

SMALL SCALE WASTE MANAGEMENT PROJECT

**The Effects of Effluents On Groundwater:
Bacteriological Aspects**

by

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ON GROUNDWATER: BACTERIOLOGICAL ASPECTS

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ABSTRACT

Counts of indicator bacteria (total coliform TC, fecal coliform FC, fecal streptococci FS) and the pathogens *Salmonella* spp., *Pseudomonas aeruginosa* and *Staphylococcus aureus* groups can serve as parameters of purification. Effluents from Small Systems treatment contained unacceptably high counts for release to surface waters without disinfection, but acceptable for subsoil release; numbers ranged TC $> 10^3 - 10^6$, FC $> 10^2 - 10^5$, FS $10^2 - 10^3$ and *P. a.* $10^2 - 10^3$ /100 mls. *Salmonella* spp. and *S. a.* were of the order of 1-2 logs less; incidence among families was low.

Data from a cross section of an absorption field of a septic tank system showed dramatic retention of these bacteria at the basal clogged zone, with $< 200/100$ g in the soil at 1-2 ft distance. Similar retention in experimental sand and silt loam columns showed greatest efficiency under partial clogging and unsaturated flow regime with low dosage. Removals of 4 log numbers or more were achieved. Less efficiency with channeling and overloading was also shown. Literature was cited showing possible movement of bacteria under some conditions.

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Introduction

In fresh raw sewage the kinds and numbers of bacteria are predominantly those of intestinal origin. The gut offers an excellent environment for growth of bacteria such as the coliform group, fecal streptococci, lactobacilli and numerous minor groups including some pathogens, if the host is currently infected. As the fresh sewage passes through treatment, conditions change and new types grow and compete with the intestinal bacteria. The microflora of sewage is thus a complex mixture of bacteria growing and dying as they find the opportunity. In the aggregate they are capable of biodegradation of numerous chemical compounds in the waste. Thus the bacteria are useful, in fact, essential to the treatment process. And in this respect, they differ from the viruses, which are merely carried along without multiplication after leaving the body.

This paper will address the topic of bacterial content of the sewage and of key stages of treatment as related to the quality of the effluents and their potential danger to

groundwater. The data presented will be taken from our project, Small Scale Waste Management Project at the University of Wisconsin-Madison, and will include on-site soil disposal where, in principle, the final step in treatment occurs as the water is absorbed and percolates to groundwater.

Bacteriological criteria

From the safety point of view determination of certain types of intestinal bacteria and of actual pathogenic bacteria will serve as criteria of quality of sewage water during and at the end of treatment. Thus counts of the fecal indicators (total coliform, fecal coliform, fecal streptococci) and such pathogens as *Salmonella* spp., *Pseudomonas aeruginosa*, and *Staphylococcus aureus* groups are presented. Needless to say, there are some problems in the interpretation of such counts, both inherent in the methods and in relation to what might be called "background" numbers of these bacteria in the wild due to their survival and possible growth and to wild animal and plant contributions. These topics have been reviewed previously 5,6,12,21.

There are also problems in applying "Standard Methods" procedures for these counts to some of our types of samples, for example, soil absorption beds where the soil microflora pre-exists with massive numbers and kinds of competing bacteria. This was particularly the problem in our tracing of pathogens, salmonellae, pseudomonads, and staphylococci. For them it was necessary to develop special procedures of enrichment culture and recovery. These methods, as now in use

in our laboratory, are given in brief in the Appendix.

Bacterial Quality of Various Wastewater Effluents

Groundwaters, used as potable water sources, may be contaminated by improperly functioning wastewater disposal systems. Many of these disposal systems are small scale for treatment of wastewater from individual homes or institutions and rely on soil as the principal medium for final purification.

Table I gives typical bacterial contents of wastewater at various stages of (small scale) treatment. The effluent bacterial counts meet acceptable levels (< 200 fecal coliforms /100 mls) for discharge to surface waters only after disinfection. However, even after disinfection, such effluents are still of questionable quality for direct discharge to groundwater which may be drawn and used without pretreatment.

Pathogenic bacteria, as *Pseudomonas aeruginosa*, have been consistently found in sewage effluents at all stages of treatment. Although *Staphylococcus aureus* and the salmonellae have a much lower incidence in the individual family system (see Table II) their demonstrated presence emphasizes the concern for proper purification of wastewaters before reaching potable waters.

Bacterial Removal from Wastewater During Percolation In Soil

It is difficult to specify a depth of soil through which wastewater must percolate to remove potentially hazardous levels of sewage bacteria. Survival and movement of sewage

Table I. Bacteriological quality of some effluents from small scale treatment units.

Effluent	Bacteria per 100 mls				
	Fecal streptococci	Fecal coliform	Total coliform	<i>Pseudomonas aeruginosa</i>	Total bacteria
	\bar{X}_g 95%	\bar{X}_g 95%	\bar{X}_g 95%	\bar{X}_g 95%	\bar{X}_g 95%
Septic tanks; 5 sampled, 2-13 day detention 2	3800 (97) 2000- 7200	420,000 (94) 290,000- 620,000	3,400,000 (91) 2,600,000- 4,400,000	8600 (33) 3800- 19,000	34 x 10 ⁷ (88) 25-48 x 10 ⁷
Aerobic units; 3 sampled, 2-4 day detention 2	3300 (70) 2300- 4800	11,000 (67) 7400- 15,000	110,000 (74) 71,000- 160,000	1200 (33) 530- 2700	18 x 10 ⁷ (80) 7.4-28 x 10 ⁷
Sand filters; 1 reported, 5 gpd/ft ² , septic tank effluent 3	90 (14) 20- 140	840 (13) 140- 4900	1400 (13) 190- 9500	55 (10) 17- 180	45 x 10 ⁵ (11) 8.6-240 x 10 ⁵
Disinfection units: Chlorination; detention= 24 hr resid. Cl= 1 mg/l HOCl dry feed ³	2 (12) 0.6- 7.0	2 (11) 0.5- 12	3 (11) 0.5- 16	3 (15) 1.8- 4.9	10 x 10 ⁴ (11) 1.3-79 x 10 ⁴
UV irradiation; flow= 3.7 gpm, turbid.= 17 JTU, TSS= 29 mg/l ⁴		48 (49) 27- 86		7.2 (31) 3.5- 15	39 x 10 ⁴ (33) 23-67 x 10 ⁴

\bar{X}_g = geometric mean; 95% = confidence interval of \bar{X}_g ; #'s in parentheses = # of samples

¹ Ziebell, W.A., unpublished data, 9 septic tanks sampled; ² From reference 28;

³ From reference 22; ⁴ Ziebell, W.A., unpublished data

Table II. Detection frequency for *Staphylococcus aureus*¹ and *Salmonella* spp.² in wastewater from 12 residences.

Residence	<i>Staphylococcus aureus</i> ¹		<i>Salmonella</i> spp. ²	
	# of samplings	# positive	# of samplings	# positive
A	16	0	8	0
B	14	1	6	0
C	5	3	1	0
D	6	0	3	0
E	8	1	10	0
F	7	1	9	1
G	4	0	—	—
H	1	0	6	0
I	1	0	7	0
J	1	0	1	0
K	—	—	2	0
L	—	—	2	1
Total # samples	63		55	
Total # positive	6		2	
% positive	9.5		3.6	
Range for +'s	10 - 1000/100 ml		3.4 - > 20/100 ml	

¹Deininger, J.F., and Elizabeth McCoy, unpublished data²From reference 18

bacteria in soil have been reviewed by Gerba et al.⁶. Soil type, temperature and pH; bacterial adsorption to soil and soil clogging; soil moisture and nutrient content; and, bacterial antagonisms are factors which affect bacterial survival and movement.

Another key factor regulating bacterial removal from wastewater during percolation is the liquid flow regime in the soil. The following data will show that higher degrees of purification can be achieved under unsaturated flow regimes, particularly in non-aggregated soils (as sands, loamy sands, etc.). Unsaturated (as compared to saturated) flow involves liquid movement through only the smaller soil pores, increasing the contact of wastewater with soil particles as well as the liquid detention time in the soil¹. Butler et al.² found the removal of bacteria during percolation to be proportional to the inverse of the particle size in sands.

Unsaturated flow can be attained by two general methods, the first being application rate: continuous ponding without clogging (as during periods of local overloading in an absorption field) results in flow conditions approaching saturation, while dosing uniformly over the field surface (particularly at low doses) provides unsaturated flow. Secondly, soil surface clogging (as created by organic material build up or smearing of the infiltrative surface) decreases the infiltration rate into the soil, promoting unsaturated flow.

Figures I and II give bacterial removal from septic tank effluent by 2 feet deep columns of Plainfield loamy sand

(C Horizon, effective size =0.14 mm, uniformity coefficient =1.99). These columns at 25 C were dosed with septic tank effluent containing an average of 5.1×10^6 fecal coliforms (FC), 7.3×10^6 fecal streptococci (FS), 1400 *P. aeruginosa* (*P. a.*) and 10^2 - 10^4 *S. aureus* (*S. a.*) per 100 ml. Loading rates (one application per day) were 10 cm/day (2.4 gpd/ft^2) and 5 cm/day (1.2 gpd/ft^2) for column 1 and 2, respectively. Chloride tracer studies indicated these application rates resulted in liquid retention times of 1.5 hours (10 cm/day dose) and 26 hours (5 cm/day dose). Soil moisture tensions 5 cm below the soil surface and outflow volumes are also given in Figures I and II.

During the first 100 days of operation a relative plateau of removal was reached by both columns. Column 1, at the high loading, removed approximately 92% of the fecal coliforms applied per day while in column 2, loaded at one half the rate of column 1, removal was about 99.9%. Similarly, the effect of high loading resulted in fecal streptococci and three isolations of *P. aeruginosa* in the effluent of column 1. These organisms were not detected in column 2 effluent. *S. aureus* was never detected in effluent of either column.

Under field conditions the most critical phase of operation corresponds to this first 100 days in the column studies. During this time high loading rates and localized overloading may effect transport of pathogenic organisms deeply into the soil. A newly constructed septic tank disposal system was implicated as the source of well water contamination (causing

60 cases of gastroenteritis) in an Illinois State Park ¹⁴.

The septic tank had been installed at the appropriate distance from the well; however, during installation groundwater was observed at 10 feet from the soil surface and the well water was reported to be turbid for a short period after installation.

After 100 days of loading a "clogging zone" developed at the top of each column. The FC count in both effluents dropped and reached a constant level (between 10-100 FC/100 ml), even though outflow volumes remained constant and ponding did not occur.

The effects of such clogging zones in trapping indicator bacteria have been reported by others ^{2,8,11,20}. The clogging plays a significant role in purification of wastewater effluents in soil. Figure III gives bacterial counts through cross section of an absorption field, demonstrating the significance of the clogging phenomenon in field systems.

Silt loam (aggregated) soils have a lower capacity (compared to non-aggregated soils) to conduct liquid and the majority of flow is through larger pores between soil aggregates ¹.

The effect of flow regime on bacterial removal in such soil was also investigated. Septic tank effluent (described above) was applied to *intact* (2 feet deep) cores of Almena silt loam, A2 and B21 horizons. Duplicate columns, designated 7 and 8, received 1 cm/day doses at 25 C. Results are given in Figures IV and V.

Under the unsaturated flow conditions associated with

the given loadings, effluent-short circuiting through large pores between soil peds (and/or those pores resulting from faunal activity or root channels) was observed for both columns.

Large numbers of indicator bacteria and *P. a.* were found in effluent of column 7 (Figure IV). The loading rate to column 7 was reduced from 1 cm/day to 3 mm/day to test the hypothesis that further reduction in loading would promote absorption of liquid into the soil peds. Consequently, bacterial removals increased drastically, reaching < 2 FS, FC and *P. a.* per 100 ml of effluent. High numbers of these organisms again passed through the 2 feet of silt loam after the loading was restored to 1 cm/day.

Fecal coliform and fecal streptococci were also observed in effluent of column 8 during the first 40 days of loading. At this time the saturated conductivity of column 8 soil fell below 1 cm/day and saturated flow (with ponding) resulted. Under the saturated conditions bacterial removal was enhanced; neither indicators nor pathogens were observed in the effluent after saturated flow occurred.

Short-circuiting could result in contamination of surface or groundwaters in close proximity to soil absorption systems in such aggregated soils, especially during dry periods when voids between peds become large due to soil drying and shrinking. Contamination of curtain drains placed 3 feet from an absorption trench in silt loam soil have been found²⁹. Also, an absorption system has been reported to cause contamination of shallow groundwater under similar soil

conditions ²⁶.

Groundwater Contamination and Associated Disease Potential

Although high degrees of purification can be achieved by relatively small depths of soil (as discussed above), cases of groundwater contamination resulting in disease are known. In 1973, contaminated, untreated groundwaters were responsible for 8 of the 24 (33%) waterborne disease outbreaks in the U.S.; resulting in 190 cases of illness ⁴. Seven of these outbreaks were associated with semi-public (5) and individual (2) water supplies.

Prevention of such disease potential requires consideration of the extent of movement and survival of pathogens in groundwater. The time required for 50% reduction in numbers of certain pathogenic and indicator bacteria, in fresh well water, ranged from 2.4 hr (*Salmonella enteritidis* ser. *paratyphi* B) to 26.8 hr (*Shigella flexneri*), and averaged 22 hr for fecal streptococci and 17 hr for coliforms ¹³. Such relatively high survival rates allow pathogens to remain in high enough numbers to cause disease even after considerable travel (time and distance) in groundwater. *Shigella sonnei* was implicated as the causative agent of 1200 cases of gastrointestinal illness in Richmond Heights, Florida, when a city well was contaminated by the septic tank disposal system of a church/daycare center 150 feet away ¹⁶.

Bacillus stearoothermophilus used as a tracer bacterium, has been found to travel 94 feet in groundwater in areas having creviced bedrock ¹⁷; this indicates that highly porous

aquifers could provide conditions in which contamination of wells may result at considerable distances from the source of pollution. Four members of a family in Yakima County, Washington contracted typhoid fever (*Salmonella typhi*) as a result of well contamination from a disposal system 200 feet from the well ¹⁵. Wells, in this low land area near the Yakima River, draw from shallow groundwater in gravel. Dye, introduced into the disposal system at the household of a *S. typhi* carrier was found in the contaminated well (and other wells in the area) within 36 hours.

The extent of bacterial movement in groundwater has also been considered in recent reviews ^{6,20}.

Discussion

While it has been shown that remarkable purification can be achieved in non-aggregated soil under conditions of established (partial) clogging and proper flow regime, it must be remembered that many soil conditions are less efficient in providing bacterial removal. During initial periods of operation (prior to clogging) conventional soil absorption systems do not provide ideal removals. Similarly, channeling, effected by voids between soil aggregates, can result in movement of bacteria to depths of 2 feet or more in aggregated soils, especially under dry conditions. Under such conditions a deep soil profile (or further treatment of effluent) must be present to insure adequate purification.

It is impossible to state with certainty the precise number of feet of soil which will retain contaminants. Three

types of soil conditions which would prevent safe soil disposal are: 1) shallow soils over creviced bedrock, 2) shallow soil over high groundwater tables, and 3) impermeable soils. Consideration should therefore be given to adequate treatment, including disinfection if necessary, before soil disposal of the effluent in such areas.

In recognition of the retention capabilities of soil, our Small Scale Waste Management Project has written guidelines to be used on an experimental basis in some problem areas of Wisconsin. These guidelines delineate procedures for installing sand-fill systems (mounds), 2 feet in depth, over a 2-5 feet minimum of naturally occurring soil. Such systems employ pressurized distribution to insure proper loadings and flow conditions.

Summary of Findings

1. The capability of soil for purification of wastewater by removal of bacteria has been demonstrated with experimental data.
2. Limitations on bacterial removal have been recognized and discussed.
3. Maximum purification of wastewater effluents should be achieved, either through adequate pretreatment or adequate adsorption in unsaturated soil, before percolates reach the groundwater table.

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Legends for Figures

Figure I. Bacterial and physical data from column 1 (sand, 25 C, 10 cm/day loading): [a] bacteria in column effluent, FC = fecal coliforms, FS = fecal streptococci; [b] X's represent soil moisture tensions 5 cm below sand surface before daily dosing, drainage curves given have the same linear time scales, each curve giving tensions for approximately 150 minutes after dosing on the day indicated; [c] daily volume of effluent leaving the column. The numbers [a], [b] and [c] have identical meaning in Figures II, IV and V. Data from reference 27.

Figure II. Bacterial and physical data from column 2 (sand, 25 C, 5 cm/day loading): LC = loading change, i.e., first change from one 5 cm/day dose to three applications per day of 1.7 cm each, and second change restoring the 5 cm/day dosage regime. Data from reference 27.

Figure III. Cross section of an absorption field in sandy soil with bacterial counts at various points in the field. Data from reference 28.

Figure IV. Bacterial and physical data from column 7 (silt loam, 25 C, 1 cm/day loading): LC = loading change, i.e., first change from 1 cm/day to 3 mm/day and second change restoring 1 cm/day. Moisture tensions [b], 5 cm below soil surface, are prior to daily dosing. Data from reference 27.

Figure V. Bacterial and physical data from column 8 (silt loam, 25 C, 1 cm/day loading). Moisture tensions [b], 5 cm below soil surface, are prior to daily dosing. Data from reference 27.

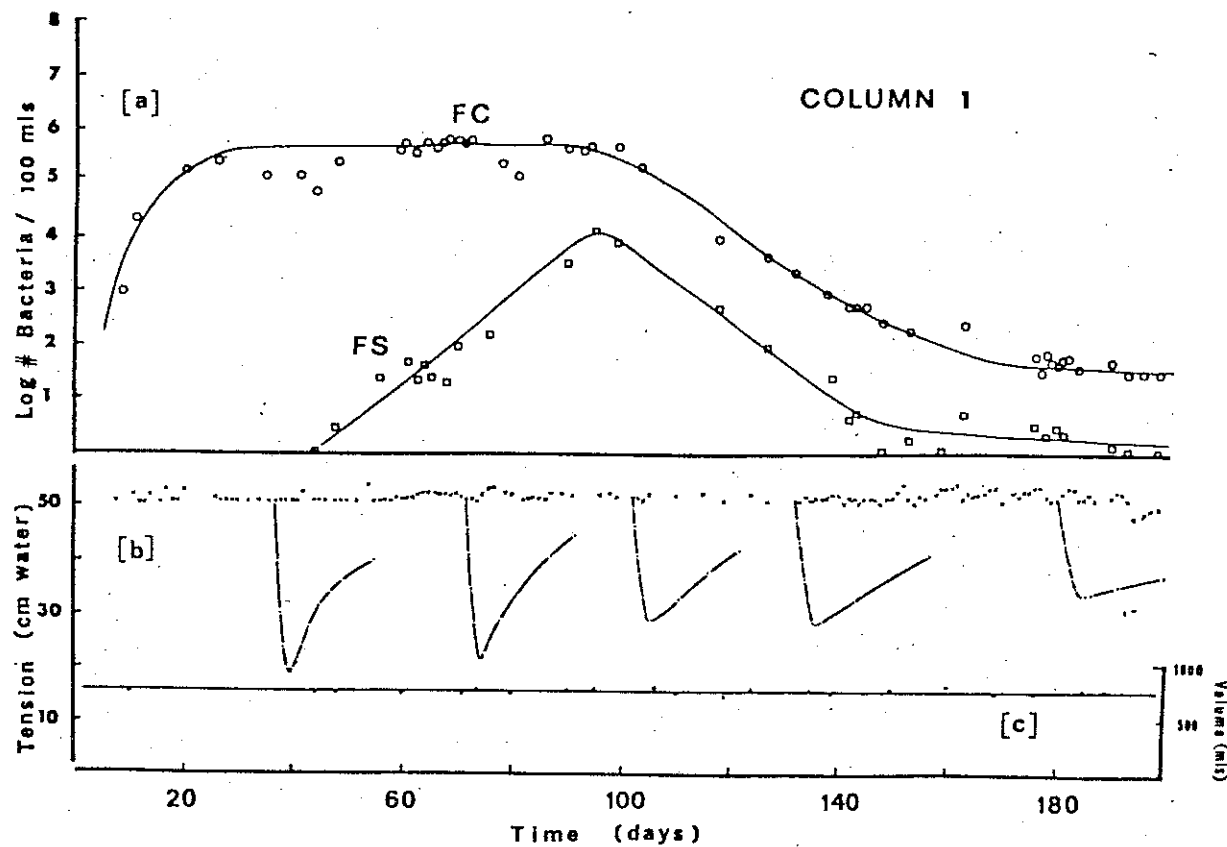


FIGURE I.

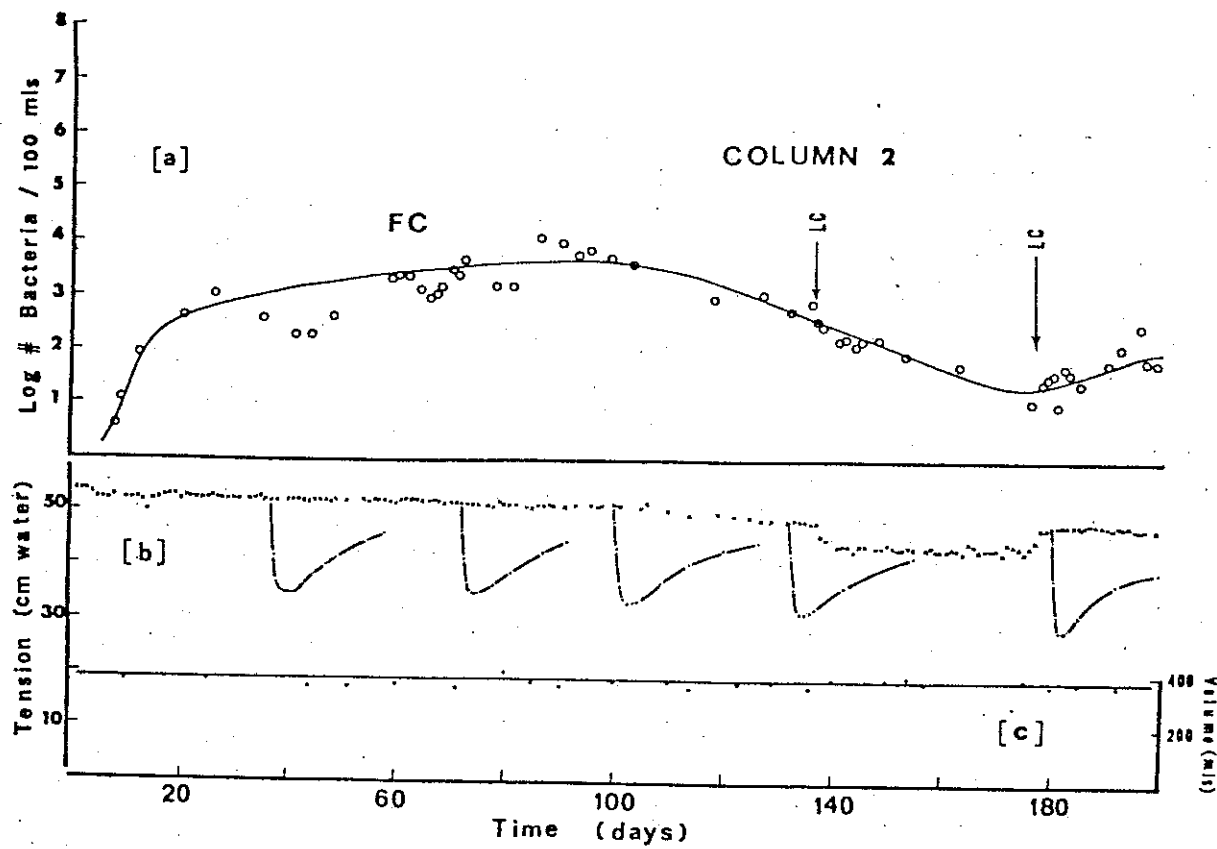


FIGURE II.

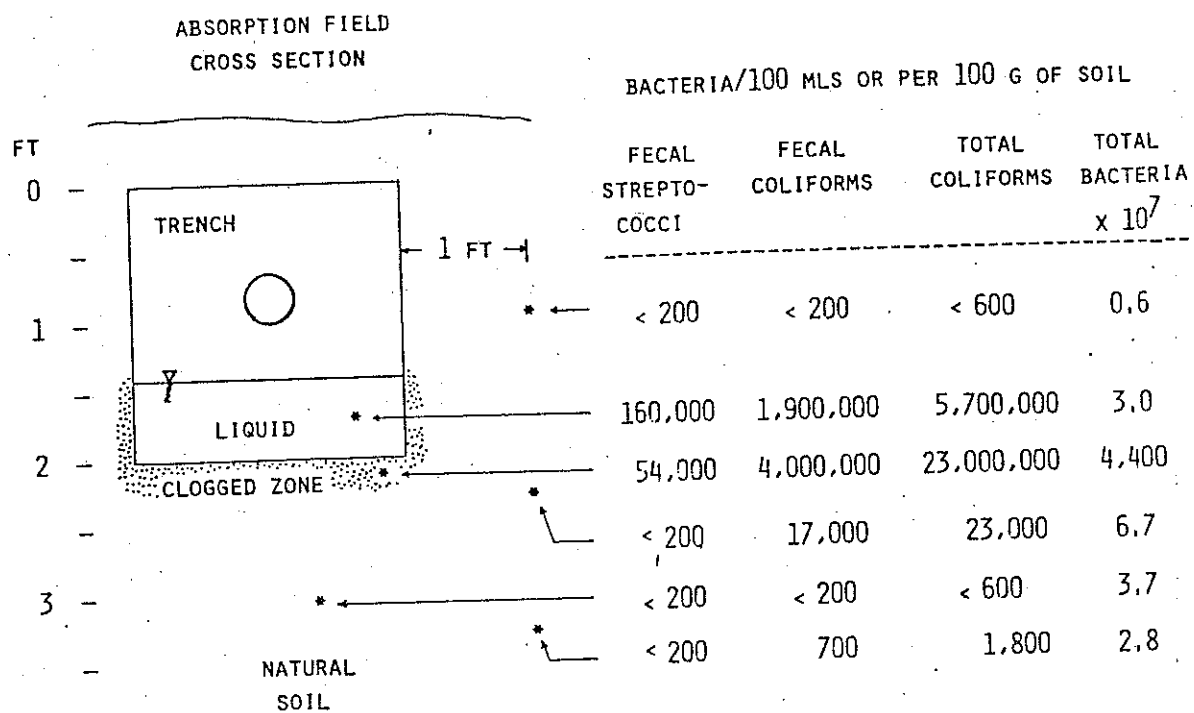


FIGURE III.

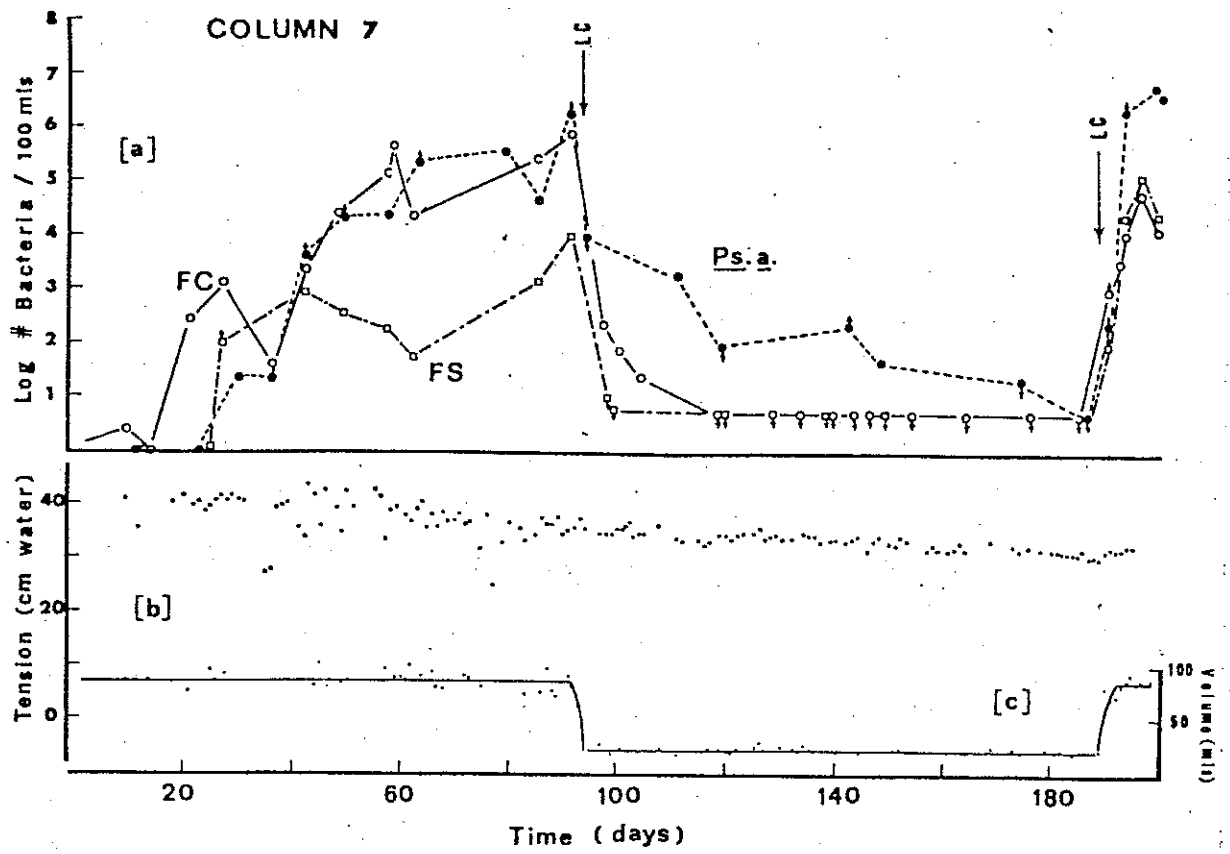


FIGURE IV.

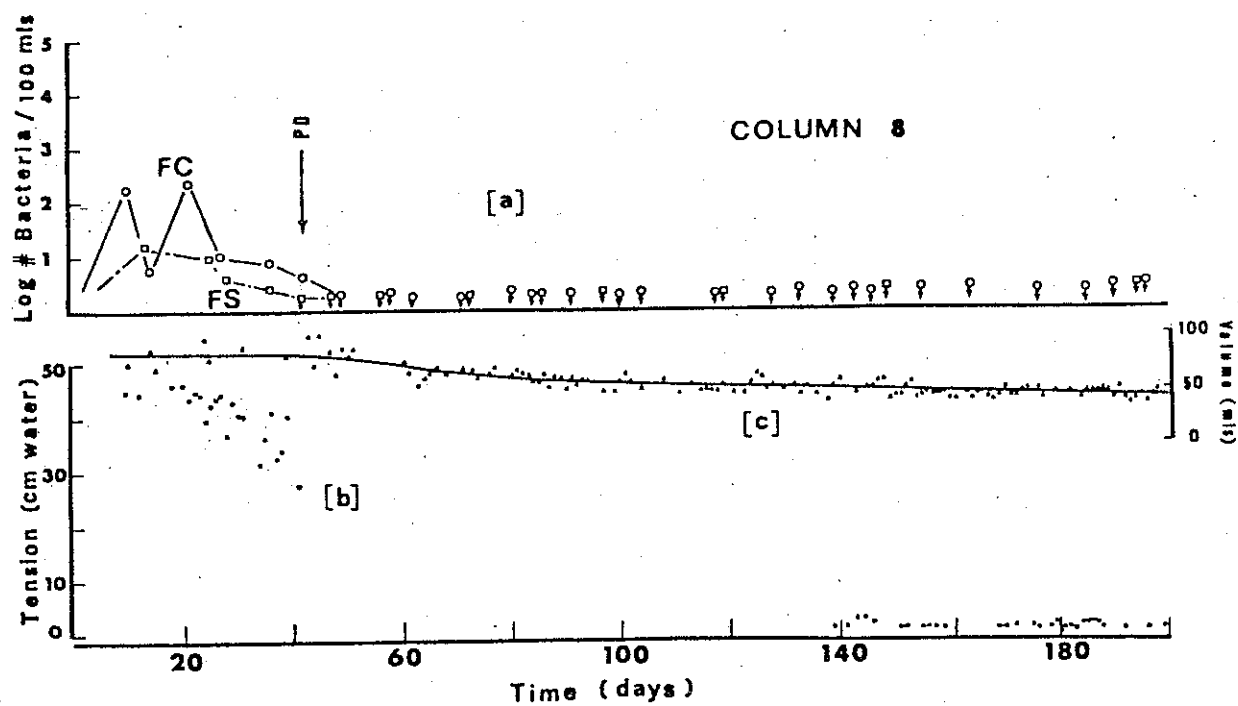


FIGURE V.

APPENDIX

Pathogens in Sewage

It is a generalization of medical bacteriology that man is subject to various enteric diseases during which pathogens are shed in the feces in tremendous numbers. Most patients cease to shed after recovery but some continue as carriers, even though apparently well. In a single family sewage there may or may not be pathogens, depending upon family history of enteric disease, but it should be emphasized that disease is potential at any time. Thus the Most Probable Number (MPN) for these pathogens in treated sewage is indicative of the potential danger to groundwater.

While Standard Methods ²⁵ offers procedures for counting the pathogens in question, it has been our experience that they lack the necessary specificity and also present mechanical problems. Especially for some of the types of samples we deal with, e.g., absorption beds and soil columns where the sewage microflora merges with the massive soil microflora, the task of detecting and counting the sewage pathogens proved very difficult. A full report on the development and testing of our methods is in preparation. Only a brief statement of the procedures will be given here as background for the counts of pathogens presented in this paper.

1. *Salmonella* spp.

Preparation of samples. Since the family is, or should be negative for salmonellae, it was necessary 1) to use large

volumes for samples, 2) to enrich for salmonellae in the most sensitive way, and 3) to confirm isolates by biochemical and serological tests.

The usual procedure of collecting the bacteria by filtration upon membrane filters was impossible for many samples; the filters clogged so rapidly that even 5-50 ml could not be passed. By centrifugation in 250 ml cups and compositing of 2-4 sediments in suitable dilution blanks, it was possible to run MPNs on 1000, 100, 10 ml of sample as needed.

Detection of salmonellae. Enrichment was in Tetrathionate broth under 1-2 cm of mineral oil (to avoid the aerobic pseudomonads) for 40 hr at 41.5 C. All Positive growth tubes were plated by streaking on Brilliant Green agar; after growth for 24 hr at 41.5 C the pink-white opaque colonies with red halos were picked to Triple Sugar-Iron agar slants. Identification of the presumptive positives was indicated 1) by red (alkaline) slants with yellow (acid) or yellow with gas bubbles in the butts, and evidence of H_2S by black iron sulfide precipitate, and 2) by agglutination with polyvalent H flagellar antiserum ²⁴. This combination of characters allows the readings to be expressed as MPN of *Salmonella* spp. Type identification was ordinarily not done but occasionally was, by courtesy of the Wisconsin Laboratory of Hygiene. The accuracy of our detection method is attested by the fact that all cultures submitted to the Hygiene Laboratory were indeed salmonellae. The method is sensitive, as shown by tests of salmonella-negative sewage with known numbers of *S. enteritidis* ser anatum added and the successful recoveries of 2-5 cell.

2. *Pseudomonas aeruginosa* group

Pseudomonas aeruginosa is considered an opportunistic pathogen, a common cause of severe ear, sinus and burn infections and some enteric disorders of infants ¹⁹. It is said to be shed by approximately 15% of all persons, and thus is more likely to be present in a single family sewage than are salmonellae. It also occurs in sufficient numbers in sewage to offer statistically valid data ⁷. For MPN counts of *P. aeruginosa* a 5 tube series is used with an Acetamide broth based on Standard Methods ²⁵ medium with its phenol red omitted plus $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5 g), KNO_3 (0.5 g), Na citrate (0.2 g) and Proteose Peptone #3 (0.2 g) added to favor *P. aeruginosa* over the saprophytic pseudomonads of sewage and also importantly to favor the blue green pigmentation. Incubation is at 40 C for 4 days with reading of MPN on the basis of fluorescence under UV illumination. High dilution positive tubes are streaked onto King's A agar ⁹ and incubated at 42 C for 24 hr to confirm the typical blue green pyocyanin pigment.

For family sewage the MPNs for *P. aeruginosa* run 10^3 to 10^4 /100 ml by this procedure.

3. *Staphylococcus aureus* group

Staphylococcus aureus is also an opportunist ¹⁹. It is often on human skin and in secondary infections following antibiotic therapy and in staphylococcal food poisoning ³.

Its detection in sewage is accomplished by 1) membrane filtra-

tion of the sample and 2) culture on m-Staphylococcus broth

plus 0.75 mM Na azide to avoid the interference of certain

bacilli ²³ and micrococci. Incubation at 37 C for 48 hr is followed by holding at 25 C for 24 hr to enhance the golden pigmentation of *S. aureus* colonies. Nevertheless more confirmation is needed and so representative colonies, including non-pigmented, are picked to Mannitol Salt agar slants, from which a check is made microscopically for staphylococcal morphology and Gram reaction. All coccal colonies are then streaked onto DNase agar and those positive on this test are picked and grown at 37 C for 24 hr in Brain Heart Infusion broth. The tube test for coagulase is then made on these cultures. The highest dilution thus confirmed as due to *S. aureus* denotes the staphylococcal count. For family sewage, the numbers usually range 10^2 - 10^3 /100 ml, if the family is positive at the time. This level of staphylococcal load in family sewage is probably an underestimate, since based upon detection of *S. aureus* alone, whereas there are now known to be several species of staphylococci on human skin ¹⁰. The significance of these non-coagulase positive staphylococci is debatable but they must soon be taken into account.

