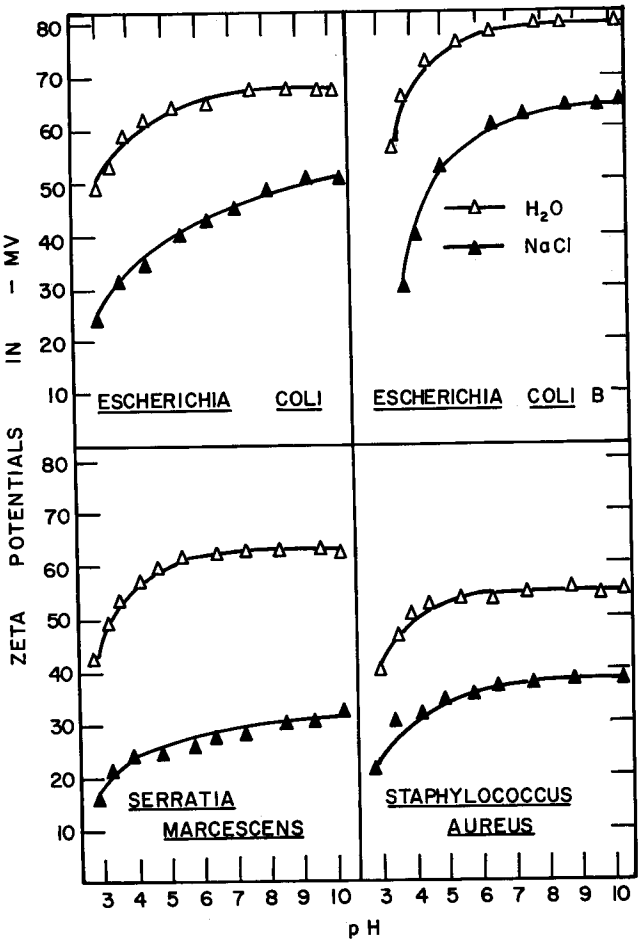


FIG. 11. EFFECT OF PH UPON ZETA POTENTIALS OF FOUR STRAINS OF BACTERIA IN 0.01 N NACL AND IN DISTILLED WATER.

The results of the electrophoretic studies using Cu^{++}, Zn^{++}, Fe^{+++}, $B_4O_7^{=}$, Ca^{++}, and Mg^{++} are given in Tables 12 and 13. It can be seen that the bacterial cells in 0.001 N Cu^{++} had zeta potentials (mv) of approximately half the values obtained in distilled water. Zn^{++}, Ca^{++}, and Mg^{++} also had a definite depressing effect on the zeta potentials of cells. The Fe^{+++} ion changed the potentials of the cells of all microorganisms tested from negative to positive.

If one were to summarize the data in a manner similar to that done for bacterial movement through particulate matter (p. 20), the following chart would result. For the purposes of this summary, the trivalent ferric ion was omitted and only divalent ions were compared. It was felt that there were too many variables to make a direct suspending solutions of di- and trivalent ions.

Organism	Rank in order of change in Zeta Potential over a distilled water control
E. coli	$Cu^{++}>Mg^{++}$, $Ca^{++}>Zn^{++}>Borate^{=}$
E. coli B	$Cu^{++}>Ca^{++}$, $Mg^{++}>Zn^{++}>Borate^{=}$
S. aureus	$Zn^{++}>Cu^{++}>Borate^{=}$
S. marcescens	$Cu^{++}>Zn^{++}>Borate^{=}$

From a comparison of the SFV (Salt Filtration Values) available (Tables 6 and 7) and the chart above, the following may be stated. There is fairly good correlation between the measured values of the zeta potential and the calculated SFV. For E. coli, only Mg^{++} is out of place. For E. coli B, Ca^{++} is out of place. The limited data available for Staphylococcus correlate well; in the case of S. marcescens, if one notes that the SFV for Cu^{++} and Zn^{++} were most similar, then these data can be considered as correlating well with the zeta potential values.

It is of interest to note that Davies, et al. (Davies, Haydon, and Rideal, 1956) working under conditions similar to ours, found comparable zeta potential values for E. coli in 0.008 N NaCl. When these workers used very dilute NaCl (0.0005 N), they calculated the zeta potential of E. coli to be about the same as the value obtained in our study in distilled water. When percolation through fine sand was performed with 0.01 N NaCl (Table 5), definite lowering of bacterial numbers was observed. However, at 0.001 N concentration (Tables 6 and 7), the effect of NaCl was not much different from distilled water percolation. Again, it appears that a correlation exists between percolation values and the zeta potential.

Table 12. *Alteration of bacterial zeta potentials in mv by selected cations at 0.001 N*

Organism	Distilled H_2O pH 6.5	Cu^{++}; pH 5.4		Zn^{++}; pH 5.8		Fe^{+++}; pH 3.3	
		R[1]	C[2]	R	C	R	C
E. coli	-65	-26	+39	-37	+28	+39	+104
E. coli B	-78	-37	+41	-54	+24	+73	+151
S. marcescens	-64	-34	+30	-42	+22	+58	+122
S. aureus	-55	-34	+21	-32	+23	+48	+103

[1] R = observed reading.
[2] C = change from reading in distilled water.

Table 13. *Alteration of bacterial zeta potentials in mv by selected ions at 0.001 N*

Organism	Distilled H_2O pH 6.5	$B_4O_7^{=}$; pH 8.9		Ca^{++}; pH 6.8		Mg^{++}; pH 6.8	
		R1	C2	R	C	R	C
E. coli	-65	-61	+ 4	-31	+34	-29	+36
E. coli B	-78	-84	- 6	-40	+38	-43	+35
S. marcescens	-64	-79	-15	--	--	--	--
S. aureus	-55	-42	+13	--	--	--	--

[1] R = observed reading.
[2] C = change from reading in distilled water.

C. Discussion

The main purpose of this work was to determine the effect of ions on the zeta potential of selected bacterial species and to correlate this data with that obtained when these bacteria were percolated through porous media. It has been shown that good correlation exists, i.e., the choice and concentration of an ion that will markedly lower the ability of a bacterial species to move through a column of fine sand will also give a definite depression of that microbe's zeta potential. This correlation holds true for most of the cases studied.

Ferric ion had far the most marked effect on bacterial zeta potentials. Although an excellent aid to particulate media filtration, it was not necessarily the very best of the ions tested. Also, the ion showed definite toxicity toward E. coli. It was the observation of Hewitt and Nicholas (1963) and Gurd and Wilcox (1956) that adsorption of di- and trivalent ions to bacterial cells involved chelate formation with groups on the cell surface, such as those present in proteins. When Fe^{+++} ions were bound at these sites, the negative sites were converted to positive ones, giving the cells a net positive charge. Adsorption of Fe^{+++} would probably occur at an intermediate step in ionization, such as occurs when the chloride ions dissociate. However, chelation may be only one of several ion adsorption mechanisms. Differences in the final positive charges of various test bacteria may have resulted from differences in the types of chelates formed. The fact that E. coli had the greatest positive charge of all the test bacteria may mean that its negative surface charges were more effectively neutralized.

It must be remembered, too that some of the ions tested, especially Cu^{++}, are well-known agents of protein denaturation. Since two of our test bacteria, S. marcescens and E. coli, exhibited toxic effects in the presence of Cu^{++}, Ca^{++}, Zn^{++}, and Cu^{++}, Fe^{+++}, Ca^{++} respectively, it is possible that denaturation of cellular protein was a factor in their changed potential.

E. coli was actively motile, while E. coli B was non-motile. Other surface differences undoubtedly could include differences in the receptive groups on the cellular surfaces. Differences in zeta potentials between these two strains were observed in all ion solutions used as suspending media.

Anions, such as the Cl^{-}, in the ion solutions had little effect on the zeta potentials of the test bacteria. Gurd and Wilcox (1956) state that this is because the dipole center of water can approach an anion

more closely than a cation; therefore, an anion would have considerably more water of hydration than would a cation of the same diameter. This would effectively separate an anion from a receptor site on the bacterial cell.

Borate$^{=}$, being large and with less concentrated charge compared to Cl^{-} , probably would get into proximity of positive charges of the cell and neutralize the depressant action of the Na^{+} ions also present in that solution. Perhaps the very alkaline pH of borate$^{=}$ solutions may be the most impressive factor in determining the action of borate$^{=}$ on zeta potential and sand percolation of bacteria.

CHAPTER V

SUMMARY AND CONCLUSIONS

The persistency (viability) of bacteria in soils has been shown to be related to several factors. Perhaps the most important for the two Larimer County soils tested are soil moisture content, presence of organic matter and/or available nutritional compounds, and the bacterial strain itself. Microbial predators were of no importance in these two soils. The microbial population explosion and its detrimental effect upon susceptible or "fragile" organisms (chiefly by competition for available carbon) appeared to be a major factor in the loss of viability of selected bacterial strains. Even so, enteric bacteria, under optimum conditions, could survive for long periods in soil.

Specific ions, in particular metallic cations and the ion common to most commercial fertilizers, NH_4^+, could, at 0.001 N concentrations, lessen or prevent the passage of bacteria through particulate matter. The "particulate matter" was, in this instance, fine sand and was used to simulate soils. Experimental evidence indicates that the presence of ions in percolating water would reduce the downward movement of bacteria through soil.

Solutions of these same cations were able to change the zeta potential (surface charge) of bacteria from that potential exhibited in a distilled water control. For a given bacterial species, the amount of zeta potential depression was shown to be, in most cases, directly proportional to the loss of bacterial mobility through porous media.

CHAPTER VI

RESEARCH IMPLICATIONS

It has become more and more apparent that the classic microbiological definition of "pollution" does not fully fill the needs of those concerned with the pollution of our water resources.

It is our suggestion that a new index of pollution or model be put forth. This "pollution index" would include other factors besides the presence of enteric indicator bacteria. In setting up a workable pollution index, we suggest that, among others, the following factors be given consideration.

1. The ability of a particular soil or environment to support microbial life - especially its ability to support the life of animal and plant pathogens as well as the enteric indicator microbes.

   a. The effect of added salts, in particular those common to fertilizers and surface waters, on the viability of microbes in a particular soil.

   b. The effect of moisture variations on a soil's ability to support microbial life.

   c. The effect of organic matter (plant debris, organic fertilizers, etc.) on a soil's ability to support microbial life.

2. By use of mobility studies, to determine if and at what rate specific bacteria may travel through the soil. A soil that can support the life of harmful microbes might not be considered a serious source of pollution if the bacteria were immobilized within the soil.

When and if suitable standards are worked out, it should be possible to state that Soil X, under normal conditions, can or cannot support the life of certain microbes, and if it can, for how long a time period. The effect of additions of moisture, carbohydrate, protein, inorganic nitrogen, etc., will be predictable.

One could even forecast the microbial leaching effect of irrigation water of known hardness. The availability of certain cations would curtail the mobility of many bacterial species. It is even conceivable that a badly polluted bit of land could be ion treated to lessen the mobility of bacteria. Eventually, many of the alien (not of soil origin) bacteria would die out in the soil and never become available as pollutants of sub-surface waters.

Whatever, there is a positive need for more definitive work on a pollution potential index of soil; the water resource scientist is in great need of such a tool.

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