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Title: Survival of Fecal Coliform Bacteria in Sludge-Amended Soils

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A study was conducted to determine the effects of temperature, solar radiation, rainfall, and saturated flow on fecal coliform numbers in sewage sludge-amended soils. To determine this, four 4.6 X 13.7 m field plots were each amended in summer and in winter with 800 liters of anaerobically-digested sewage sludge, and fecal coliform numbers monitored. In winter and in summer, fecal coliform numbers declined from $10^5$ to $<10^2$ and $<10^3$ cells/g of soil, respectively, in 13 weeks. In winter, saturated flow conditions accelerated fecal coliform decline by causing movement of the bacterial cells into the soil and groundwater. Under nonsaturated conditions, fecal coliform growth after a rainfall was observed for both sampling periods. Results suggest that growth was dependent on nutrient availability as well as moisture. The effects of solar radiation and tem-

perature could not be determined in winter because of fecal coliform losses to saturated flow, however their effect during the summer was slight. No differences in the die-off rate constants among the four field sites in winter and in summer were observed. The results suggest that movement of fecal coliforms during conditions of saturated flow should be considered in landspreading of sludge.

In a separate study, the effect of sludge crusting was studied. The formation of a sludge crust on a Dayton silt loam caused fecal coliform numbers in the 0-2.5 cm soil depth to remain at a constant $10^3$ cells/g of soil for 11 weeks while numbers in the sludge crust declined from $10^5$ to $10^3$ cells/g of soil during the same period. The results suggest that the sludge crust serves either as a continuing source of nutrients or fecal coliforms or both for the soil below.

A most-probable-number (MPN) microtitration technique for isolating fecal coliforms from soil was developed. A correlation coefficient of 0.86 was obtained when this technique was compared to the standard elevated-temperature fecal coliform MPN procedure. The advantages of this microtechnique are a substantial savings in time and media.

# Survival of Fecal Coliform Bacteria in Sludge-Amended Soils

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## INTRODUCTION

### A. General

Sewage sludge is the final precipitated solid matter produced by sewage treatment processes. It is usually a dark brown to black liquid containing 4-6% solids that, if thoroughly digested, is inoffensive in odor (Metcalf and Eddy, 1972). In 1970, the United States produced over 3 million metric tons of sewage sludge, and is projected to produce over 7 million metric tons by 1985 (CAST, 1976). In 1976, it was estimated that 20% of the 5 million dry metric tons of sewage sludge that was produced was applied to cropland (Bastian, 1977).

Land application of sewage sludge is not new: sewage "farming" in the United States was practiced in Wyoming, Colorado, California, Utah, and Montana as early as the late 1800s (Sepp, 1963). By 1973, over 1000 communities were landspreading sewage wastes (Sullivan, 1973). While sewage sludge has benefits as a soil conditioner and a source of many plant nutrients, it has several liabilities in that it is bulky and thus expensive to transport, and it may contain high concentrations of heavy metals and pathogens (Loehr et

al., 1979).

Pathogens are organisms capable of causing disease in humans and livestock. In sewage sludge, pathogens belong to the viruses, bacteria, protozoa, and helminths (Burge and Marsh, 1978). Although sewage treatment may reduce the number of all types of pathogens, sewage sludge still contains appreciable levels (Kabler, 1959; Foster and Engelbrecht, 1973).

Except in one case, no record exists in the United States of a disease outbreak definitely attributable to the application of secondary activated, chemically treated, or anaerobically-digested sewage sludge or effluent (Dunlop, 1968; Hoadley and Goyal, 1976; Menzies, 1977; Elliott and Ellis, 1977). In the one exception, 2500 cases of gastroenteritis were recorded when an overloaded sewage treatment plant irrigated a field with sewage effluent, some of which travelled down a gopher hole and contaminated a poorly-protected municipal water supply well (Browning and Mankin, 1966). Outside of this unusual case however, disease outbreaks have occurred only when raw wastes are placed on crops that are eaten raw too soon after waste application, or in the case of animals, on areas grazed too soon after raw waste application (Sepp, 1971; Geldreich and Bordner, 1971; Elliot and Ellis, 1977). Thus, only following lapses in good sanitation have problems occurred.

This study is only concerned with bacterial pathogens. The major bacterial pathogens in sewage are Salmonella spp.,

Shigella spp., and Mycobacterium tuberculosis (Burge and Marsh, 1978). In this study fecal coliforms were selected to represent bacterial pathogens. Fecal coliforms are a heterogeneous group of bacteria that ferment lactose at 44.5°C, and are found fairly specifically in the feces of warm-blooded animals (APHA, 1971). They are used as indicators of pathogenic bacteria because of the methodological difficulty in obtaining actual bacterial pathogens and because bacterial pathogens are usually in low numbers in the environment. Fecal coliforms, on the other hand, are easy to isolate and are considered the best indicator of bacterial pathogens (Geldreich, 1970; Geldreich and Bordner, 1971). Although fecal streptococci are also used as indicator organisms (Geldreich and Kenner, 1969) and were considered for this study, recent evidence suggests caution in the use of these organisms because of the difficulty in distinguishing indigenous streptococci from those of fecal origin (Kibbey et al., 1978). Evidence also suggests that fecal streptococci survive longer or shorter than fecal coliforms depending on the season (Van Donsel et al., 1967). Thus, only fecal coliforms were used here.

It is important that indicator organisms fulfill two functions: that they are a reliable guide in determining the absence or presence of a bacterial pathogen, and second, that their survival is relatively the same as that of the pathogen.

Of the three major bacterial pathogens found in sewage sludge, the most important are the Salmonella spp., which are responsible for several diseases including typhoid, paratyphoid and salmonellosis. Shigella spp. are rare in sewage (Foster and Engelbrecht, 1973) and concentrations of Mycobacterium tuberculosis are only 1% of the numbers of Salmonella spp. (Foster and Engelbrecht, 1973). While it is estimated that 1% of the human population excretes salmonellae at a given time (Anon., 1965), this percentage fluctuates with the rates of disease in the community (McCoy, 1971) as well as with season (CDC, 1974). Quantitative ratios between the presence of salmonellae and the presence of fecal coliforms have not been established for waste-amended soil, but have been established for sediments. Van Donsel and Geldreich (1971) were able to recover salmonellae from two-thirds of bottom sediment samples when the level of 200 fecal coliforms per 100 mls was reached in the overlying water. Smith and Twedt (1971) report similar findings in the Saline and Huron rivers of southeastern Michigan, as do Smallbeck and Bromel (1975) in animal waste lagoons.

The evidence that fecal coliforms survive the same as salmonellae in soil is contradictory. Salmonellae survival in soil or pasture is highly variable, ranging from less than a day (Bell, 1976) to 280 days (Mair and Ross, 1960) and even longer in some cattle slurry-soil studies (Thunegard, 1975). Thus, the consensus for soil (Morrison and Martin, 1977) is similar to that for water (McFeters et al.,

1974): some Salmonella strains have identical survival characteristics to fecal coliforms, while other strains are more resistant and other strains less resistant. Considering that fecal coliforms are generally thought to survive 2-3 months in the environment (Gerba et al., 1975), the quantitative ratios between fecal coliforms and salmonellae, and the high infective dose of samonellae necessary to cause illness ($10^5$ cells, DuPont and Hornick, 1973), it is the opinion of some authors (Morrison and Martin, 1977) that 2-3 months is sufficient time for bacterial pathogens to become negligible as well.

B. Factors affecting fecal coliform numbers in soil

The potential health risks associated with landspreading sewage have been extensively reviewed (Rudolfs et al., 1950; Benarde, 1973; Elliot and Ellis, 1977; Menzies, 1977; Burge and Marsh, 1978) and it is not the intent here to re-review them. The objective here is to detail the environmental factors responsible for survival of bacterial pathogens as represented by fecal coliforms.[1] An understanding of these factors will allow an understanding of the length of time that is necessary before sludge-amended land

[1]In considering each factor, it was recognized that the term "fecal coliform" is a recent development, and would preclude older literature which does not use this term. Because fecal coliforms are about 90% E. coli (IMViC++--, Geldreich et al., 1962a; Geldreich, 1966; Mishra et al., 1968), old and new literature referring to E. coli survival was considered as well. Other non-E. coli fecal coliforms were not considered.

is considered safe for human or livestock use, and potentially reduce this time is conditions are optimal for die-off.

The factors believed to affect fecal coliforms in soil are shown in Table 1. These factors have been superficially reviewed before (Gerba et al., 1975; Parson et al., 1975; Wray, 1975; Morrison and Martin, 1977) and, in two cases, have been used in predicting pathogen die-off (Crane et al., 1980; Reddy et al., 1981). These factors are now considered individually.

1. Biotic factors

a. Antibiotics and toxic substances

Depite claims to the contrary, no conclusive evidence exists that antibiotics are produced in nonsterile soil, and it is unlikely that this factor has any major effect on fecal coliform numbers. Waksman and Woodruff (1940a, 1940b) observed that E. coli numbers increased in sterile soil but decreased in nonsterile soil. The latter was accompanied by an increase in antibiotic-producing organisms, notably actinomycetes. Although laboratory medium filtrates had a bactericidical effect on E. coli, this was never demonstrated in nonsterile soil. Likewise, Soulides (1965) demonstrated the production of streptomycin, terramycin, and aureomycin in two clay loam soils inoculated with the appropriate Streptomyces species, but antibiotic production was only demonstrated in sterile soil with various organic

TABLE 1. FACTORS AFFECTING FECAL COLIFORM NUMBERS IN SOIL.

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|---|
| 1. Biotic |
| a. antibiotics and toxic substances |
| b. parasitism and predation |
| c. competition |
| 2. Abiotic |
| a. moisture |
| b. soil texture |
| c. sunlight |
| d. temperature |
| e. pH |
| f. nutrients |
| g. waste application |
| h. leaching and runoff |
| i. outside sources of contamination |

amendments. In the same nonsterile, unamended soils, no antibiotics were detected. Similar results were also obtained by Grossbard (1952) in a sterilized amended soil inoculated with fungi.

In the case of toxic substances, Hatsuda et al. (1965) reported that psoralen, benzoic, salicylic, vanillic, and p-coumaric acids, all derived from plant roots, inhibited E. coli growth at minimum concentrations of 0.5 to 1.0 mg/ml in vitro. However, Guenzi and McCalla (1966) observed that various extracts of soil yielded p-coumaric, p-hydroxybenzoic, and vanillic acids only in concentrations of <1 μg/g. Thus, only small amounts of these phenolic acids would exist freely in the soil and are therefore unlikely to be of any consequence in fecal coliform survival.

b. Predation and parasitism

Although microbial predation and parasitism are major factors in the biological control of fecal organisms in marine sediments (Mitchell et al., 1967; Enzinger and Cooper, 1976; McCambridge and McMeekin, 1980, 1981), this has not been conclusively shown for soil. In two studies, Klein and Casida (1967a, 1967b) observed no major role for bacteriophage or Bdellovibrio bacteriovorus in eliminating E. coli from soil whether the soil was amended with glucose, sewage effluent, or left unamended. Tate (1978) implicates protozoan predation in the decline of E. coli in histosols but no supporting data are shown. Protozoan predation in

soil is in any case probably prey density-dependent (Habte and Alexander, 1975) and it is probable that the physical separation in the soil as well as the soil type affords protection. In aquatic sediments, Roper and Marshall (1978) showed that montmorillonitic clays partially inhibited the parasitism of E. coli by Bdellovibrio because the clay formed a protective colloidal barrier. The same case is likely to exist in soils because the same protective effect has been demonstrated for Rhizobium and Bdellovibrio (Keya and Alexander, 1975).

c. Competition

The role of competition is probably important in the survival of fecal coliforms in soil, but the role is difficult to separate from nutrient availability because all the studies on competition are comparisons of sterile and nonsterile soils. Furthermore, except in one case, competition is not distinguished from parasitism and predation. In the one exception, Boyd et al. (1969) compared survival of $10^9$ E. coli cells/g of soil in a nonsterile sandy loam and the same soil sterilized by autoclaving. After 14 days, numbers of E. coli had declined to $10^8$ and <10 for the sterile and nonsterile soil, respectively. Attempts to enumerate protozoa, Bdellovibrio, and other predatory organisms in the nonsterile soil were unsuccessful. Therefore, in the sterile soil, the difference in survival was attributed to increased nutrient availability and lack of competition.

Until more definitive studies are done however, the actual importance of the role of competition remains unclear.

2. Abiotic factors

a. Moisture

Moisture is one of the largest single factors affecting fecal coliform survival in soil. With one exception, optimum survival is in moist soil; flooded or dry soils cause marked declines in fecal coliform numbers. Young and Greenfield (1923) noted that E. coli numbers dropped rapidly in sterile and nonsterile soil at 100 and 60% saturation, but dropped more slowly at 40, 20, and 10% saturation. Similar results were obtained by Chandler and Craven (1980) who observed optimal survival of E. coli in a loam soil with moisture levels between 15 and 60%. Extreme moisture (greater than 90%) or extreme dryness (less than 8% moisture in a soil with a wilting point at 11% moisture accelerated the decline of numbers rapidly. One exception is the survival of E. coli in histosols: anaerobically-grown E. coli survives much better in flooded soils than in moist soils, while aerobically-grown cells survive about the same in both (Tate, 1978).

While it is clear that fecal coliform numbers decline rapidly when applied to dry soil, the surviving fecal coliforms tend to persist for long periods once they are in a desiccated state and moisture remains unavailable. Evidence for this was first noted by Harry (1964) in the dust of

poultry houses. In this study, E. coli showed no appreciable decline from $10^5$ cells/g of dust for 32 weeks if the relative humidity remained low (8.3 to 11.4%). When the relative humidity was increased to near saturation levels, however, the numbers were reduced by 90 to 98% within 10-14 days. A follow-up study showed similar results (Harry and Hemsley, 1964). In soil, Chandler and Craven (1978) observed a decline from $10^5$ to $10^2$ cells/g of soil in one day when E. coli was applied to dry soil. During the dry weather that followed however, the remaining cells persisted for an additional eight weeks before disappearing. Desiccation might also explain the long survival periods of E. coli in the moisture experiments of Young and Greenfield (1923). Because of the interruption of World War I, the moisture contents of their soils were not adjusted and, after the war, although all the soils were "very dry," E. coli could still be isolated.

The number of cells surviving desiccation in soil is probably directly related to the rate of drying. Although this has not yet been conclusively demonstrated for fecal coliforms, the rate of drying is critical in the survival of Pseudomonas in soil (Zechman and Casida, 1982) and fecal coliform survival was prolonged when poultry manure formed a crust on soil and decreased water evaporation (Crane et al., 1980). This might also explain the differences in fecal coliform survival rates between sandy and heavier-textured soils when the soils are allowed to dry: fecal coliforms

die faster in sandy soils because the rate of drying is faster.

b. Soil texture

The data on the effect of soil texture on fecal coliform survival are contradictory unless soil moisture is considered. In experiments where moist soils were allowed to dry, soil texture was important in that it governed moisture holding capacity and probably the rate of drying as well. Soils with high clay or organic matter, which have higher moisture-holding capacities (Brady, 1974), show better fecal coliform survival. However, in experiments where moisture or desiccation is held constant, differences in soil texture disappear.

In studies where the soil was allowed to dry, Mallman and Litsky (1951) observed that coliforms survived about the same in one muck and three loam soils after 11 weeks, but survival was much better in these soils than in a sandy soil run concurrently. In another study involving two sterile soils, E. coli declined from $10^7$ to approximately $10^2$ cells per g of soil in 4 months in a loam soil compared to 6 months in a sandy loam (Papavassiliou and Leonardopoulos, 1978). Subsequent soil analysis showed that the soils were similar except that the sandy loam had a higher organic matter content. In a study where soil moisture was kept constant, Glathe et al. (1963) noted no difference in survival of E. coli between a sandy soil and a loam soil. Moisture

in this experiment was held at 60% of field capacity. In a field study, Temple et al. (1980) observed no differences in the survival of E. coli inoculated into feces and buried in three mineral and two organic soils. In this particular study, the soils had low moisture levels. In a soil with more organic matter, Tate (1978) showed that the survival of E. coli was threefold greater in a histosol than in a fine sand, although the soil moisture levels remained at close to 0.1 bar soil moisture tension for both soils because of the short 8-day incubation period. Although this seems to contradict the idea that soil textural differences are irrelevant if soil moisture is held constant, a closer examination of the data reveals that the death rates of E. coli in the two soils are similar and that the apparent differences are related to an intial 3-day period of no mortality for the E. coli population in the histosol. This is more likely to be a case of higher nutrient availability in the histosol than differences in soil type. Finally, in a field study where moisture fluctuated as well as remained constant for long periods, Van Donsel et al. (1967) observed no differences in E. coli survival between a coarse loam soil and a dense clay soil during the winter and spring months when both soils were consistently moist, however in summer and autumn E. coli survived better in the loam soil than in the clay. In this case, the loam soil had a consistently higher moisture content during these periods than the clay soil. Thus, soil textural differences may be soil

moisture-holding differences.

c. Sunlight

Although visible and near-ultraviolet light are known to have mutagenic and lethal effects on bacteria (Eisenstark, 1971), it is difficult to single visible and ultraviolet light out as responsible for bacterial mortality because the amounts of light are small, particularly in temperate climates (Wray, 1975). It is more probable that the effect of sunlight is through rapid drying. This would explain a large portion of contradictory data. Fecal coliforms, then, would die rapidly on exposed plant tissue, but would persist if the plant had sections where moisture was conserved, or if the bacteria succeeded in reaching the soil, where the soil would slow moisture loss. The evidence for this is good.

Falk (1949) noted no difference in coliform numbers on normal tomatoes under exposed and shaded conditions. However, when the tomatoes were "abnormal," that is, had cracked stems, the number of coliforms was higher in the shaded tomatoes than the exposed ones. Rudolfs et al. (1951) obtained similar findings. Taylor and Burrows (1971) noted that on pasture sprayed with cattle manure slurry inoculated with $10^6$ E. coli cell per ml, no E. coli could be detected after one day on grass greater than 7.6 cm tall, after three days on grass between 2.5-7.6 cm tall, after seven days on grass between ground level and 2.5 cm, and

after nine days in soil. Similar results were observed by Bell (1976) where fecal coliforms declined from $10^3$ to <2 cells per g dry plant tissue on sewage-irrigated alfalfa following 10 hours exposure to bright sunlight. This time was extended to 50 hours for reed canary grass (Bell and Bole, 1976). In the latter case, the leaf sheaths of this grass presumably collect and protect the effluent from sunlight, although this was not tested. The top 2.5 cm of soil under the sewage-irrigated grass however still contained 5 X $10^3$ fecal coliforms/g of soil on the second day. These findings were reiterated in a study by Chandler and Craven (1978) who observed that E. coli declined slowly from 3 X $10^4$ to 8 X $10^3$ cells per g in three weeks on surface soil under pasture sprayed with piggery effluent while numbers declined to undetectable levels on the grass above. Magnusson (1974) noted that coliforms remained viable for approximately 14 days on plant samples during dry weather compared to 7 days during rainy weather. Although this appears to be a contradiction to the effect of sunlight, the discrepancy is because of the washing action of rainfall on the vegetation. Unfortunately, this cannot be ascertained conclusively because the soil beneath the vegetation was not sampled.

In soil, Van Donsel et al. (1967) showed that E. coli survived twice as long on a protected hillside site during summer and autumn compared to an exposed lawn site. Because the survival of E. coli was similar for both sites during

the winter and spring, they suggested that reduced solar radiation during the winter and spring was a partial explanation. This is probably not the case; more important is their mention that the soil moisture of the hillside site was consistently higher and subject to less fluctuation than the lawn site during the summer, but little difference was observed during the other seasons. This would explain why Chandler and Craven (1978) observed no differences between shaded and unshaded clay loam sprayed with E. coli inoculated piggery effluent: the moisture content of the soil was the same for both field plots.

Thus, the major effect of sunlight is probably one of desiccation rather than bactericidal effect of ultraviolet light.

d. Temperature

In water between 0 and 15°C, the survival of E. coli is inversely related to temperature, however above 15°C, this relationship is less critical (McFeters and Stuart, 1972). This relationship also appears to be the case for soil. Ostrolenk et al. (1947) observed that in an unfertilized soil, E. coli survived 7 and 66 days at room temperature and 7.2°C, respectively. Nutrients afford greater protection: in the same soil that was fertilized, E. coli survived 66 and 109 days, respectively. This finding was also noted by Klein and Casida (1967b) who observed that the die-off rates of E. coli increased with an increase in incubation tempera-

ture, but that the addition of 0.25% glucose afforded some temperature protection, particularly at 37°C. In sterile soil, survival also increases with decreasing temperature. Thus, E. coli with an initial inoculum of $10^7$ cells per g of soil survived 6 months at 30°C, but 24 months at 12°C (Natvig and Nilsen, 1955).

Although lowered temperature such as winter conditions usually lead to lengthened survival, the effect of freezing temperatures is deleterious. Unfortunately, actual temperatures are usually not given. On decaying wood amended with feces, E. coli declined from $10^5$ to 0 cells per g of wood in 61 days in winter, but the same numbers persisted 153 days in summer (Tonney and Noble, 1931). The authors attribute the increase in mortality in winter to "very cold" conditions. Although often cited as evidence of the environmental effects of freezing on E. coli, the work of Weiser and Osterud (1945) was done in peptone or peptone-buffer medium and not representative of soil. Their results however are similar in that freezing or freeze-thaw cycles always resulted in some E. coli mortality. Similar results of E. coli in water have been reported (Ray and Speck, 1972, 1973). In sterile soil subjected to "severe frost," E. coli numbers declined rapidly compared to soil kept at 12°C (Natvig and Nilsen, 1955). Van Donsel et al. (1967) also observed a decline in coliform numbers under subfreezing conditions. Hirte (1977b) observed that E. coli declined at a slower rate at 4°C than at 20°C, but if the soil was

frozen to -2°C and then warmed, E. coli numbers dropped rapidly without recovery.

Thus, the effect of freezing temperature is deleterious, while at above-freezing temperatures, survival is inversely proportional to temperature.

e. pH

The consensus is that fecal coliform survival is better in neutral pH soils than in soils with relatively higher or lower pHs. Cuthbert et al. (1955) demonstrated that E. coli died out rapidly in an acidic peat of pH 2.9-3.7, but if the pH was increased to pH 5.6-7.3 with the addition of chalk, then E. coli persisted for 110 days. In the case of waste amendment, Crane et al. (1980) did not observe any differences in the survival of fecal coliforms in two soils heavily amended with poultry manure despite changes of pH 4.44 to pH 6.51 for one soil, and pH 5.45 to pH 8.21 for the other. In a study with a declining soil pH, the addition of 1% glucose every three days to a neutral soil inoculated with E. coli resulted in a stable E. coli population until pH 5.7 was reached, whereupon E. coli numbers began to decline (Klein and Casida, 1967b). Van Donsel et al. (1967) also noted that soil pH changes in the range of pH 7.2 to 8.5 had little effect on fecal coliform survival.

f. Nutrient availability

Nutrient availibility is another one of the largest single factors affecting fecal coliform survival in soil. Revis (1910) first observed that E. coli survived <30 days when inoculated into sterile "virgin" soil, but in the same soil amended with sterile (a) cow dung, peat, and $CaCO_3$, (b) cow dung, or (c) feces, E. coli survived 150, 180, and 300 days, respectively. Although Revis was not concerned with E. coli survival, it is likely that the availability of nutrients was responsible for lengthened survival.

It is apparent that nutrient availability refers to a readily metabolizable source of organic carbon rather than other nutrients or inorganic constituents. Thus, in a non-sterile silty clay loam to which E. coli was added, Klein and Casida (1967b) observed that E. coli declined from $10^6$ to $<10^2$ cells/g of soil at 28 days if no glucose was added, but declined to $10^4$ cells/g of soil if either 0.25 or 0.50% glucose was added. The addition of ammonium nitrate had no effect. Similarly, Boyd et al. (1969) reported that the additions of lactose and glucose to a sandy loam were conducive to E. coli survival, but not the addition of phosphate or yeast extract. In another experiment, no differences in survival of E. coli was noted in two sandy loams differing widely in sulfate, magnesium, sodium, or potassium ions even upon addition of ammonium sulfate and calcium nitrate (Boyd et al., 1969). So while it is apparent that the concentration of inorganic constituents is important to the survival

of E. coli in natural waters (McFeters and Stuart, 1972), soils probably contain sufficient ions for cell maintenance.

It was suggested by Klein and Casida (1967b) that the inability of E. coli to survive in soil was because of its inability to step down its metabolic rate to meet the lower levels of available carbon in unamended soils. This was based on the difference in the growth rate of E. coli recovered from soil compared with the growth rate of the same strain kept in laboratory medium. However, the differences are small and no statistical data are presented to show that the growth rates are significantly different. Chandler and Craven (1980) observed no differences in the survival of E. coli in a loam soil if the cells were stored for 7 days at 4°C before inoculation (low metabolic rate) versus cells stored for 7 days at a higher temperature (high metabolic rate). Thus, while it is clear that E. coli survives better when sources of carbon are available, it is unclear whether or not the cells cannot survive under low carbon availability because their metabolic rates cannot be stepped down to avoid starvation.

g. Waste application rate

From studies of nutrient availability, it is reasonable to assume that high application rates of sludge would be conducive to fecal coliform survival. The evidence is contradictory.

On one hand, Dazzo et al. (1973) noted an inverse relationship between the death rates of fecal coliforms and the previous rate of cow manure irrigation, so that fecal coliforms had the highest death rate in unamended soil, while stabilizing in soil that received continued waste application. Smallbeck and Bromel (1975) observed similar findings: the death rates of E. coli were reduced in half upon reinoculation of a wastewater-amended clay soil. On the other hand, McCoy and Crabtree (1970) noted no difference in coliform survival among field sites that received the equivalent of 33.6, 179.2, or two sequential 33.6 metric tons of cow manure/ha, as did Hirte (1977a) with E. coli in soil receiving the equivalent of 40, 1720, 2600, or 3520 metric tons of cattle manure/ha, regardless of whether these applications were repeated or not. Crane et al. (1980) noted no differences in the death rate constants of fecal coliforms in two soils amended with the equivalent of 36.5 and 164 metric tons of poultry manure/ha. What is noteworthy in the cases of McCoy and Crabtree (1970) and Crane et al. (1980) is that the application rates were such that the authors commented on the formation of a crust on the soil surface. It is possible that the formation of a crust minimizes moisture loss, thus minimizing differences in bacterial survival.

Given the contradictory evidence of fecal coliform survival depending on application rate, it is not surprising that the effect of fecal coliform inoculation density is

also contradictory. Thus, the death rate of E. coli was 100-fold faster at an initial density of $10^7$ cells/g of soil than at $10^5$ cells/g of soil after 6 days in a histosol (Tate, 1978), while no differences were reported in the rates of decline of E. coli in nonsterile soil amended with cattle manure slurry with an initial cell density of $10^8$, $10^6$, $10^5$, or $10^4$ cells/g of soil (Hirte, 1977b).

h. Leaching and runoff

Losses of fecal coliforms through runoff and leaching have been well-summarized elsewhere (Reddy et al., 1981). These are now considered separately.

In leaching, the removal of bacteria is inversely proportional to the particle size of the soil, regardless of soil moisture (Butler et al., 1954). This removal is a combination of filtration and adsorption, the soil particle-cation-microbe bonding. Filtration is reasonably well understood, but adsorption is not. Adsorption appears to be related to the character, quality, and surface area of the soil particulate matter, the type and concentrations of the soil ions, and the bacterial strain itself (Boyd et al., 1969). Thus, Chudiakow (1926) observed that of seven different species of bacteria, E. coli had the lowest adsorption in soil (11.5%) compared to the higher adsorption (40.5 to 95.5%) for the other six species. Similar low adsorption for E. coli was observed by others (Guy and Visser, 1979). The distinction between filtration and adsorption is usually

not made however, because microorganisms in nonsaturated soils are effectively trapped in the soil within a few meters of the soil surface in any case. Mărculescu and Drocan (1962), in studies using $^{32}P$-labelled coliforms, observed that 92-97% of the bacteria were retained in the top 1-cm soil layer, and 3-5% in the 1-5 cm layer. McCoy and Crabtree (1970) observed >95% of the coliforms in a manure-amended silt loam were retained in the top 36 cm of soil. In a percolation study, coliform bacteria on the ground surface were reduced from 582 cells/100 ml to an average of 1 cell/100 ml at the 52- and 102-cm soil depth (Glantz and Jacks, 1967).

Movement of bacteria under saturated flow conditions is also well summarized elsewhere (Romero, 1970; Gerba et al., 1975; Hagedorn et al., 1981), however these studies are concerned with the movement of bacteria in soil under septic tank effluent loading or in sewage wastewater infiltration beds rather than agronomic uses of landspreading sludges where application rates and fecal coliform densities are much lower. Thus, movement of bacteria under the latter conditions is largely unknown.

Basically, under saturated flow, an instability in the filter zone occurs, causing a cascade of organisms to advance at the front of the saturated zone (Gerba et al., 1975). The microorganisms continue to be removed at a rate that is inversely proportional to the particle size of the soil until the capacity for straining is reached whereupon

only sedimentation is operative (Romero, 1970). Therefore, under saturated conditions, microorganisms can move great distances. Rahe et al. (1978) observed movement of E. coli up to 1,500 cm/hr injected into the subsurface horizon of one saturated soil, and Hagedorn et al. (1978) showed similar findings.

Runoff, like adsorption, is also poorly understood (Reddy et al., 1981), however it appears the loss of microorganisms through runoff is slight, varying according to length of time since waste application and season. Van Donsel et al. (1967) were able consistently to isolate E. coli from an inoculated hillside site during periods of heavy rainfall during the spring, but only sporadically during the summer and autumn. No numbers were given. When the soil was allowed to dry out, or when bacterial numbers dropped to $<10^4$ cells/g of soil, no organisms were obtained in the runoff. Similar findings were also reported by Dunigan and Dick (1980). In the latter study, fecal coliform losses were a maximum 1.0 to 1.2% for the first two to three weeks after sludge amendment and then declined sharply. The percentages are of interest since Chandler and Craven (1978) report similar maximum losses of 2.5 to 3.5% of E. coli in runoff water from piggery effluent-amended field sites. Again, maximum losses occurred early, in this case, 24 hours after application.

i. Outside sources of contamination

In field trials involving waste amendment, it is important to identify outside sources of contamination. It is apparent however that this contamination is slight. Undisturbed soil rarely contains E. coli (Fraser et al., 1956) or fecal coliforms (Geldreich et al., 1962b). Glantz and Jacks (1967) suggested that birds might be attracted to field sites amended with wastewater effluent because of lush vegetation and high moisture, but they did not observe transmission of any E. coli serotypes from wild birds trapped in the disposal area. In another study, Geldreich et al. (1964) checked the incidence of fecal coliforms on 152 species of plants and 40 samples of insects. Plants had only low levels of fecal coliforms, whereas values for insects ranged from <20 to 79,00 fecal coliforms/g. The latter value however was only observed in Coleoptera (beetles). Given the likely low density of Coleoptera in the field however, the general conclusion is that numbers of fecal coliforms derived from insects would be small. Chipmunks, rats, rabbits, dogs, cats, and raccoons are also carriers of fecal coliforms (Geldreich et al., 1968) but their effect on field trials is unknown. It is reasonable to assume, that unless numerous, their effect would be small.

C. Growth of fecal coliforms in soil

Under certain conditions, fecal coliforms may show growth in soil; these conditions are in 1) sterile soils, 2)

in soils newly-amended with wastes or nutrients, and 3) in soils following a rainfall.

In sterile soils, there are numerous reports of regrowth of E. coli or fecal coliforms. Because sterile soils are virtually nonexistent in nature, these cases are not considered here. It is noteworthy that in these studies regrowth was always attributed to the lack of bacterial competition or increased nutrients, or both.

In soils amended with fresh sludge, E. coli increased slightly from 8 X $10^5$ to 2.3 X $10^6$ cells/g after 30 days for both a loamy soil and a sandy soil (Glathe and Makawi, 1963). Similarly, McCoy and Crabtree (1970) noted a slight temporary growth of coliforms on field sites three days after amendment with cow manure. Although the authors did not comment on it, it is noteworthy that 38 mm of rain fell the second day following amendment, thus high moisture conditions existed. E. coli increased slightly in numbers in a sandy soil amended with sterile manure (Hirte, 1977b). Crane et al. (1980), in a laboratory study involving a clay loam amended with poultry manure kept under constant moisture, reported a regrowth of fecal coliforms 7 days after an initial die-off period. All of these studies point to the necessity of moisture: increases are only observed directly after bacterial application when moisture conditions were high or if moisture conditions are kept constant. There are no reports in the literature of E. coli or fecal coliforms showing growth in desiccated soils.

With regard to rainfall, Van Donsel *et al.* (1967) and Chandler and Craven (1978) observed increases in coliforms or *E. coli* following a rainfall. Temple *et al.* (1980) observed initial regrowth of *E. coli*-inoculated feces in all five different field sites where the feces was buried before a decline in cell numbers occurred. Although the field sites differed widely in soil moisture, the authors suggested that the feces, in the form of a slurry, had sufficient moisture to nullify water differences among the sites and thus regrowth was possible.

With two exceptions, it is apparent from the aforementioned examples that growth of *E. coli* or fecal coliforms usually occurs within a week or two of soil amendment. This suggests that nutrient availability is critical; once nutrients are low, regrowth is not observed. The two exceptions to this are an unusual soil amendment or soil moistening after a period of desiccation. Van Donsel *et al.* (1967) observed regrowth of coliforms after a rain as much as two or three months after inoculation. An examination of their methods shows that laboratory medium (Brain Heart Infusion) was used to amend the soil, so perhaps this is atypical. In a second exception, Chandler and Craven (1980) noted a 13-fold increase in *E. coli* from $1.9 \times 10^3$ to $2.6 \times 10^4$ cells/g of soil in 2 days after the soil was dried for 14 days and then remoistened. Although the authors offer no explanation, it is possible that rewetting the soil causes organic carbon to be released and thus high nutrient conditions

exist. Such an observation has already been made for Rhizobium in desiccated soils (Pena-Cabriales and Alexander, 1983).

D. Methodology in studies of fecal coliforms

Fecal coliforms are clearly capable of surviving several months in waste-amended soils. In some cases, the effect of an environmental factor is clear (e.g., pH), while for another it is contradictory (e.g., waste application rate) or yet inconclusive (e.g. predation). Unfortunately, understanding these variables is further complicated by methodology. The relative importance of each environmental factor is difficult to assess under field conditions, so most investigations are conducted under laboratory conditions involving only a few variables. Of 34 investigations involving the survival of fecal coliforms or E. coli in soil or pasture, 24 (71%) were conducted in the laboratory (Table 2). Of these studies, 17 (71%) used water or laboratory medium; only 7 used actual waste. Eight studies, most before 1963, involved only sterile soil. Even of 10 investigations involving field conditions, 3 used water or laboratory medium in place of waste. Van Donsel et al. (1967), who used laboratory medium to inoculate soil under field conditions, caution that survival of bacteria grown in artificial medium might be different from wild-type strains found in feces. Precedence for this exists: Tonney and Noble (1931) reported that E. coli isolated from fecal

TABLE 2a. SELECTED CHRONOLOGICAL LITERATURE REVIEW OF THE SURVIVAL OF E. COLI OR FECAL COLIFORMS IN SOIL.

| Author | Year | Lab or field | Waste | | Soil | | Log no. cells/g of soil | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | type | amount | s/n[†] | amount | initial | final | days |
| Horrocks | 1903 | lab | water | NG[‡] | n | NG | NG | NG | 90 |
| Savage | 1905 | lab | tapwater | NG | s | NG | 4-5 | 2-3 | 42 |
| Revis | 1910 | lab | water | 5 cc | s | 1/2 test tube | NG | NG | 150 |
| Koser | 1924 | lab | distilled water | 1 cc | s | 1/2 test tube | 6 | 2-4 | 120 |
| Young and Greenfield | 1923 | lab | saline | 1 cc | n | 200g of soil | NG | NG | 517 |
| Jordan | 1926 | lab | feces | NG | n | NG | 9 | 0 | 133 |
| Skinner and Murray | 1926 | lab | cow manure | 2 g | n | 200g | 5 | 0 | 136 |
| Kulp | 1932 | lab | laboratory medium | 1 cc | s | 1/2 test tube | NG | NG | 1305 |

[†]sterile/nonsterile; if both, then nonsterile listed

[‡]not given

(continued)

TABLE 2a (CONTINUED).

| Author | Year | Lab or field | Waste | | Soil | | Log no. cells/g of soil | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | type | amount | s/n | amount | initial | final | days |
| Kline | 1935 | lab | water | 1000 ml | s | 5 X 610 cm pipe | NG | NG | 410 |
| Waksmann and Woodruff | 1940a,b | lab | tapwater | NG | n | 1000 g | 3 | few | 10 |
| Ostrolenk et al. | 1947 | lab | chicken manure | NG | n | 2.26 kg | 7 | 2 | 160 |
| Bardsley | 1948 | field | water | 350 ml | n | 2 $m^2$ | 7 | 0 | 120 |
| Mallman and Litsky | 1951 | lab | sterilized sewage sludge | NG | n | 100 g | 5 | 3 | 77 |
| Cuthbert et al. | 1955 | lab | Ringer's solution | NG | n | 100 g | 5-6 | 7 | 110 |
| Natvig and Nilsen | 1955 | lab | nutrient broth | 5 ml | s | 14 g | 7 | 0 | 730 |
| Glathe et al. | 1963 | lab | water | 50 ml | n | 500 g | 3 | 0 | 120 |

(continued)

TABLE 2a (CONTINUED).

| Author | Year | Lab or field | Waste | | Soil | | Log no. cells/g of soil | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | type | amount | s/n | amount | initial | final | days |
| Klein and Casida | 1967b | lab | tapwater | 4 ml | n | 100 g | 7 | 7 | 7 |
| Van Donsel et al. | 1967 | field | laboratory medium | 1500 ml | n | 1 $m^2$ | 6-8 | 1-3 | 65 |
| Boyd et al. | 1969 | lab | distilled water | NG | n | 10 g | 10 | 4 | 40 |
| McCoy and Crabtree | 1970 | field | cow manure slurry | equiv. 180t/ha | n | approx. 0.05 $m^2$ | 5 | <3 | 21 |
| Dazzo et al. | 1973 | both | cow manure slurry | 5.08 cm | n | 0.40 ha | 5 | 2 | 56 |
| Miller | 1974 | lab | freeze-dried sewage sludge | equiv. 224t/ha | n | NG | 3 | 0 | 90 |
| Smallbeck and Bromel | 1975 | field | lab. medium + sterilized lagoon effluent | 500 ml | n | 0.19 $m^2$ | 10 | 1 | 31 |

(continued)

TABLE 2a (CONTINUED).

| Author | Year | Lab or field | Waste | | Soil | | Log no. cells/g of soil | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | type | amount | s/n | amount | initial | final | days |
| Hirte | 1977a | lab | cattle manure slurry | equiv. 3,500 t/ha | n | 200 g | 8 | 0 | 56 |
| Hirte | 1977b | lab | tapwater | NG | n | 250 or 500 $cm^3$ | 8 | 0 | 56 |
| Papavassiliou and Leonardopoulos | 1978 | lab | saline | 5.0 ml | s | 100 g | 6-8 | 2 | 180 |
| Tate | 1978 | lab | mineral salts | NG | n | 10 g | 7 | 5 | 8 |
| Guy and Visser | 1979 | lab | laboratory medium | NG | s | NG | 7 | 8 | 60-90 |
| Chandler and Craven | 1980 | both | 0.1% peptone | 0.1 ml | n | 100 g | 5 | 4 | 21 |
| Crane et al. | 1980 | lab | diluted poultry manure | 0.33 kg | n | 0.09 $m^2$ | 7 | 6 | 30 |

TABLE 2b. SELECTED CHRONOLOGICAL LITERATURE REVIEW OF THE SURVIVAL OF *E. COLI* OR FECAL COLIFORMS IN PASTURE.

| Author | Year | Lab or field | Waste | | Pasture Size | Log no. cells/g of soil or plant tissue | | |
|---|---|---|---|---|---|---|---|---|
| | | | type | amount | | initial | final | days |
| Taylor and Burrows | 1971 | field | cattle manure slurry | 37.9 liter | 8.36 $m^2$ | 6 | 0 | 7 |
| Bell | 1976 | field | sewage lagoon effluent | 7.5 cm/ plot | 150 $m^2$ plot | 3 | 0 | 0.4 |
| Bell and Bole | 1976 | field | sewage lagoon effluent | 4.5 cm/ plot | 150 $m^2$ | 3 | 0 | 2.1 |
| Chandler and Craven | 1978 | field | piggery effluent | 400 kl | 0.2 ha | 5 | 3 | 13 |

suspensions survived 61 days when put on decaying wood, whereas E. coli from culture survived only 9 days under similar conditions. Also, Tate (1978) demonstrated that anaerobically-grown E. coli survived better than aerobically-grown E. coli in a flooded histosol. Furthermore, factors like nutrient availability and the protective effects of waste against moisture loss, factors that increase survival, would not exist. Further criticism is probably warranted for field trials involving abnormally high inoculation densities ($10^{10}$ cells/g of soil, Smallbeck and Bromel, 1975), very small field plots (0.05 $m^2$, McCoy and Crabtree, 1970), or other anomalies (freeze-dried sludge, Miller, 1974).

Thus, little or no standardization in methodology exists. Interdependence between variables, such as between moisture and sunlight or moisture and soil texture, is often ignored. It is understandable then, that some authors (Elliott and Ellis, 1977; Morrison and Martin, 1977) state that methodological differences make it difficult to determine how each environmental factor affects each other.

## E. Purpose of sludge-amended soil experiments

The consensus on factors affecting fecal coliform or E. coli numbers in waste-amended soil is shown in Table 3. It is noteworthy that many of the primary factors for fecal coliform survival exist during the winter months in the western regions of the Pacific Northwest: rainfall is heavy

TABLE 3. CONSENSUS ON FACTORS AFFECTING FECAL COLIFORM OR *E. COLI* NUMBERS IN WASTE-AMENDED SOIL.

| Primary factors | Optimal conditions |
|---|---|
| competition | sterile soil |
| moisture | moist (not dry or flooded) |
| nutrient availability | maximum organic carbon |
| pH | pH 6-7 |
| runoff and leaching | no saturated flow |
| soil texture | high clay or organic matter |
| sunlight | minimum |
| temperature | cool but not freezing |
| **Secondary factors or yet unclear** | |
| antibiotics and toxic substances | |
| outside sources of contamination | |
| parasitism and predation | |
| waste application rate | |

so the soils are moist, temperatures are cool but rarely below freezing, and sunlight is low. Sewage sludge applied during this period to near neutral pH soils that were not sandy and located on relatively flat land where runoff was minimal should have conditions for optimal fecal coliform survival. On the other hand, saturated flow, a condition ignored in field studies of this type, could also exist. This, in turn, could cause fecal coliform loss and point to possible groundwater contamination problems. Therefore, a study was conducted to determine the effects of these factors by amending four field sites in the Willamette Valley in the Pacific Northwest with sewage sludge in the summer and in the winter and monitoring fecal coliform numbers. Rainfall, air and soil temperature, solar radiation, and groundwater tables were recorded, and in sites with sufficiently high water tables, loss of fecal coliforms through saturated flow was assessed by sampling ground water from *in situ* sampling wells. To determine if sampling wells were adequate in precluding surface water contamination, a preliminary experiment was conducted using sampling wells mounted in soil cores and amending the soil cores with sludge. Also, to determine the effect of sludge crusting of soil on fecal coliform survival, a field site was amended with sewage sludge and fecal coliform numbers assessed in and below the sludge crust.

## F. Purpose of micro-MPN experiments

A method commonly used for enumerating microorganisms from soil is the most-probable-number (MPN) technique. The MPN technique is tedious and time-consuming, so a micro-MPN technique was developed.

### 1. Diluent experiment

The accuracy of any MPN counting method is based on two assumptions: the organisms are randomly distributed throughout the sample, and the culture medium will show growth when the sample contains one or more organisms (Cochran, 1950). In the case of the former assumption, sludge-amended soil sample must be sufficiently homogenized to ensure bacterial randomness. Sewage sludge is, however, normally in a flocculated state, and moreover, most soils containing bacteria contain sufficient organic matter and clay to resist dispersal. Because chemical surfactants do not release the bacteria in sewage flocs (Yin and Moyer, 1968), several authors recommend ultrasonication for sewage floc dispersal (Williams *et al.*, 1970, 1971; Pike and Carrington, 1972). Pike *et al.* (1972) showed that optimal counts of viable bacteria could be obtained by the use of a sodium tripolyphosphate diluent sonicated in an ultrasonic bath. Ultrasonication was relatively nonlethal for times less than 2 minutes. To determine if the use of unsonicated 0.1% peptone diluent as recommended by *Standard Methods* (APHA, 1971) for MPN procedures was sufficient to ensure

bacterial randomness of sludge-amended soil samples, an experiment was conducted comparing unsonicated 0.1% peptone diluent to sonicated 0.1% peptone and to sonicated sodium tripolyphosphate diluent.

2. Micro-MPN assay

Several authors recommend the use of various micro-MPN techniques as alternative to the standard MPN technique. Curtis et al. (1975) determined numbers of nitrifying bacteria in sediments by serial dilutions in test tubes and transferring aliquots to a 25-microwell "repli-plate." Darbyshire et al. (1974) estimated bacterial and protozoan populations and Rowe et al. (1977) enumerated soil ammonium-oxidizing populations in soil using a 96-well microwell plate (12 dilutions, eight replicates/dilution). To enumerate fecal coliforms in soil, a micro-MPN technique was developed using a 96-microwell plate (12 dilutions, 8 replicates/dilution) and this method compared to the elevated temperature fecal coliform MPN as outlined in Standard Methods (APHA, 1971).

Because the medium in the micro-MPN technique differed from the medium in the standard MPN technique, the media variation between the two techniques was also assessed.

# MATERIALS AND METHODS

## A. Sludge-amended soil experiments

### 1. Sampling wells

Three undisturbed soil cores, each 25 cm high by 30 cm in diameter, were excavated from a Dayton silt loam. Cores were placed in a 38-cm high by 42-cm diameter clay pot containing 1 cm of clay pot shards and 5 cm of sand (#8, Bristol Silica Co, Rogue River, Or.). A 1-cm layer of bentonite (Western Yellowstone bentonite, Dresser Minerals, Houston, Texas) was added around the base of the soil core and a 1:10 bentonite-water slurry added to ground level. As water evaporated from the bentonite slurry, fresh slurry was added until the bentonite remained at the ground surface and the core was immobilized. Each core contained three sampling wells, one each at 5, 10, and 20 cm. Each sampling well consisted of a 16 mm (i.d.) heavy glass wall tube cut to the appropriate length plus 5 cm. The additional 5-cm length protruded above the ground surface after installation. One end of the glass tube was sealed and eight 2-mm holes blown less than 3 cm from the sealed end. The tubes were placed in holes of the appropriate depth that were previously augered with a 1.9 cm soil probe (Art's Mfg. & Supply, American Falls,

Idaho). Holes were filled with fine crushed rock to above the 2-mm holes and followed by a heavy inner-tube rubber gasket. A 1-g portion of bentonite was added to the hole and a bentonite-water slurry added to ground level. More slurry was added daily until the slurry stabilized at ground level. The tubes were checked for drainage with sterile tapwater and sealed with polypropylene caps. Each clay pot was placed in a 120-liter polyethylene container and tapwater added until the level of water coincided with the ground surface of the soil core. After several days to allow equilibrium and dechlorination to occur, a 10-ml aliquot of water from each sampling well was removed and tested for the presence of fecal coliforms using the confirmed 5-tube MPN procedure of Standard Methods (APHA, 1971). Two soil cores were each amended with 1 liter of fresh anaerobically-digested sewage sludge and the third core was amended with 1 liter of the same sludge sterilized by autoclaving. An aliquot of nonsterile sludge was taken to determine fecal coliform numbers. A 10-ml aliquot of water from each sampling well was removed hourly from each sampling well for 12 hours and once a day thereafter for two weeks. Samples were treated as described above. Dechlorinated tapwater was added periodically to maintain the water level with the ground surface of the soil core.

2. Winter and summer sludge applications

a. Study area

The Willamette Valley is located in northwest Oregon and occupies a broad structural depression 30 to 50 km wide by 160 km in length. The valley is oriented on a north-south axis between the Oregon Coast Range to the west and the Cascade Range to the east. The valley has a moderate, relatively uniform climate with a strong marine influence. Summers are warm and dry while winters are cool and wet.

Corvallis, Oregon is located on the main, broad, nearly level, Late Pleistocene valley floor terrace of the southern Willamette Valley (Balster and Parsons, 1969). The climate is representative of the Willamette Valley: the average annual precipitation is 1030 mm, of which only 2% occurs in July and August, while 60% occurs in the form of rain during November through February (Johnsgard, 1963). The average temperature in July is 19.4°C, while the average January temperature is 3.9°C (Johnsgard, 1963). The ground is seldom frozen.

b. Site selection and soils used

Four field sites were selected, all located within 10 km of Corvallis, Oregon. Each field site was a different soil series; these were: Amity, Cloquato, Dayton, and Woodburn. Amity soil is classified as a fine-silty, mixed, mesic Argiaquic Xeric Argialboll, Cloquato as a coarse

silty, mixed, mesic Cumulic Ultic Haploxeroll, Dayton as a fine, montmorillonitic, mesic Typic Albaqualf, and Woodburn as a fine-silty, mixed, mesic Aquultic Argixeroll. Site selection was based on similar soil texture and runoff potential, and dissimilar drainage and soil permeability (Table 4). Selected soil properties of the A horizon of the four soil series were also determined (Berg and Gardner, 1978) (Table 5).

Cloquato and Woodburn field sites had 0-3% slopes. Amity and Dayton field sites were located in the bottom of shallow swales and 0-3% slopes in the center with varying small slopes at the periphery. Amity, Dayton, and Woodburn field sites were previously used for pasture and were covered with a mixture of annual and perennial grasses. Cloquato was previously cultivated with corn (Zea mays (L.)) but had lain fallow for the last two years. None of the sites had received herbicides or pesticides within a 2-year period. None of the sites was shaded. Elevation at the Woodburn site was 180 m above sea level (U.S. Environmental Data Service, 1977) and differences in elevation were less than 100 m for all sites.

c. Site preparation

Each field site consisted of four 4.6 X 13.7 m plots, two plots each for summer and winter sludge applications. One plot of each of the two seasonal plots was designated as a control. Plots were separated from each other by a bound-

TABLE 4. SOIL TEXTURE, RUNOFF POTENTIAL, DRAINAGE, AND PERMEABILITY OF THE SOILS USED.[†]

| Soil series | Soil texture | Runoff potential | Drainage | Permeability |
|---|---|---|---|---|
| Amity | silt loam | slow | somewhat poorly drained | moderately slow |
| Cloquato | silt loam | slow | well-drained | moderate |
| Dayton | silt loam | slow to ponded | poorly drained | very slow |
| Woodburn | silt loam | slow to medium | moderately well-drained | slow |

[†]table adapted from Knezevich et al., 1975

TABLE 5. SELECTED SOIL PROPERTIES OF THE SOILS USED.

| Series | pH | Organic matter (%) | Exchangeable cations cmol ($p^+$)/kg soil | | |
|---|---|---|---|---|---|
| | | | Ca | Mg | Na |
| Amity | 7.1 | 5.0 | 17.3 | 3.2 | 0.2 |
| Cloquato | 6.2 | 4.2 | 17.8 | 6.1 | 0.2 |
| Dayton | 5.8 | 4.7 | 11.8 | 1.0 | 0.1 |
| Woodburn | 5.8 | 3.9 | 7.7 | 1.1 | 0.1 |

ary of at least 2 m. Pits were dug on one of the control sites of each soil series and the soil profiles matched against the taxonomic description (Knezevich et al., 1975). The four soil series all matched their description.

Each plot was rototilled to a depth of 15 cm by a conventional tractor-mounted rototiller. Rototilling served to reduce runoff.

Each control site was equipped with a self-registering maximum-minimum thermometer (Taylor Instruments, Arden, N.C.), rain gauge (Victor Rain-Gage Co, Berryville, Ark.), 7-day recording thermometer (Pacific Transducer Corp., Los Angelos, Ca.), and 1-m, self-registering maximum-minimum water table gauge. The maximum-minimum thermometer and rain gauge were mounted on a 1.2 m wooden post facing north. The 7-day recording thermometer was wrapped in polyethylene and buried to a 5-cm depth. The dimensions and installation of the water table gauge are described elsewhere (Hammermeister, 1978). Solar radiation was recorded only at the Woodburn field site with a pyroheliometer (Science Associates, Princeton, N.J.) connected to a strip-chart recorder. Readings were taken daily except for the recording thermometer readings which were taken weekly.

Each field plot designated for sludge amendment was equipped with three 100-cm sampling wells to assess fecal coliform movement into the water table. The wells were arranged in a row, 1 m apart, centered on the longitudinal

axis of the plot. The tubes were 120 cm in length, so the additional 20 cm protruded above ground level after installation. Installation was the same as described earlier except that the sampling well holes were augered with a 5-cm soil auger (Art's Mfg. & Supply). Fine crushed rock and dry bentonite were also increased to accommodate the larger hole dimensions as appropriate.

d. Sludge amendment

Primary anaerobically-digested sewage sludge was obtained from the Corvallis, Oregon wastewater treatment plant and contained in a mobile 1900-liter tank. The tank was equipped with a recirculating pump to ensure sludge homogeneity. Each site received 800 liters of sludge applied as uniformly as possible with a 4.6-m, hand-operated, wheel-mounted sprayer. A 500-ml aliquot of each sludge load was obtained to determine physical and chemical sludge properties. Physical and chemical properties, as well as heavy metal concentrations, were determined as described elsewhere (Hemphill et al., 1982), except percent solids which were determined as described in Standard Methods (APHA, 1971) (Tables, 6, 7). Because of the sludge application rates, one tank-load of sludge was sufficient for only two sites. In the case of the summer application where the physical and chemical properties of the sludge were significantly different, the loads are shown separately. In the winter application where the

TABLE 6. SELECTED PROPERTIES OF THE SEWAGE SLUDGE USED.[†]

| | Solids | N | P | K | Ca | Mg |
|---|---|---|---|---|---|---|
| | ---------- | ---------- | ---------- | % ---------- | ---------- | ---------- |
| Winter | 5.0 | 6.4 | 1.7 | 0.4 | 2.6 | 0.6 |
| Summer #1[‡] | 2.5 | 4.0 | 1.3 | 0.3 | 2.8 | 0.5 |
| Summer #2[§] | 6.2 | 7.6 | 1.9 | 0.5 | 2.2 | 0.6 |

[†]all analyses except % solids performed by D. Hanson

[‡]applied to Dayton and Cloquato field sites

[§]applied to Amity and Woodburn field sites

TABLE 7. HEAVY METAL ANALYSES OF THE SEWAGE SLUDGE USED.[†]

| | Cd | Cr | Cu | Fe | Mn | Mo | Ni | Pb | Zn |
|---|---|---|---|---|---|---|---|---|---|
| | μg/g dry sludge | | | | | | | | |
| Winter | 19 | 262 | 432 | 20,500 | 501 | 100 | 53 | 1.3 | 1630 |
| Summer #1[‡] | 17 | 249 | 401 | 22,300 | 531 | 51 | 61 | 2.4 | 1550 |
| Summer #2[§] | 20 | 268 | 446 | 19,700 | 487 | 123 | 48 | 0.8 | 1660 |

[†]all heavy metal analyses performed by D. Hanson

[‡]applied to Dayton and Cloquato field sites

[§]applied to Amity and Woodburn field sites

properties of the sludge were identical, the data were combined. The lack of homogeneity in the summer sludge loads was because the anaerobic digester was being emptied at the time of application, thus the first load was the top layer of the digester whereas the second load was the sediment. Control sites were not sludge-amended. All sludge-amended sites were amended within 4 hr.

e. Site sampling

Three walkways of plywood, each 0.6 X 61 X 122 cm, were placed across each field plot at each sampling period to allow soil sample collection without cross-contaminating between the control and sludge-amended plot. Each plot had its own walkways which were removed after sampling.

Six soil corings, each 1.9 X 15 cm, were removed from each plot at each sampling period with an ethanol flame-sterilized soil probe and placed in a sterile polyethylene bag. Two random corings were taken from each third of the plot. Coring holes were not filled in. All sites were sampled the day before as well as the day of sludge application. After sludge application, the sites were sampled weekly or biweekly as necessary except the winter application which was sampled twice in the first week. If the water table was sufficiently high, a 35-ml water sample was collected weekly from each sampling well using a sterile, 7 mm (o.d.) glass tube connected to a sterile

sampling reservoir. Suction was provided by a battery-operated vacuum pump. The water table well of the control site was sampled as a control. Both bags of soil and sampling well water were kept at ambient temperature and processed within 1 hr.

To determine fecal coliform numbers in the soil, corings from each plot were uniformly mixed for several minutes by hand. A 10-g soil sample was removed from the polyethylene bag under laminar flow hood conditions and placed in a 160-ml milk dilution bottle containing 90 ml of 0.1% peptone (Difco Laboratories, Detroit, Mich.). The bottle was shaken for 1 min and allowed to settle for 40 sec. A confirmed 5-tube MPN determination for fecal coliforms, as outlined in Standard Methods (APHA, 1971), was then conducted. Water from the sampling wells was combined and the same procedure was used without the initial dilution.

f. Microbial die-off

Fecal coliform die-off rate constants (k) were determined from the equation

$$M_t = M_o^{-kt}$$

where

$M_t$ = number of fecal coliforms at time, t;

$M_o$ = initial number of fecal coliforms after sludge application;

k = first-order die-off rate constant for fecal coliforms, $day^{-1}$;

t = time, in days (Reddy et al., 1981).

3. Sludge crust

A field site on Dayton silt loam was prepared identically to the site preparation used for the summer and winter sludge applications except that the two plots were not rototilled. The experiment began in February and was concluded in mid-April. The sludge plot was amended with 895 liters of anaerobically-digested sewage sludge. Percent solids was determined as described in Standard Methods (APHA, 1971). One week was allowed to elapse for the sludge to form a crust. The plots were sampled at 1, 4, and 11 weeks after sludge application. The sludge crust was sampled by cutting an 8 X 8 cm section with an ethanol flame-sterilized spatula. This section was gently lifted from the soil surface and placed in a sterile polyethylene bag. Soil samples directly under the sludge crust were taken using a 5-cm diameter bulk density soil corer. The corer was sterilized by ethanol-flaming and two sterile aluminum rings (5-cm diameter, each 2.5 cm high) inserted. The unit was driven into the soil with a hammer until the rings were full. The corer was removed, and the rings separated and put into labelled sterile polyethylene bags. This sampling then represented the 0-2.5 cm and 2.5-5.0 cm soil corings. In addition, the height of the sludge crust directly adja-

cent to the coring site was measured. Two sludge crusts and corings were randomly taken from each third of the plot at each sampling period. Coring samples were subsequently removed from the aluminum rings under laminar flow hood conditions, aseptically sectioned lengthwise and the center of the core carefully removed. In this manner, all six corings for each depth were collected and placed in a separate polyethylene bag. Sludge crust samples were not resectioned. Fecal coliform numbers were determined as described earlier.

B. Micro-MPN experiments

1. Diluent test

Sodium tripolyphosphate (Pfaltz and Bauer, Stamford, Conn.) diluent was made to a concentration of 5 mg/L in distilled water, adjusted to pH 6.8 with 1.0 N HCl, and sterilized by autoclaving as recommended (Ministry of Technology, 1971). Peptone (Difco) was prepared using standard methods (APHA, 1971). Soil samples from the winter sludge application field sites were collected as described earlier and processed within 1 hr. Sampling continued for 10 weeks, with soil corings from the sludge-amended and control plots from a different field site each week. In this manner, each of the four field sites was sampled either 2 or 3 times. Fecal coliform numbers were determined as described earlier except bottles receiving sonication were sonicated for 1 min at 50-55 kHz in an ultrasonic cleaning

bath (Model 220, Branson Cleaning Equipment Co., Shelton, Conn.). At the end of each confirmed test, fecal coliform MPNs were compared against the unsonicated peptone diluent MPNs.

2. Micro-MPN assay

Concurrent with the summer sludge application, the same soil core samples used for the standard fecal coliform MPN were used for the microwell MPN. Therefore site sampling was the same as described earlier except that an additional 10 g of soil was removed for moisture correction.

Microwell plates each 8 X 12.5 X 1.3 cm, 8 X 12 microwells, 0.225 ml maximum volume per microwell, were drilled from polycarbonate blocks and designed to fit inside a glass 15-cm petri dish for autoclaving. Used plates were washed and then rinsed with distilled water for reuse. Repeated autoclaving had little effect on the polycarbonate. Polycarbonate plates were compared to prepackaged sterile polystyrene plates (Linbro Scientific, Hamden, Conn.).

Soil samples were processed as described earlier. Microwell plates were placed on a damp paper towel in the laminar flow hood to eliminate static charge accumulation (Conrath, 1972). Lactose broth was poured into a sterile 10-cm petri plate and transferred to the microwell plate with an 8-channel micropipet (Flow Laboratories, Inc., Inglewood, Cal.). The micropipet delivered 100 µL of

double-strength lactose broth/microwell to the first row of eight wells and 100 µL of single-strength lactose broth/microwell to all the remaining wells. A pipet dropper (Cooke Laboratory Products, Alexandria, Va.) was also used for loading the microwell plates but discontinued because it was too time-consuming to use. Soil samples were shaken vigorously for 1 min and allowed to settle for 40 seconds. A 30-ml aliquot was poured into a sterile 10-cm petri plate for the microwell MPN and the rest of the sample was used for a confirmed 5-tube fecal coliform MPN as outlined in Standard Methods (APHA, 1971). Microwell plates were oriented with the initial dilutions furthest from the laminar airflow to minimize aerosol contamination (Darbyshire et al., 1974). The micropipet discharged 100 µL of sample/microwell into the first row of eight microwells. The wells were mixed ten times by drawing up and discharging the micropipet gently to minimize splashing. In this manner, 11 rows were serially diluted; the 12th row was left uninoculated as a control. The Cooke microtiter system (Cooke Laboratory Products) was also tried but was unsatisfactory since organic debris from the soil samples became entrapped in the vanes. To determine the minimum incubation times for lactose broth in the microwell plates, the first soil samples taken from each of the four sludge-amended field sites were incubated at 35°C and aliquots transferred with an ethanol flame-sterilized multi-point inoculator (Fung and Miller, 1970) at 4, 5, 6, 7, 8, 9, 10, and 24 hrs

incubation to a surface-dried, 15-cm, m-FC agar (Difco) plate. Surface-drying the plates minimized spreading-type colonies. The m-FC agar contained no rosolic acid since this is the medium of choice in isolating fecal coliforms (Presswood and Strong, 1978; Grabow et al., 1981). An attempt was made to transfer aliquots with the multi-point inoculator to another 96-microwell plate containing EC broth (Difco) but this required an Amojell-mineral oil seal to visualize gas production (Fung and Miller, 1970) and was too messy for routine use. The m-FC agar plates were wrapped in polyethylene bags and incubated underwater in a coliform incubator bath (Precision Scientific, Chicago, Ill.) at 44.5±0.2°C. The plates were examined hourly until characteristic blue colonies were clearly visible. Blue colonies were converted to MPN values using the 3-column MPN code of Rowe et al. (1977). These values were then compared to the MPN values obtained by Standard Methods.

3. Media variation

An aliquot of each microwell of incubated lactose broth was inoculated into a 15 X 150 mm test tube containing a Durham tube and 10 mls of EC broth. Test tubes were incubated for 48 hours at 44.5±0.2°C in a fecal coliform incubator bath and Durham tubes assessed for the presence or absence of gas. Results were then correlated with results obtained on the m-FC agar plates.

# RESULTS

## A. Sludge-amended soil experiments

### 1. Sampling wells

The sewage sludge applied to the soil cores contained $4.9 \times 10^5$ fecal coliforms/100 ml. All the sampling wells, including those of the control, remained negative for fecal coliforms for the entire two-week period. Thus, the method of sealing the sampling wells was effective in preventing surface water contamination.

### 2. Winter and summer sludge applications

#### a. General

Fecal coliforms declined from $10^5$ to $<10^2$ cells/g of soil in 13 weeks in winter (Fig. 1) and from an average of $10^5$ to $<10^3$ cells/g of soil in 13 weeks in summer (Fig. 2).

During the winter, the first order die-off rate constants for fecal coliforms on the Amity, Cloquato, Dayton, and Woodburn field sites were 0.046, 0.046, 0.042, and 0.042/day, respectively, and during the summer, the constants were 0.034, 0.039, 0.026, and 0.034/day, respectively. The constant for the Dayton field site during the summer was based on a 61-day time period because of a sheep break-in. Thus, the die-off rate constants are similar among field sites for summer and winter sludge applications.

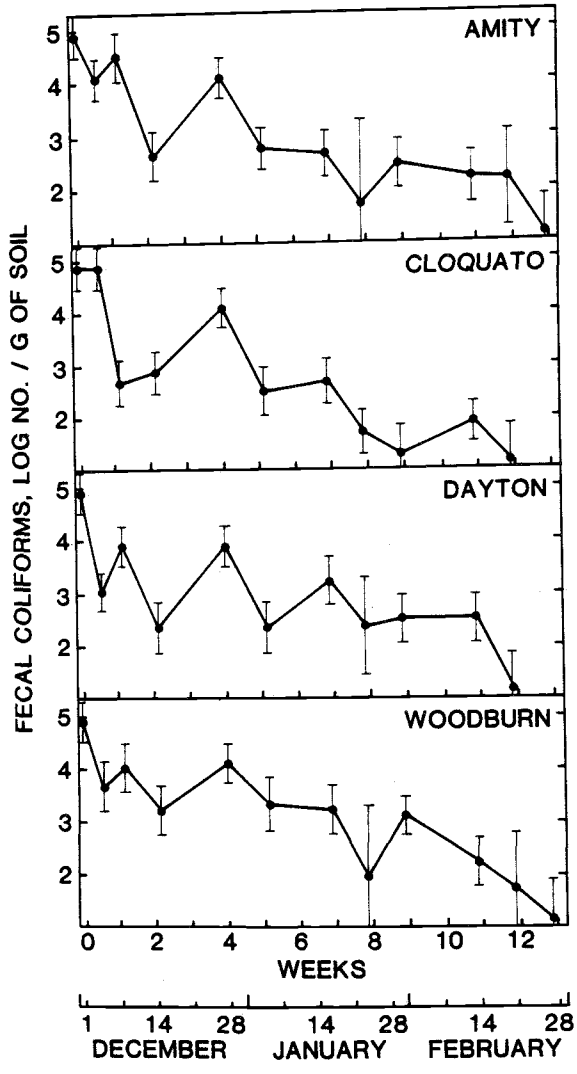


Figure 1. Survival of fecal coliforms in four sludge-amended soils during the winter. Error bars designate 95% confidence interval. Numbers of fecal coliforms were below detectable levels (10 cells/g of soil) in all control plots throughout the sampling period.

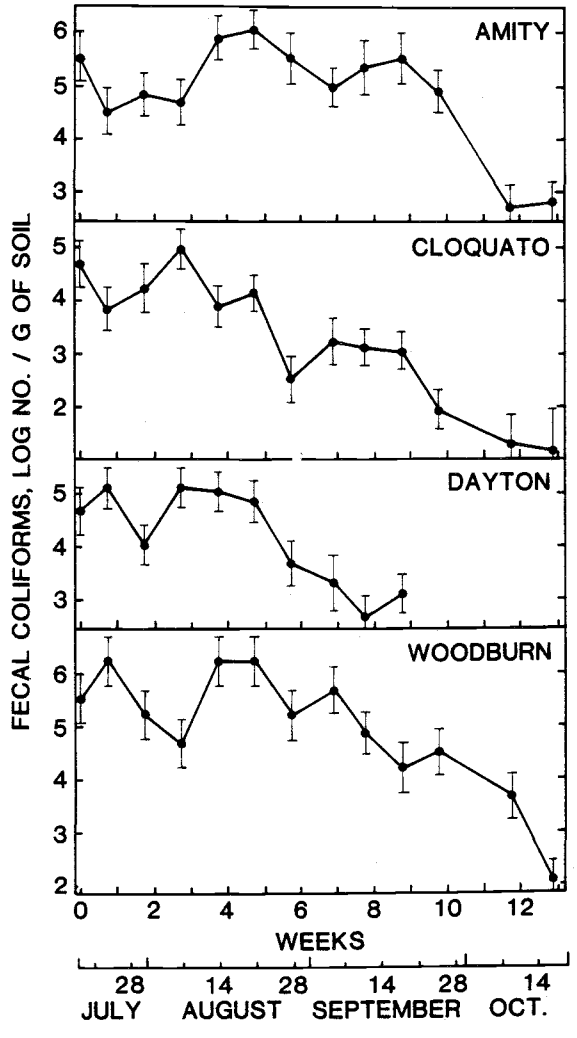


Figure 2. Survival of fecal coliforms in four sludge-amended soils during the summer. Numbers of fecal coliforms were below detectable levels (10 cells/g of soil) in all control plots throughout the sampling period.

Variation in rainfall and temperature were only minor from field site to field site (data not shown), so the values shown for each represent the combined average of the four field sites.

All field sites before sludge amendment were negative for fecal coliforms. All control plots were negative for fecal coliforms throughout both sampling periods.

b. Rainfall

During the winter sampling period, measurable precipitation occurred on 74 of 91 days (81%) for a total of 576 mm of rain. No snowfall was recorded. Four major rainfall periods, arbitrarily defined here as rainfall resulting in a 50-cm rise in the water table of the Amity field site, were recorded: before Week 1 (Dec. 1-7), Week 1 (Dec. 10-28), Week 5 (Jan. 3-22), and Week 9 (Jan. 31-Feb. 9) (Fig. 3). During these periods, a rise in the water table of the Dayton field site was also observed, while on the well-drained Cloquato and Woodburn field sites, the water tables remained at >100 cm below ground level for the entire sampling period.

A relationship between rainfall and fecal coliform numbers was observed. The first major rainfall occurred with a concomitant rise in the water tables of the poorly-drained Amity and Dayton field sites. This was a period of saturated flow and was accompanied by a decline in fecal coliform number at three of the four field sites. The most

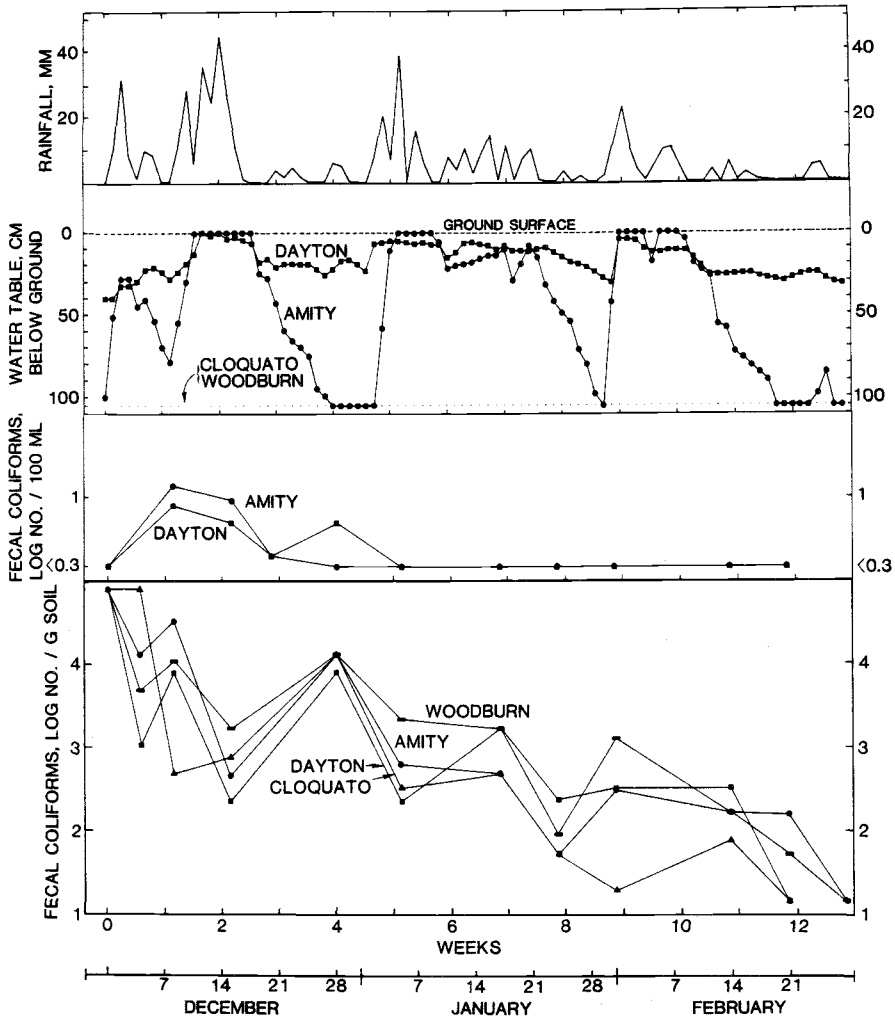


Figure 3. Comparison of rainfall (top), water table depth (middle top), log no. of fecal coliforms recovered from 100-cm sampling wells (middle bottom), and fecal coliform survival in Amity (circles), Cloquato (triangles), Dayton (squares), and Woodburn (bars) soils during the winter. Error bars deleted from fecal coliform survival lines for clarity.

well-drained field site, Cloquato, showed no decline. Following the cessation of rainfall (Dec. 8-9), saturated flow ceased, and a brief period of fecal coliform growth was observed at three of the four sites; no growth was observed at the Cloquato site. During this period, an average of 10 fecal coliforms/100ml were isolated from the 100-cm sampling wells of the Amity and Dayton field sites. With the beginning of the second rainfall period (Dec. 10), saturated flow occurred again, and numbers of fecal coliforms declined at three of the four field sites; a slight increase was observed for the Cloquato site. An average of 7 fecal coliforms/100ml were isolated from the Amity and Dayton 100-cm sampling wells. At the cessation of the second major rainfall (Dec. 19), saturated flow ceased, and fecal coliform numbers increased at least one logarithmic order of magnitude at all four field sites. During the second saturated flow period, fecal coliforms in the sampling wells declined and then rose slightly at the Dayton site, while declining to below detectable levels at the Amity field site. At the beginning of the third rainfall period (Jan. 3), saturated flow occurred, and fecal coliform numbers declined at all four field sites. Fecal coliforms were not detected in the sampling wells nor in any subsequent samplings. Water tables remained high at the Amity and Dayton field sites for three weeks and fecal coliform numbers remained stable at three of the four field sites, while increasing slightly at the Dayton field site. Following the third rainfall period

(Jan. 24), the pattern among rainfall, saturated flow, and fecal coliform numbers ceased, and fecal coliform numbers declined at all the field sites. At the beginning of the fourth major rainfall period (Feb. 1), fecal coliform numbers recovered somewhat on the Amity and Woodburn field sites, showed a slight increase on the Dayton field site, and declined on the Cloquato field site. At the cessation of the fourth major rainfall, fecal coliform numbers declined at three of the four sites; numbers remained steady at the Amity site for a week before declining. No clear relationship between the magnitude of rainfall and fecal coliform growth was observed.

In contrast to the winter sampling period, measurable rainfall during the summer sampling period was recorded on only 27 of 91 days (31%) for a total of 146.2 mm. No rainfall was recorded for the first 23 days (July 19-August 11) (Fig. 4) compared to 265 mm of rain for the first 23 days of the winter sampling period. Two rainfall periods occurred: before Week 4 (August 12-26) and before Week 7 (Sept. 4-18). Rainfall was insufficient to cause the water table on any site to rise within 100 cm of the ground surface and therefore water table and 100-cm sampling well data are not shown. Inoculation densities differ because the sludge was from different layers of the anaerobic digester.

No clear pattern of survival is evident for the first four sampling periods until fecal coliform numbers stabi-

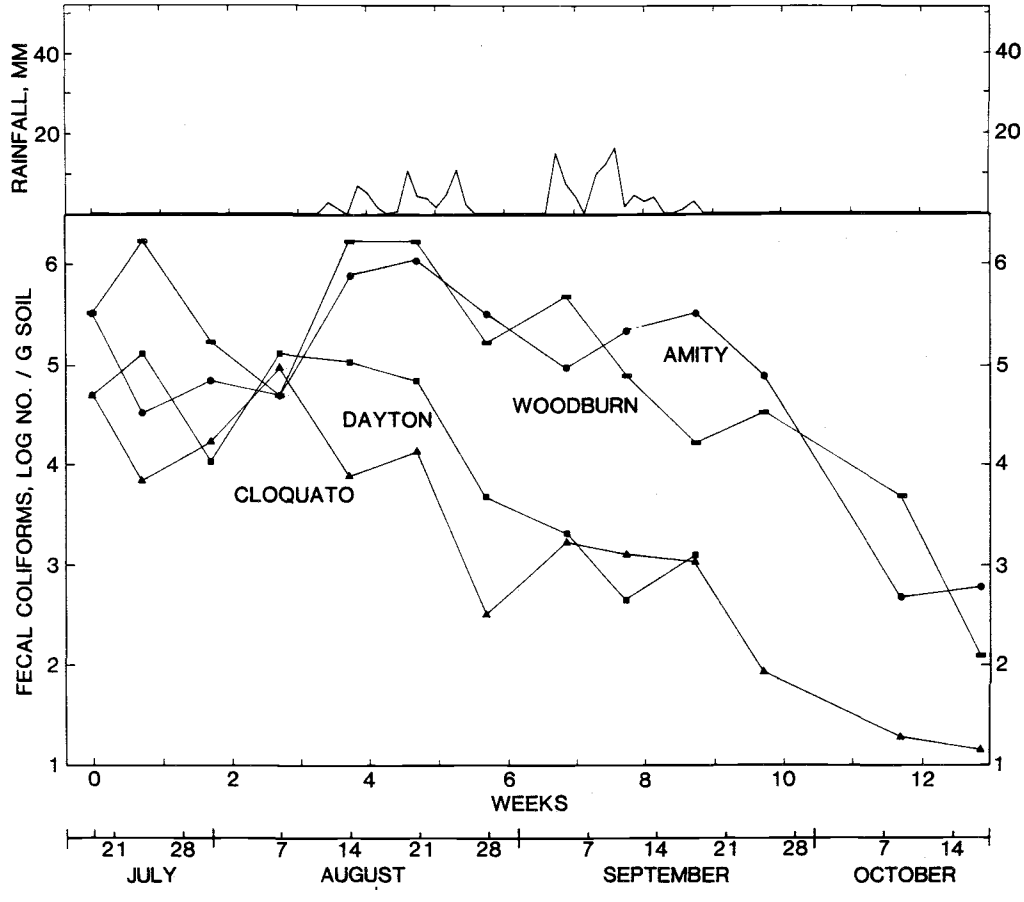


Figure 4. Comparison of rainfall (top) and survival of fecal coliforms in four sludge-amended soils (bottom) during the summer.

lized at $10^5$ cells/g of soil. Following the first measurable rainfall however, a significant increase in cell numbers per g of soil occurred for the Amity and Woodburn field sites; no significant increase was observed for Cloquato and Dayton field sites. Fecal coliform numbers remained relatively unchanged until the first rainfall period ended, whereupon fecal coliform numbers declined at all the field sites. Between the first and second rainfall period, a small increase in fecal coliform numbers was observed for the two well-drained sites, Cloquato and Woodburn; fecal coliform numbers continued to decline at the Amity and Dayton field sites. Following the beginning of the second rainfall period, fecal coliforms declined at three of the four field sites, and the Amity field site showed an increase. Thus, the trend of fecal coliform growth occurring with the onset of moisture within the first five weeks of sludge application is true not only for the winter sampling period, but also for the Amity and Woodburn field sites during the summer. Thereafter growth may or may not appear with the onset of rainfall.

c. Temperature

During the winter sampling period, the average daily minimum air temperature was 3.7°C. Below-freezing temperatures were recorded on nine days with a maximum low temperature of -3.3°C (Fig. 5). Soil temperatures at the 5-cm soil depth were less variable and warmer; the average

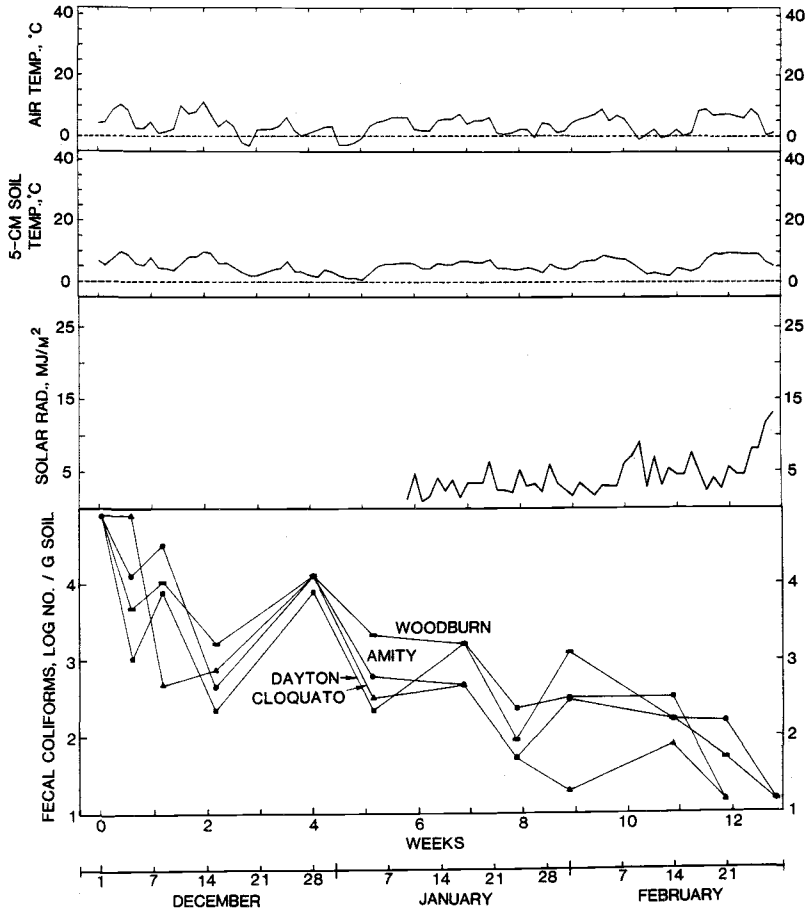


Figure 5. Comparison of minimum air temperature (top), 5-cm soil depth minimum temperature (middle top), solar radiation (middle bottom), and survival of fecal coliforms in four sludge-amended soils (bottom) during the winter.

daily low temperature was 5.3°C and no freezing temperatures were recorded. No discernible relationship between freezing temperatures and fecal coliform die-off was observed. Thus, although fecal coliform numbers declined during the freezing period of the fourth week (Jan. 2-5), fecal coliform numbers increased during the freezing period of the second week (Dec. 20-21).

During the summer sampling period, the average daily maximum air and 5-cm soil temperatures were 24.6 and 25.2°C, respectively (Fig. 6). The first 23 days had an average daily maximum air temperature of 32.1°C compared to 21.9°C for the rest of the sampling period. Despite this temperature difference, no discernible difference in fecal coliform die-off was observed between the two periods.

d. Solar radiation

An instrument failure occurred during the first six weeks of the winter sampling period; for the rest of the sampling period, the average daily solar radiation was 4.3 $MJ/m^2$ with a range of 0.1 to 13.1 $MJ/m^2$ (Fig. 5). During the summer sampling period, the average daily solar radiation was 14.5 $MJ/m^2$ (Fig. 6). The first 23 days had a daily average of 22.3 $MJ/m^2$ compared to a daily average of 11.6 $MJ/m^2$ for the rest of the sampling period. No discernible difference in fecal coliform die-off was observed for either winter or summer sampling periods, or between the first 23

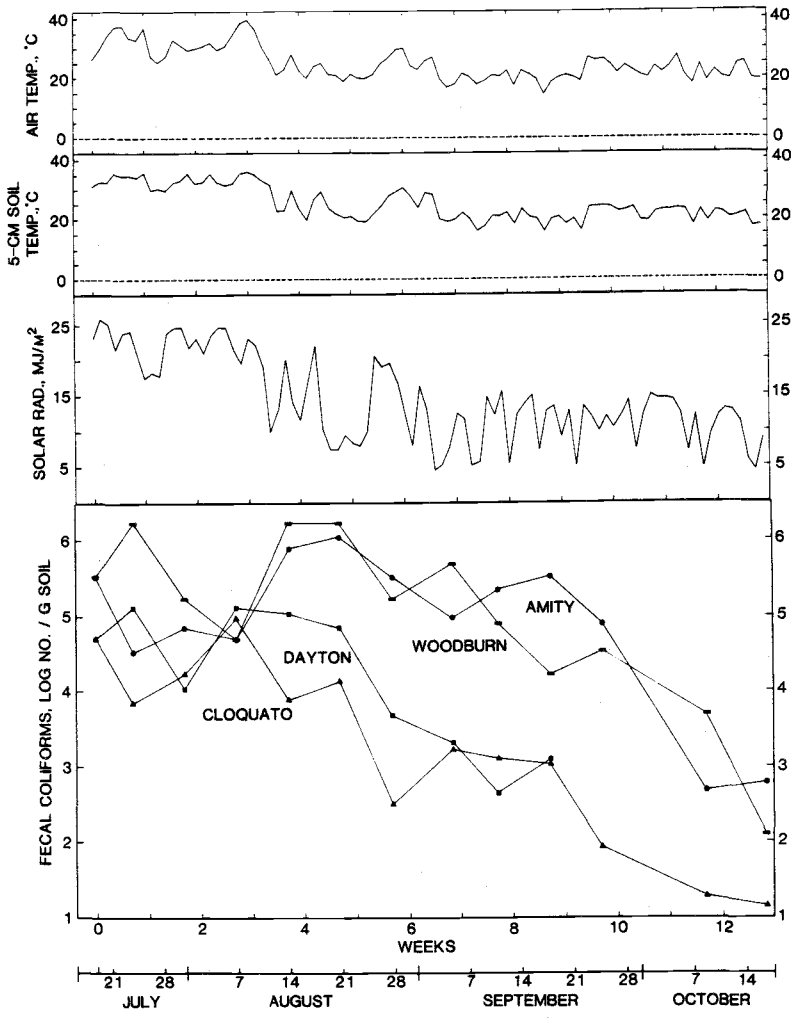


Figure 6. Comparison of maximum air temperature (top), 5-cm soil depth maximum temperature (middle top), solar radiation (middle bottom), and survival of fecal coliforms in four sludge-amended soils (bottom) during the summer.

days of the summer sampling period and the rest of the summer sampling period.

3. Sludge crust

The average daily low temperature for air and 5-cm below the soil surface was 1.6 and 4.9°C, respectively. During the 77-day period, 21 days occurred with air temperatures lower than 0°C; of these days, 20 were between -2.2 and 0°C, and one day, the first day after sludge application, was -5.0°C. No freezing temperatures occurred at the 5-cm soil depth. Thus, temperatures were cool, and freezing periods, when they occurred, were not severe.

Measurable rainfall during the first 18 days following sludge application was 1.8 mm, with rainfall recorded on 3 of 18 days (16.7%). In contrast, rainfall was 180.1 mm for days 19 through 40, with 29.5 mm of rain in the two days prior to the third week sampling. Measurable rainfall was recorded on 19 of 21 days (90.5%) during this period. Rainfall was sporadic after day 40 with 38.4 mm recorded. No snow occurred during the entire sampling period. Thus, conditions were dry during the first 18 days, then seasonally wet.

The sludge applied to the field plot had 5.9% solids. The average thickness of the sludge crust was 8.7 mm with a range from 4 to 15 mm. After one week, 99.5% of the fecal coliforms were present in the sludge crust with the remaining percentage in the 0 to 2.5 cm layer (Table 8). No fecal

TABLE 8. FECAL COLIFORM SURVIVAL IN SEWAGE SLUDGE CRUST AND DAYTON SILT LOAM.

| Sample | Week | | |
|---|---|---|---|
| | 1 | 3 | 11 |
| | -log no. fecal coliforms/g of soil- | | |
| Sludge crust | 5.85 | 6.23 | 3.52 |
| Soil, 0-2.5 cm depth | 3.52 | 3.69 | 3.66 |
| Soil, 2.5-5.0 cm depth | <2.0 | 2.90 | <2.0 |

coliforms were detectable in the 2.5 to 5.0 cm depth. After sampling on the 21st day, fecal coliform numbers did not decline in the sludge crust and in the 0-2.5 sampling depth, and fecal coliforms were detected at the 2.5-5.0 cm depth. By Week 11, fecal coliform numbers had declined significantly in the sludge crust, but showed no change at the 0-2.5 cm soil depth and were no longer detectable at the 2.5-5.0 cm soil depth. The first-order die-off rate constant for fecal coliforms in the sludge crust was 0.033.

Despite the rainfall during days 19-40, no water table above 100-cm soil depth was recorded during the 11-week sampling period. Rodent activity was observed on the control plot, but the plot remained negative for fecal coliforms throughout the sampling period.

B. Micro-MPN experiments

1. Diluent test

The correlation coefficient between the logarithmic numbers of fecal coliforms per g of soil obtained by sonicated and unsonicated 0.1% peptone was 0.95 (Fig. 7) and is described by the line

$$\log y = 0.774 \log x + 0.753$$

with a standard deviation of y about the regression line equal to 0.41. The correlation coefficient between the logarithmic numbers of fecal coliforms/g of soil obtained by sonicated sodium tripolyphosphate was also 0.95 (Fig. 8) and

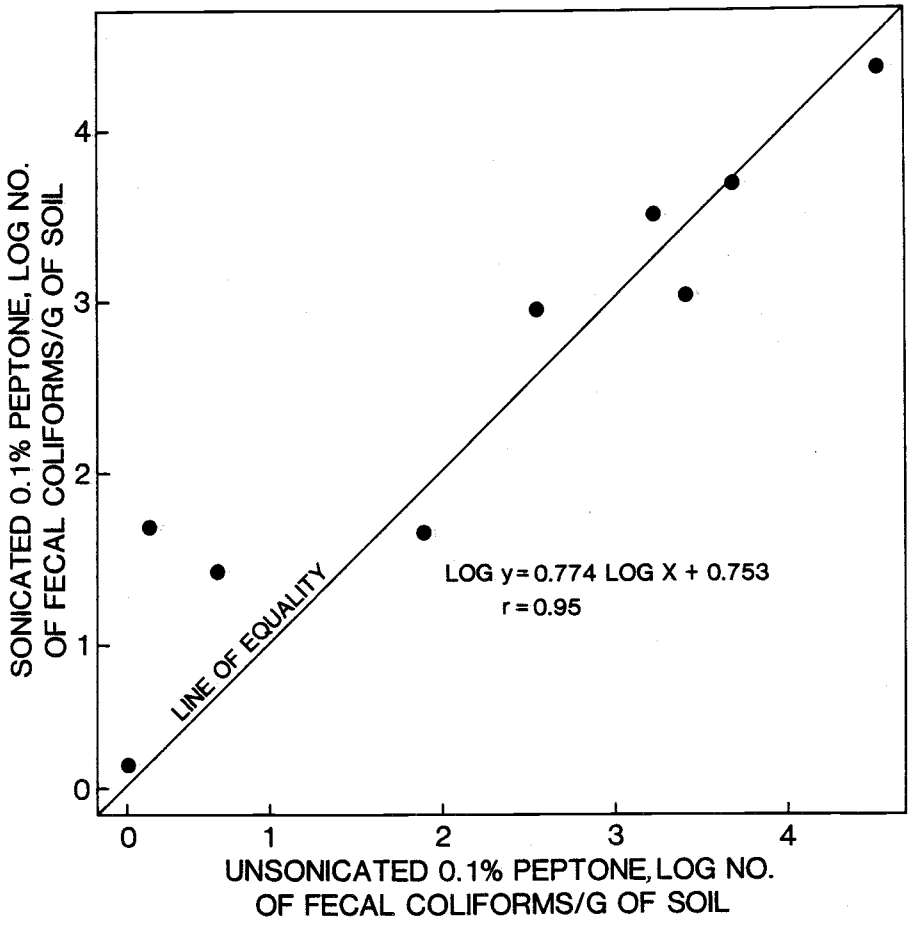


Figure 7. Comparison of log number fecal coliforms isolated per g of soil using sonicated versus unsonicated 0.1% peptone.

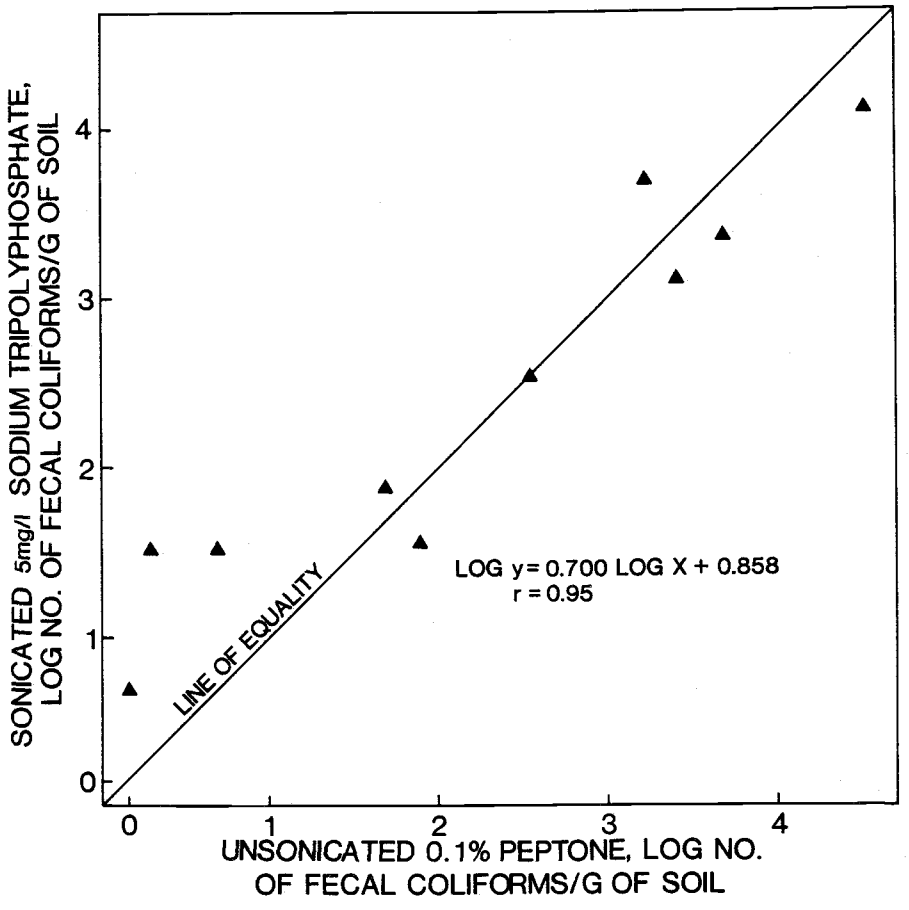


Figure 8. Comparison of log number fecal coliforms isolated per g of soil using sonicated sodium tripolyphosphate versus unsonicated 0.1% peptone.

is described by the line

$$\log y = 0.700 \log x + 0.858$$

with a standard deviation of y about the regression line equal to 0.37. Neither of the sonicated diluents were significantly different from the unsonicated 0.1% peptone ($p \leq 0.05$). Thus, neither changing diluents or subjecting the diluents to sonication gave significantly higher fecal coliform counts from sludge-amended soils than the current Standard Methods (APHA, 1971) procedure. The MPNs for all the control sites were negative.

2. Micro-MPN method

A comparison of MPNs between the polycarbonate and prepackaged sterile polystyrene plates showed no significant differences (data not shown).

Incubation times of less than eight or nine hours resulted in a loss of fecal coliform counts on the m-FC plates, while incubation times longer than nine hours had little or no effect (Table 9). As incubation times increased beyond eight hours in lactose broth however, the time required to count the m-FC plates decreased linearly to a minimum of 10 hours. Thus, lactose broth incubations of 8, 10, 12, 18, and 24 hours resulted in countable m-FC agar plates in 16, 14, 12, 10, and 10 hours, respectively.

A correlation coefficient of 0.86 was obtained when the micro-MPN was compared to the Standard Methods (APHA, 1971) fecal coliform MPN (Fig. 9). The line of best fit is

TABLE 9. EFFECT OF LACTOSE BROTH INCUBATION TIMES ON m-FC MOST-PROBABLE-NUMBERS.

| | Hours of lactose broth incubation | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 24 |
| | ----- log no. fecal coliforms/g of soil ----- | | | | | | | |
| Amity | 3.7 | 4.6 | 4.8 | 5.1 | 5.1 | 5.1 | 5.1 | 5.0 |
| Cloquato | 1.5 | 2.2 | 2.3 | 2.4 | 2.6 | 2.7 | 2.5 | 2.5 |
| Dayton | 3.0 | 3.9 | 4.1 | 4.1 | 4.2 | 4.2 | 4.2 | 4.2 |
| Woodburn | 3.9 | 4.7 | 4.9 | 5.1 | 5.1 | 5.1 | 5.1 | 5.2 |

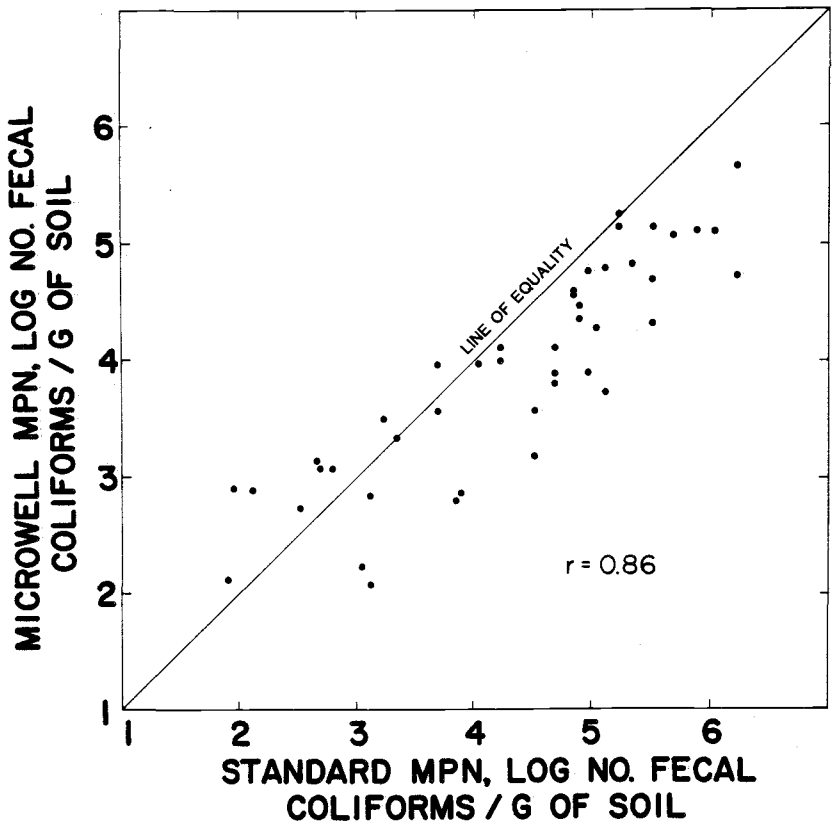


Figure 9. Comparison of log number fecal coliforms isolated per g of soil using the microwell MPN versus the standard MPN. Each point represents the combined corings of one sample site.

described

$$\log y = 0.69 \log x + 0.95$$

with a standard deviation of y about the regression line equal to 0.45.

3. Media variation

Of 3872 microwells, 3733 (96.4%) were m-$FC^+/EC^+$ or m-$FC^-/EC^-$, 139 (3.6%) microwells were m-$FC^+/EC^-$, and none was m-$FC^-/EC^+$. Entire plates were further assessed for m-$FC^+/EC^-$ variation. No discernible variation of m-$FC^+/EC^-$ microwells either over time or among the four sampling sites was observed (Table 10). All the control microwells were negative.

TABLE 10. NUMBER OF m-FC$^+$/EC$^-$ MICROWELLS PER MICROWELL PLATE.

| | Week | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| Dayton | 1 | 12 | 0 | 1 | 8 | 3 | 0 | 0 | 0 | 3 | ND[†] | ND |
| Cloquato | 3 | 8 | 2 | 3 | 4 | 1 | 3 | 1 | 4 | 5 | ND | ND |
| Woodburn | 0 | 7 | 0 | 0 | 13 | 2 | 1 | 10 | 2 | 7 | 3 | 4 |
| Amity | 1 | 2 | 0 | 0 | 2 | 2 | 1 | 1 | 10 | 3 | 4 | 2 |

[†]Not determined

# DISCUSSION

## A. Sludge-amended soil experiments

### 1. General

Soil is a complex ecosystem. Because of this complexity, it was necessary to exclude some environmental factors as unlikely to have any significant impact; these were: pH, soil texture, sludge type and amount, runoff, and outside sources of contamination. *E. coli* survives well in water with a pH range of pH 5 to 8 (McFeters and Stuart, 1972), and Cuthbert *et al.* (1955) observed that *E. coli* persisted in soils of pH 5.6, so the soil pHs among the four field sites were not considered low enough nor different enough to substantially affect fecal coliform numbers. It is noteworthy that acid soils are unlikely to be sludge-amended in any case because the acidity would exacerbate heavy metal toxicity (CAST, 1976) and U.S. federal regulations require sludge-amended soils to be $\geq$pH6.5 if the sludge Cd concentration is $\geq$2.0 mg/kg (USEPA, 1979). The latter would apply to the sludge used here. All four field sites had the same soil texture for the A horizon (Knezevich *et al.*, 1975), and all soil sampling was done in this horizon, so the effect of soil texture should be relatively the same for all. All the field sites received the same amount of anaerobically-digested sewage sludge, so the type and

amount of sludge is not in question although the composition is. Runoff was severely restricted in these experiments by selecting soils that had slow runoff potentials, by rototilling each site, and by locating the two poorly-drained plots, Amity and Dayton, in shallow swales. Given that appreciable numbers of bacteria are only lost to runoff under periods of high rainfall and when indicator organisms exceed $10^4$ cells/g of soil (Van Donsel et al., 1967), conditions which only existed in the winter sampling period for a short time, it is probable that runoff was negligible in these experiments. It is noteworthy that sludge is often injected or tilled into the soil because of odor and aesthetic objections, and these treatments would also limit runoff. The absence of fecal coliforms from the field sites before sludge amendment as well as the control sites during the sampling periods also suggests that fecal coliform contamination from outside sources was unlikely.

Although Geldreich et al. (1962) recommend the completed MPN when examining soils for fecal coliforms because of false positives, only the confirmed test was performed here because all the field sites were negative for fecal coliforms before sludge amendment.

2. Winter sampling period

In studies of fecal coliform movement in subsurface soil horizons under conditions of saturated flow, Hagedorn et al. (1978) observed that rapid movement of fecal coli-

forms occurred under saturated flow with only a 2% surface gradient, and that fecal coliform movement was closely associated with a water table rise following major rainfall periods. Movement of fecal coliforms was greatest during the first major rainfall period and less for subsequent periods. These observations were also noted here. Several other points are noteworthy. First, it is apparent that fecal coliforms can move from the soil surface under saturated flow similar to that for subsurface soil horizons. Second, the movement of microorganisms through soil is in two distinct steps: first, movement downward with percolating water, and second, lateral movement once the microorganisms have reached the groundwater (Romero, 1970). In this study, the sampling wells only assessed groundwater. Thus, if bacterial movement occurred principally during the first rise of the water table, then the critical sampling period during the first few days of winter rainfall was not assessed, and moreover, the lateral movement of the groundwater could have caused considerable fecal coliform dispersion. This might explain why fecal coliform numbers were low in the sampling wells.

The fluctuation of fecal coliform numbers during the sampling period suggests not only a relationship between fecal coliform numbers and rainfall, but also between nutrient availability as well. Three of the four major rainfall periods are marked by a pattern of fecal coliform regrowth and decline during nonsaturated and saturated flow

conditions, respectively. Saturated flow conditions cannot be conclusively demonstrated for the Cloquato and Woodburn field sites however because the water table levels were not recorded, although water tables at <100 cm depth should have occurred (Boersma et al., 1972; Simonson and Boersma, 1972). Nonetheless, the circumstantial evidence is strong that saturated flow did occur on these sites because, with one exception, the pattern of decline in fecal coliform numbers is identical to that of the poorly-drained Amity and Dayton sites. The one exception, the delay in decline of fecal coliform number during the first rainfall period on the most well-drained field site, Cloquato, may reflect the delay in achieving saturated flow. Following cessation of saturated flow, regrowth of fecal coliforms occurred. Given the conditions necessary for growth to occur, this must be a period of high nutrient availability. It is unlikely that these increases are a result of soil dilution because fecal coliforms were observed in the sampling wells and regrowth following a rainfall has been noted before (Van Donsel et al., 1967). This cycle continues until the end of the third major rainfall period whereupon the pattern ceases. This latter period probably reflects low nutrient availability such that strong regrowth is no longer possible during nonsaturated flow, yet numbers of fecal coliforms are sufficiently low that filtration and absorption prevent their loss when saturated flow does occur.

Solar radiation was of little consequence during the winter. It is impossible to assess if cooler temperatures lengthened fecal coliform survival because of fecal coliform loss through saturated flow, but freezing temperatures were probably of insufficient severity both in relative and temporal terms to have any effect.

3. Summer sampling period

The summer sampling period was also typical for the Willamette Valley with warm, dry weather. Therefore, in contrast to the winter sampling period, sunlight and high temperatures were expected to have a pronounced effect on fecal coliform survival. They did not.

Reddy et al. (1981) predicted, based on the data presented by several workers, that the die-off rate of an organism approximately doubled with a 10°C rise in temperature. Discounting the one period of regrowth during the third and fourth weeks of the sampling period, the die-off rates during the first 23 days are clearly not that dissimilar from the die-off rates during the last 68 days although the two periods differ in temperature by 10.2°C. The most reasonable explanation for this is that the first 23 days is a period of high nutrient availability, and this has already been shown to have protective effects (Ostrolenk et al., 1947; Klein and Casida, 1967b). Moisture was also optimal.

Solar radiation also had little effect although the radiation levels were almost twice as high during the first 23 days compared with the rest of summer sampling period. This agrees with Van Donsel et al. (1967) who noted that a reduction of coliforms during dry periods was not a function of solar radiation because declines in cell numbers were similar on shaded and unshaded sites.

The pattern of fecal coliform survival was variable during the first 23 days. The reason for this is unclear, but may be because of lack of a predominant variable such as rainfall that occurred during the winter.

Van Donsel et al. (1967) and Chandler and Craven (1978) noted growth of coliforms or E. coli on inoculated field sites following a rainfall. A similar observation was made here following the first rainfall for two of the four field sites. Two features are noteworthy. First, rainfall did not bring about a decline in fecal coliform numbers similar to that of the winter sampling period. This is probably because saturated flow conditions did not exist, although the evidence is not conclusive because water tables on the sites were beyond measurement. As in the winter sampling, the results agree with Van Donsel et al. (1967) that the magnitude of bacterial growth is not proportional to the rainfall amount, because high rainfall in the winter gave comparable fecal coliform increases to that of the summer when rainfall amounts were smaller. Second, fecal coliform growth did not occur on the Cloquato and Dayton sites.

This is because of nutrient availability. Although all field sites received the same amount of sludge, the percent solids of the sludge applied to the Amity and Woodburn field sites was 2.5-fold higher than the sludge applied to the other two sites, and comparable to the 5.0% solids of the sludge used in the winter application. The effect of a higher percent solids would be higher nutrient availability. During the second rainfall period, fecal coliform growth is negligible, similar to the response during the winter application. Again, it is likely that nutrient availability is low. Thus, only under conditions of high nutrient availability and high moisture (but not saturated flow) can fecal coliform growth occur.

4. Sludge crust

McCoy and Crabtree (1970) and Crane et al. (1980) suggested the effect of sludge crusting was one of lowering the impact of various adverse environmental variables such that bacterial survival was prolonged. Similar results were observed here. Fecal coliform numbers remained relatively constant in the 0 to 2.5-cm soil depth of the Dayton silt loam, thus it is likely that the sludge crust served either as a continuing source of nutrients or fecal coliforms or both for the soil below.

The retention of virtually 100% of the fecal coliforms in the sludge crust and 0 to 2.5-cm soil depth suggests that Dayton silt loam was highly effective in preventing fecal

coliform movement and agrees with others (Mărculescu and Drocan, 1962; McCoy and Crabtree, 1970) that soil is an effective filter.

5. Summary

Many of the factors affecting fecal coliform survival are interdependent and constantly changing, so it is impossible to define the factors completely. The results agree with the consensus that two to three months is sufficient to reduce bacterial pathogens to negligible numbers (Gerba et al., 1975).

The use of first-order die-off rate constants for fecal coliforms for modelling purposes as suggested by others (Crane et al., 1980; Reddy et al., 1981) is premature. Reddy et al. (1981) give a range of die-off rates for fecal coliforms ranging from 0.08 to 9.1/day. The rates obtained during the winter and summer sludge applications as well as the sludge crust experiment in this study were below the low end of this range. The reason for this was that fecal coliform growth occurred. Growth under any circumstances violates the assumption of the simple logarithmic model which proposes that bacterial death is continuous and proportional to the number of viable cells present. Furthermore, some evidence exists (Chandler and Craven 1978) that fecal coliform death is biphasic, with an initial sharp decline followed by a slower decline.

The loss of fecal coliforms through saturated flow suggests that this is another variable which must be ascertained when determining bacterial pathogen survival. Furthermore, it raises questions of groundwater contamination that must be addressed if landspreading of sludge is to continue.

B. Micro-MPN experiment

1. Diluent test

Because neither the use of sodium tripolyphosphate nor sonication gave statistically higher fecal coliform counts from the sludge-amended field sites than the unsonicated 0.1% peptone, and because the latter does not involve the cost of a sonicator, 0.1% peptone remains the diluent of choice for enumerating fecal coliforms from soils. One possibility for the failure of sodium tripolyphosphate is that autoclaving results in the hydrolysis of tripolyphosphate to the orthophosphate (Pike et al., 1972). The latter is known to be an inferior diluent (Straka and Stokes, 1957).

2. Micro-MPN method

A nine-hour lactose broth incubation period was selected for use with the microwell plates. The underestimation of fecal coliform numbers in lactose broth incubated less than seven or eight hours is probably the result of insufficient time for the fecal coliforms to recover from

environmentally-induced physiological injury. The need for recovery time for environmentally-stressed E. coli has been noted before (Bissonnette et al., 1975).

The statistical difference between the micro-MPN and the standard fecal coliform MPN is most likely because of the increased endpoint accuracy of 2-fold serial dilutions over 10-fold serial dilutions. Both Cochran (1950) and Rowe et al. (1977) discuss this in detail. Furthermore, a 3-column MPN code was used instead of a $\geq$4-column code. Although a 4-column code would give greater precision, for example, a 3-column code was used because of its simplicity. On the other hand, the use of the slightly less restrictive m-FC agar would increase MPNs.

The major disadvantage of this microtechnique is the initial cost of materials. The cost of sterile polystyrene plates was minimized by substituting reuseable polycarbonate plates. A simple glove box works well instead of a laminar flow hood (D. Y. C. Fung, personal communication). The polypropylene tips of the 8-channel micropipet can also be reused.

The advantages of this microtechnique are a substantial savings in both time and media. The technique is especially worthwhile for nonfilterable environmental samples.

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