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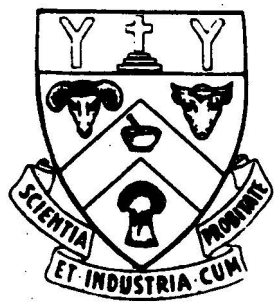

SOIL CONTAMINATION

BY SEPTIC TANK

EFFLUENT

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ABSTRACT

A survey of literature relating to septic tanks and the movement of micro-organisms through soil was made. The septic tank still appears to be the most satisfactory method of treating household wastes. Little experimental evidence is available concerning the movement of micro-organisms through soil, but work which has been done indicates that soils vary greatly in their filtering ability. Furthermore soils appear to change in capacity to retain micro-organisms which subsequently die at different rates depending on the nature of the soil. The travel of viruses through soil has received negligible attention.

Investigations undertaken at two sites in the Tai Tapu Area, near Christchurch, indicated that pollution of the soil by faecal organisms can take place from septic tanks and can extend over quite a wide area. The bacteria may remain viable in the soil for some months, both near the surface and at depth. From samples obtained by drilling holes in the soil it was found that bacteria could apparently move laterally through the soil at 2.0 to 3.0 m depth for considerable distances.

Two main conclusions can be drawn from this work. First, soil can become contaminated by septic tank effluent over a wide area. Second, once the soil is contaminated the micro-organisms can persist for long periods.

I REVIEW OF LITERATURE

Septic Tanks

"Domestic waste disposal in rural areas is specifically a problem of disposing of individual household wastes at the least annual cost without creating a health hazard and without violating land and water quality standards" (Gates 1967).

There are two main types of sewage treatment used for individual households, aerobic and anaerobic systems. According to Gates (1967) aerobic devices have been found to require more maintenance than is normally practical at the household level. Also failure of these aerated systems causes disastrous results, and practical experience with these units has been generally unsatisfactory although improvements are being made all the time. Therefore the most commonly used method is still the anaerobic septic tank together with a below ground soil absorption system, e.g. boulderpit, or seepage pit.

Two processes are carried out simultaneously in the septic tank; sedimentation and biological degradation of the waste (Pelzar and Reid, 1972). The second part of the system, the seepage pit is designed to allow aerobic biological oxidation of organic solids as well as recharge of ground water.

Septic tanks can be installed where the subsoil is permeable enough to absorb the effluent at the proposed rates, but not so permeable as to allow pollution of the ground water. The bottom of the seepage pit should also be at least 60 cm above the maximum ground water level and 1.5 above rock (Gates 1967).

According to Thomas et al., (1960) dissatisfaction with the septic tank method derives from four main sources. Firstly, maintenance by the householder is often erratic or completely lacking and adequate inspection by health authorities is difficult and expensive. Secondly, most soils eventually become clogged with particles and the seepage pit will therefore have to be replaced. Thirdly, the design of seepage pits is not always entirely satisfactory. Design is made difficult by the fact that in many soils there is a low correlation between the observed short uptake capacity for fresh water as measured by the standard test and the long term, more or less, steady state percolation capacity needed for septic tank effluent. The fourth source of dissatisfaction is that often the system is installed without adequate attention to placing because exploration for suitable soils and subsoils for the seepage pit is expensive. Because of these problems unhygenic practices have often occurred in many communities.

Operation of Septic Tanks

Septic tanks are normally constructed of concrete or steel and have an average retention of two to four days sewage flow. The tank is usually so designed that heavy solids will settle out and scum will float to the surface. These components will therefore not be discharged with the effluent. Failure to remove these two components periodically will cause the discharge of solids into the seepage pit and the clogging of the soil.

In most anaerobic systems two processes occur. Firstly the conversion of sewage compounds into organic acids and secondly the formation of methane from the organic acids. The second phase is not important in the septic tank system because the methane forming bacteria are very delicate and slow growing and are very susceptible to changes in flow rate caused for example by people going away for holidays. Thus the evil smelling organic acids and some of the micro-organisms which formed them may be periodically washed out into the seepage pit.

The effluent which comes out of a septic tank compares very unfavourably with effluents from other types of treatment plants as can be seen in the following tables.

TABLE A. Data taken from Bailey and Wallman (1971)

Parameter	Raw waste water mg/l	Effluent mg/l	Percentage removal
BOD	150	75	50
COD	310	160	48.4
Suspended Solids	185	50	73

TABLE B. Comparison of methods of waste treatment. Data taken from Pelzar and Reid (1972).

Disposal System	Suspended Solids % removal	m^3 of sludge per 10^6 m^3 sewage	% removal bacteria	% removal BOD
Septic Tank Effluent	40-75	500-1500	40-75	25-65
Activated Sludge	70-97	10,000-30,000	95-99	70-96

TABLE C. Characteristics of Septic Tank Effluent.
Data taken from Popkin and Bendixen (1968).

Parameter	Average mg/l	Range mg/l
COD	152	238-90
NH_4 - N	24.6	35.2-14.4
NO_2	Trace	
NO_3	Trace	
Organic N	5.6	9.3-2.9
Total Suspended Solids	41	96-12

According to Bailey and Wallman (1971) capacity and hydraulic design are the most important factors influencing the purification of the effluent. Capacity is important to create conditions which favour sedimentation of suspended solids. Capacity must also be sufficient to dilute any harmful chemicals and to even-out irregular flows from various household appliances. Additional capacity is needed for the storage of the digesting solids so that there is a minimum of maintenance required. The hydraulic design determines the storage efficiency and the extent to which the effluent can short circuit.

Modifications

There have been many modifications suggested for the design of septic tanks. One such given by Bailey and Wallman (1971) consists of a fibreglass tank containing two chambers which allows the separation of wash waters from sanitary and kitchen wastes. The sanitary and kitchen wastes are held in an upper compartment for longer periods than the wash water which is fed into a lower compartment. This means that the bacteroicidal side effects of household detergents and household cleaners used in the wash waters will have less effect on the microbial activity taking place in sanitary and kitchen wastes. After a time the waste water of the lower compartment is mixed with the upper compartment. Thomas et al., (1960) suggested that improvements could be made in the conventional septic tank. For instance, the reshaping of the tank with the utilisation of upward flow, mechanical mixing and the application of small amounts of heat might improve the system. These modifications would, however, make the tank more difficult to maintain.

Absorption System

The seepage pit is an intergral part of the septic tank system which must be carefully designed. The main problem in using septic tanks lies in the disposal of effluent into those soils which have poor drainage characteristics. The effluent will not receive the proper aerobic treatment and may rise to the surface only partially treated, causing a definite health hazard. On the other hand, if the soil under the seepage pit is too permeable, waste water may travel too quickly and not receive sufficient treatment before being withdrawn at the nearest well.

The seepage pit may provide additional storage time for processing the effluent. This may improve the filterability of the effluent and result in less soil clogging. Particles from the seepage pit may move into the soil, clogging the soil pores. Micro-organisms decompose particles aerobically and thus unblock the pores. The rate at which oxygen can diffuse into this zone may influence the rate at which soil pores become blocked (Thomas et al., 1960).

Health Problems Associated with the Use of Septic Tanks

Water contaminated with human excreta is dangerous because of the possible presence of pathogenic micro-organisms. The potential water borne pathogens include members of the Salmonella and Shigella genera, Vibrio comma, Entamoeba histolytica, and enteric viruses (Gates 1967). The danger of septic tank effluent will depend on whether the effluent comes to the surface, whether the soil is insufficiently permeable to retain the micro-organisms or whether the ground water moves too freely to allow time for the micro-organisms to die out before they reach a well.

Bacteria

The Coliform bacteria which are excreted from the intestines of warm blooded animals in large numbers may be used to indicate the possible presence of pathogenic micro-organisms which may be excreted at the same time. Because these indicator organisms are more resistant than most pathogenic species in the alien environments of soil and water they will exist until most of the pathogenic forms have died out. The septic tank system reduces the number of bacteria as shown in Table B. but the effluent still contains too many micro-organisms to be safe. Hence the importance of proper functioning of the seepage pit and soil treatment.

Since the threat of disease depends on the survival time of microbial pathogens in the soil and water, the rate of water movement through the soil, the filtering ability of the soil and the time interval from release of effluent until it comes into contact with humans, these factors will be considered in the following discussion.

1) Survival time of bacteria in soil

Studies of this type have not been numerous; Klein and Casida (1967) showed that survival of coliforms in natural soil was markedly influenced by temperature and the presence of food materials. In their study they found that numbers declined in 20 days from approximately 5×10^6 to 10^6 at 10°, 10^5 at 26° and 10^1 at 37°. Some differences in rate of die-off were found when nutrients were added.

Assuming that conditions in the soil were the same as Klein and Casida's experiment (10°C and 0.25% glucose) then by extrapolation of their graph it would take approximately 100 days for all the coliforms in the effluent to die out. This result is supported by Gates (1967) who stated that enteric bacteria could be recovered months after their introduction into soils.

2) Rate of movement of water in soil

The rate of flow of water seeping from the pit and its lateral movement in the ground water depends on many factors including the size of the soil particles and the hydraulic gradient. For example, Schiff and Muckel (1967) have reported the average velocities of water movement in granular materials set out in Table D.

TABLE D. Movement of water in soil.
Data from Schiff and Muckel (1967).

Soil type	Grain size mm	Average velocity cm/day with 1% gradient
Silts and loess	0.005 - 0.25	1.98
Sand Stone + medium sand	0.25 - 0.5	35.3
Coarse and + sandy gravel	0.5 - 2.0	192
Gravel	2.0 - 10.0	914

The Ministry of Housing (1961) reported that the average rate of flow of ground water was variable, but averaged 30 cm/day. In Canterbury Oborn (1962) suggested that groundwater moved at speeds ranging from a few inches to a few tens of feet per day.

If bacteria survived for 100 days and moved at 9 m/day they would be able to move a considerable distance. However, this may be an overestimate except in coarse shingle.

3) Filtering action of the soil

The filtering action of the soil particles depends on many factors including the size of the particles, whether or not the soil is saturated, the state of any "biological defense layer" and other factors (Gould 1970). The effect of soil particle size was shown by the Ministry of Housing (1961) report (Table E on separate page).

McCoy (1969) showed that soil is effective in removing potentially infectious bacteria. For example, 35 cm of silt loam removed all the inoculum of coliforms (10^5/ml) and enterococci (10^6-10^7 ml).

Under the conditions of the experiments undertaken at Lodi (Butler et. al, 1954) bacterial disappearance in percolating water was very rapid in the first 25 cm of soil and they were only occasionally found at a depth of greater than 125 cm though the applied wastes at times had coliform counts of greater than 5 x 10^6/100 ml. Furthermore the number of coliform organisms penetrating 30 cm or more as measured by tests on the liquid was essentially independent of the intensity of pollution of the waste water applied.

The experiments at Whittier showed a reduction of coliforms from 1.1 x 10^5/100 ml to 4x10^4/100 ml in 1.0 m of soil with none appearing in the lower horizons (California State, 1954). In a coarser sand at Azusas 1.2x10^5 coliforms/100 ml were reduced to 60/100 ml approximately 1 m below the surface of the soil (McGauhey 1968). These results show that water containing bacteria is filtered efficiently through a fine soil layer and numbers are reduced rapidly.

Experiments conducted by the University of California on five types of soil held in lysimeters (1 m deep) showed the effect of the formation of a "biological defense layer". The influent settled sewage water had a coliform concentration of greater than 10^8/100 ml.

The first sample (Oakley sand) had an effective soil particle size of 0.015 mm and was subjected to an infiltration rate of 3 cm/day. This soil gave an effluent with a fairly consistent coliform concentration of 2.2x10^5 /100 ml for the first six weeks. The concentration then dropped to approximately 2.4x10^2/100 ml thereafter. Yoho sandy loam with an effective size of 0.0155 mm (infiltration rate 9 cm/day) produced an effluent with a coliform concentration which fluctuated between 2.4x10^4-4.8x10^3/100 ml. With observations tending generally towards the higher value there was no indication in this case of the formation of a "biological defense layer". The three other soils all yielded effluent coliform concentrations of less than 45/100 ml with only slight variation except that occasional extremely high counts appeared. This may have been due to a periodic unloading of organisms in the soil and was therefore of considerable interest (Gould, 1970).

TABLE E. Reduction in numbers of Escherichia coli as water passed from a rubbish pit through horizontal filters composed of different sized particles. Results are expressed as percentage frequency of counts per ml of effluent.

Ranges of counts per ml.	Concentration of bacteria initially in rubbish pit	Coarse substratum				Medium substratum				Fine substratum			
		1.8m	3.7m	5.5m	7.3m	1.8m	3.7m	5.5m	7.3m	1.8m	3.7m	5.5m	7.3m
0	0	2	5	2	7	4	4	24	24	39	53	77	98
0.01-.1	7	3	10	18	13	11	13	21	22	25	18	9	2
0.1 - 1	19	25	25	35	35	18	25	24	24	20	16	9	0
1 - 10	33	25	32	23	25	34	29	22	14	9	11	5	0
$10 - 10^2$	19	18	13	10	10	20	21	2	7	7	2	0	0
$10^2 - 10^3$	13	13	8	10	8	9	4	7	7	0	0	0	0
$10^3 - 10^4$	8	2	5	0	2	4	4	0	2	0	0	0	0
$10^4 - 10^5$	1	2	2	2	0	0	0	0	0	0	0	0	0

Coarse substratum particles average size 30-40 mm
Medium " " " " 10-20 mm
Fine " " " " 1-2 mm

The build-up of a "biological defense layer" was also demonstrated in the Dutch sand dune infiltration basins (Gould, 1970). Under a 15 year old spreading basin the bacterial penetration was only half as much as it was under a basin one year old.

The effect of the degree of water saturation of the soil was demonstrated at Flushing Meadows in Arizona by Bouwer (1968). Effluent containing coliform bacteria at a concentration of 10^6/100 ml which was spread out over a soil consisting of 1 m of sandy loam showed a reduction after travelling 9 m downward and 3 m laterally to 0.5 coliforms/100 ml if aerobic conditions existed in the topsoil or to 8-33/100 ml when the topsoil was kept more anaerobic. This however, is not a marked difference.

Coliforms have been observed to travel further in ground water than they do when percolating through an unsaturated zone (Gould, 1970). This effect has been demonstrated on several occasions. When sewage (coliforms 2.4×10^6/100 ml) was injected 30 m into a aquifer consisting of a band of sand and pea gravel (effective size 0.2-0.3 mm) coliforms were detected at a distance of 30 m in the direction of the groundwater flow after 33 hours. They were not detected at 60 m even after 46 days. Bacterial pollution was greatest at the beginning of the period and subsequently became less showing that even in gravel a "defense mechanism" of some kind may build up. Neither prolonged recharge, increased concentration of bacteria, nor greater injection rates caused the bacterial pollution to extend beyond the initial distance of travel (Krone et al., 1957)

Furness (1956) working in the United Kingdom studied the spread of pollution from a refuse pit and found that coliforms were absent in a 3.5 m deep well 70 m down stream from an experimental pit whereas ammonium levels increased showing that coliforms were being filtered out.

When sewage (2.4×10^6 coliforms/100 ml) was injected directly into an underground aquifer at Richmond, England, the decrease in numbers with distance travelled was shown to be:

3 m from source	-	$2 \times 10^3 - 2 \times 10^4$
15 m from source	-	2×10^2
30 m from source	-	0

On the other hand there is evidence that bacteria can travel long distances on occasions. This is probably most likely to occur where there are cavities or fissures in the ground through which the ground water runs freely. Merrell et al., (1967) found that sewage water still had a coliform concentration of $1\text{-}5 \times 10^3$/100 ml after travelling 60 m through a mixed alluvium channel. After further travel to a total of 750 m it still had a concentration in the range of 5-20/100 ml.

In Washington there were complaints of well contamination up to 450 m from a lagoon with no outlet. Faecal coliforms were obtained from samples collected at a distance of 75 m from the lagoon (Bogan, 1961). Reports of epidemic surveys show that in many cases bacteria have travelled further than in experiments. It has been postulated that travel rate and distance may be increased by the lowering of surface tension produced by the use of synthetic detergents. (Mallman et al., 1961).

Viruses

Unfortunately unlike bacteria, there are no viruses excreted in large numbers by warm-blooded animals which can be readily detected. This absence of indicator viruses makes them more of a threat because, although they cannot be readily detected they may cause illness at low concentrations. Coliform bacteria have often been suggested as sufficient indicators of viruses but the absence of coliforms may not necessarily mean the absence of virus particles since these two types of micro-organisms can behave very differently.

Enteric viruses multiply in the human gastrointestinal tract and as a result domestic waste water may contain large numbers of virus particles (Gates, 1967). For instance the average concentration of viruses in samples of waste water from a number of communities in Israel was 100/100 ml Shuval (1971).

There is little information about the effect of sewage treatment processes on the survival of viruses but some information given is summarised in Table F.

TABLE F. Reduction of Virus Numbers.

Method of Treatment	Reduction
Activated sludge	90-99%
Trickling filter	40%
Thermophilic anaerobic digestion for 30 days	Coxsackie B5 survived
Oxidation ponds	Good removal because of long retention times

Survival and Movement of Virus Particles

The survival of viruses is dependent on environmental factors. For example at 4° in domestic sewage a virus (ECHO 6) survived in almost undiminished numbers for seven days, while in fresh water at the same temperature it was completely inactivated in five days. In sea water kept in the laboratory some viruses (ECHO 6 and POLIO 1) survived with diminishing numbers at 5° for 140 days. However, in the estuary survival time was reduced to 20 days at temperatures of between 1° and 10° (Metcalf and Stiles, 1965).

Holden (1970) has reported that enteroviruses may survive in soil for periods of up to 170 days; survival being longer at low temperatures (3-10°) and at pH 7.5 than at higher temperatures and under more acid conditions. Survival was longer in moist than in dried soil, and in a loamy soil the die-off was quicker than in sandy soil.

From outbreaks of infectious hepatitis cases it appeared that water travelled anywhere from a few feet to a few hundred feet through the soil to enter the water supplies which ranged from shallow to deep wells. (Drewry et al., 1968; Merrell & Ward 1968) reported that after water had travelled horizontally through natural sand and gravel strata for 450 m no virus could be detected.

In contrast to these distances it has been shown by Drewry et al., (1968) using a bacterial virus that 99% of the virus could be removed in a short soil column. Adsorption by the soil particles however, depended on physical factors such as pH. The virus used by Drewry et al., was a large complex bacterial virus and in a study using a smaller virus Gilcreas and Kelly (1955), found percolation through 1 m of garden soil ineffective in removing this virus. The effect of percolation rate was demonstrated by Robeck et al., (1962) who found that vertical flow rates of less than 1.2 m per day would be likely to be effective in removal of viruses from ground water when percolating through fine sand. It seems therefore that, depending on the type, soil may either aid the process of purification or provide access for pollution.

Once a virus is in groundwater it may be able to survive for long periods, especially at low temperatures. For example, when poliovirus in the form of a faecal suspension was added to river water and stored at 4° the virus survived for at least 188 days. The survival of the virus causing infective hepatitis for at least ten weeks in a relatively clean water has been demonstrated since human volunteers contracted the disease after drinking contaminated well water stored for six weeks in a well and for a further four weeks in the laboratory.

Except for an apparently paradoxical case of prolonged survival in heavily sewage-polluted waters, it appears that the cleaner the water and lower the temperature the longer the period of survival. Furthermore it would appear that entero viruses may survive longer than coliforms.

Since soil is generally effective in filtering bacteria and suspended solids, water from a septic tank which passes through soil may be free of most impurities except viruses. Thus it is possible that conditions in the groundwater may be suitable for the long-term survival of enteric viruses.

II CONCLUSIONS FROM THE LITERATURE

1. The septic tank and soil absorption still appears to be the most satisfactory method for disposing of sewage from small installations especially isolated rural buildings provided care is taken in the design of the system.

2. Soil is an effective filtering medium. Most reports of long travel by micro-organisms seem to be associated with fissures in the underlying substratum. Unfortunately it is difficult to prove that there are no fissures in an area.

3. As the gap between total volume of waste water generated and the land area available for all uses narrows, the risk of soil and water pollution increases.

4. Soils and subsoils vary in their drainage characteristics and although there is little mention of upward flow into surface soil this is a possibility under conditions of poor drainage.

5. Soils seem to change in their ability to hold and cause the death of bacteria.

6. Very little is known about the travel of micro-organisms and especially viruses through the soil and subsoil.

III EXPERIMENTAL

The Tai Tapu Problem

Christchurch City is peculiar in that it is situated on the edge of a plain made up of gravels. These gravels extend to great depths and because near the coast they are interlayered with mud and sand they may contain aquifers.

Although much can be inferred from the disappearance of water from the Waimakariri near Halkett and fluctuations in the height of water in wells, the way in which the soil water moves beneath the Canterbury plains remains almost completely shrouded in mystery.

Because this water is used, both for domestic supplies and for factories as well as irrigation it is most important that this ground water never becomes contaminated.

There appear to be three main ways in which contamination can occur. First is the deliberate recharge of the aquifers by pumping used water into the soil. This is a reckless measure which is to be strongly deprecated under all circumstances. The second would be contamination due to seepage of irrigation water into the aquifers and thirdly the introduction of micro-organisms and other pollutants into the soil water from soak pits connected to septic tanks.

The problems associated with the disposal of water from septic tanks in low lying areas around Christchurch is too well known to need amplification here, but on the other hand the constant pressure from people wishing to make a profit by sub-division and local bodies who would like the increased rates from sub-divided land is also very obvious. Thus, sooner or later, some decisions will have to be made about the allowable density of housing in these areas.

In the Tai Tapu area the soil profile varies very greatly over short distances, but it would appear that most of the surface and probably some sub-surface water finds its way to the Halswell stream.

Because little relevant information is available from the literature about contamination of ground water and movement of micro-organisms in the soil, a series of experiments was conducted, designed first to delimit the problem, and second find ways of studying it in the Tai Tapu area.

Immediately the problem was considered it became clear that there were major technical difficulties to be overcome in the collection of bacteriologically unaltered samples of soil from the soil profile. Hand digging and the use of a simple soil cover is quite satisfactory at depths down to one m but below this a percussion rig is required and in wet areas such as Tai Tapu the well drilled must be cased albeit temporarily which adds to the cost.

Since it is not possible to estimate the contamination level of the samples obtained from depth, full bacteriological testing must be undertaken on every one and this means considerable laboratory facilities are required.

The first series of experiments concerned a septic tank and boulder pit that were known not to have been functioning properly in that the effluent had not been seeping from the boulder pit and some surface flooding had occurred. The second experiment was aimed at finding out more about the distribution of micro-organisms from a boulder pit through the soil.

It is presumed that in properly functioning seepage pits the effluent which has been introduced into the centre of the pit about one m below ground level flows downwards and moves out into the soil in all directions when it meets the water table.

This being the case two problems immediately arise in the Tai Tapu area, one is that the water table in the winter may be very close to the surface and the other is that "it is said" that there is a movement of ground water towards Lake Ellesmere so that all the effluent may be concentrated in one area and at times close to the surface of the soil.

Site I

The area selected was a property on the Tai Tapu-Lincoln Road near the Christchurch-Akaroa Main Highway. The first samples were taken on August 22nd, 1972.

It was understood that this was a relatively new installation, consisting of a septic tank feeding into a boulder pit. In this locality there is a layer of loamy soil of good quality about 30 cm thick under which there is a layer of sandy clay to about 60-80 cm where there is a relatively dense impervious layer, and under this there is water-saturated sand down to at least 3 m. When building a boulder pit, it is usual to dig through any impervious layers until sand or shingle is reached; this usually requires a hole 2.5 to 3 m deep in this area.

In this preliminary investigation it was decided to take samples from four sites at two depths, starting over the boulder pit and working outwards.

Sampling Area

The site of the pit had been earlier planted in pine trees but these had been removed and replaced with small shrubs. The soil had been cultivated to a shovel's depth.

Site 1	Over the top of the boulder pit.
Site 2	One m from the centre of the pit just outside the area disturbed during digging the pit.
Site 3	Two m from the centre of the pit.
Site 4	Three m from the centre of the pit.
Site 5	Seven m from the centre of the pit.

Sampling Method

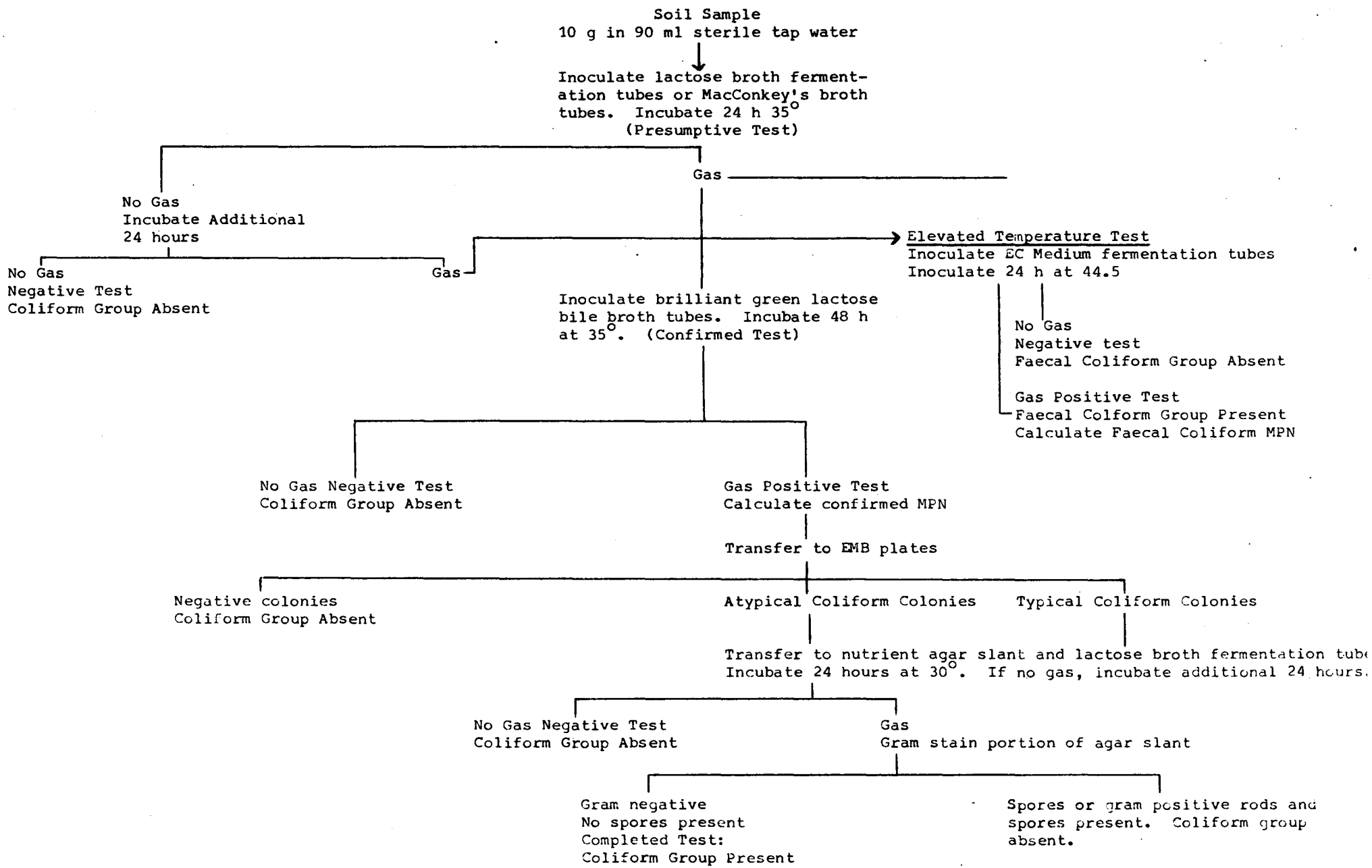
Small holes were dug with a spade about 25-30 cm deep and cores were taken from the bottom of each hole with a tubular soil sampler. Subsequently the holes were deepened to 40-50 cm and further cores taken from the bottom. The cores were placed in new plastic bags and transported to the laboratory where they were put into a refrigerator at approximately 4° until they were sampled.

Enumeration of Micro-organisms

There are numerous difficulties in the enumeration of micro-organisms and a number of different methods have been devised to cope with these problems.

The first cause of error lies in the size of the sample taken. In sampling soil a quantity, usually 20 g, is taken to represent a site which may be many square metres in area; this small sample may or may not be representative of the whole.

GURE 1. Schematic Diagram for the Detection of the Coliform Group and the Faecal Coliform Group



The next problem to be faced is how to enumerate those species and individuals present. A commonly used method is to make a suspension of the soil in water which, when diluted, will contain few enough propagules to allow each to be counted when grown on a suitable nutrient medium. Of course it is not possible to devise a nutrient medium that will allow every species to grow and although some such as tryptone glucose extract agar (T.G.A.) may be considered to be all purpose media there are some types of organisms that will not grow on it.

The next decision to be made is at what temperature the micro-organisms are to be grown. Two temperatures, 24^{o} and 37^{o}, are most often used. It is presumed that a 24^{o} soil inhabiting species will be encouraged, whereas 37^{o} (blood heat) will stimulate those that are normally inhabitants of the gut and other organs of warm-blooded animals including man.

By using special techniques and media it is possible to encourage certain species to grow selectively. Thus members of the Coliform group, Faecal Streptococci and Staphylococci can be enumerated using suitable media.

The Coliform group of bacteria are frequently used as indicators of pollution. Some types of these bacteria are able to grow only in the gut of warm-blooded animals, and their presence in soil, water, milk etc. is generally considered sufficient evidence to presume that contamination by faeces has taken place recently. The methods used for detection of Coliform bacteria are set out in Figure 1. During the first stage of this investigation not all of the steps were used, but the samples from the second site were fully examined.

By using a number of tubes of broth inoculated with known amounts of soil suspension in the first step of the procedure it is possible to obtain an assessment of the numbers present. This is known as the'Most Probable Numbers test' (M.P.N.) and is based on the statistical probability of the occurrence of the bacteria in certain volumes of the diluted suspension of soil.

Another group of bacteria that may indicate pollution are the Staphylococci, in particular Staphylococcus aureus, an organism that may cause boils, food poisoning etc. The medium chosen to test for this organism was Vogel & Johnson's agar. This medium is designed to permit the detection and enumeration of coagulase and mannitol positive strains of Staphylococci typical of Staphylococcus aureus. On this medium colonies of these bacteria are shiny and black.

Faecal Streptococci are bacteria that live in the gut of warm-blooded animals and may be used as indicators of faecal pollution in a manner similar to Coliform organisms.

However, because of difficulties in finding a suitable medium for growing them their use has been somewhat limited. Improved media are now available and one of these was used with the samples from Site 2.

Preparation of Cultures

Twenty g samples were taken from the centre of the cores, using sterile techniques. Dilutions were prepared and plates poured according to the standard plate count method. Tubes of MacConkey's broth were also inoculated to ascertain the most probable numbers of coliform organisms present.

Results

Site I

Samples were collected from this site on:

22nd August

23rd November

7th December

The samples were tested for:

(1) Total number of micro-organisms growing at 24° incubation temperature.

(2) Total number of micro-organisms growing at 37° incubation temperature.

(3) Most probable number of Coliform organisms, and the presence of Faecal Coliform organisms.

(4) Staphylococcus aureus (22nd August sample only)

Site II

This site was in Perryman's Road close to the Christchurch -Akaroa Main Highway. Samples were collected from this site on only one occasion, 12th February, 1973, by drilling holes with a Ministry of Works rig.

They were tested for:

(1) Total number of micro-organisms growing at 24° incubation temperature.

(2) Total number of micro-organisms growing at 37° incubation temperature.

(3) Most probable number of Coliform organisms and Faecal Coliforms.

(4) Faecal Streptococci.

Site I - Tai Tapu - Lincoln Road

Results of 22nd August sampling

Test I

This test was designed to find the total numbers of micro-organisms growing at 24° incubation with tryptone glucose agar (TGA) as the nutrient medium.

Using this technique a wide range of soil micro-organisms was encouraged to grow. The results are shown in Table I.

Table I Number of micro-organisms per g of soil. Medium TGA, incubation 24° for 3 days.

Site	Depth	Number of Organisms per g of soil
	25-30 cm	
1		2.2×10^7*
2		9.3×10^6
3		4.0×10^5
4		6.7×10^5
	40-50 cm	
1		1.6×10^6
2		5.0×10^6
3		4.0×10^5
4		2.3×10^4

From the results it is clear that there were more micro-organisms in the top layer of soil, which is generally expected and that the numbers are less further from the pit. This indicates that contamination of the soil from the pit may have occurred.

Test II

In this test the nutrient medium was the same but the samples were incubated at 37° for two days to encourage the growth of those organisms that might have originated from man or warm blooded animals.

*N.B. The results given in the tables are set out as a whole number multiplied by ten to a power, thus 10^6 is 1 million, 10^7 is 10 million etc. In experiments of this nature, differences of less than a power of ten are unlikely to be significant.

Table II Numbers of micro-organisms per g of soil. Medium TGA incubation 37°C for two days.

Site	Depth	Number of Organisms per g of soil
	25-30 cm	
1		7.5×10^7
2		1.9×10^6
3		1.5×10^7
4		4.8×10^6
	40-50 cm	
1		9.9×10^6
2		2.0×10^6
3		3.2×10^6
4		3.2×10^6

These results are remarkably high and there is no drop-off with increasing distance from the pit. In water samples taken from streams one would expect in unpolluted water to find many more organisms growing at 24° than 37°. However this is not the case here.

Test III

This test was designed to ascertain the most Probable Number of Coliform organisms per g of soil. These organisms are considered to be presumptive indicators of faecal pollution. The method used was the MPN test with 1x50 ml, 5x10 ml, and 5x1 ml tubes incubated at 37°. MacConkey's Broth was the medium.

Table III MPN of Coliform using MacConkey's Broth at 37° for 24 hours. Presumptive test.

Site	Depth	Coliforms per g of soil M.P.N.
	25-30 cm	
1		8.0×10^1
2		9.2×10^2
3		0.7×10^1
4		0.5×10^1
	40-50 cm	
1		2.4×10^2
2		5.4×10^2
3		2.2×10^1
4		1.3×10^1

A further test was applied to the tubes of MacConkey's broth that showed a positive reaction. This was to grow organisms from test tubes on Eosin Methylene Blue agar (EMB) at 37° for 24 hr.

Close to 50% of the tubes gave a positive result indicating that the Coliform organisms detected belonged to the Faecal group. From this could be concluded that recent contamination of the soil by faeces had occurred.

These results confirm the suspicion shown in Test II that there were large numbers of micro-organisms of faecal origin in all samples. The high numbers present confirm that considerable contamination was present. Even in the sample taken 3 m from the pit pollution indicating bacteria were present.

Test IV

In this test one tenth ml samples from the dilutions prepared for Experiment I were spread on to the surface of Vogel and Johnson's agar to see if Staphylococci were present.

Table IV Number of black colonies typical of Staphylococcus aureus occurring on Vogel & Johnson's agar. Incubation 36 hours at 37°.

Site	Depth	Number of Organisms per g wet weight soil
	20-30 cm	
1		3.0×10^4
2		3.0×10^3
3		7.0×10^2
4		1.4×10^2
	40-50 cm	
1		1.5×10^3
2		1.2×10^2
3		4.9×10^2
4		9.0×10^1

Further tests on organisms isolated from typical black colonies on Vogel & Johnson's agar, showed that these were not Staphylococcus aureus but were apparently soil organisms able to grow on this special medium. This indicated that Vogel & Johnson's agar was not satisfactory for differentiating between Staphylococcus aureus and other species occurring in the soil environment and its use was consequently abandoned.

Results of 23rd November Sampling

Test I

Following on from sampling done on 22nd August, a further set of results were obtained from soil samples from the same area using the methods as described for the 22nd August sampling.

Table V Number of micro-organisms per g dry weight of soil. Medium TGA incubation 24° for 3 days.

Site	Depth	Number of Organisms per g of soil
	10-20 cm	
1		1.8×10^7
2		1.5×10^7
3		1.9×10^7
4		3.3×10^6
	23-35 cm	
1		7.3×10^6
2		1.8×10^7
3		4.8×10^6
4		3.9×10^6

Test II

Table VI Number of micro-organisms per g oven dry weight of soil. Medium TGA incubation 37° for 1 day.

Site	Depth	Number of Organisms per g of soil
	10-20 cm	
1		2.2×10^6
2		1.8×10^6
3		7.1×10^6
4		1.9×10^6
	23-35 cm	
1		1.6×10^6
2		2.0×10^6
3		1.5×10^6
4		1.2×10^6

Test III

Table VII M.P.N. of Coliform organisms using MacConkey's broth at 37° for 24 hours per g oven dry soil.

Site	Depth	Total Coliforms per g of soil
	Top Sample 10-20 cm	
1		> 22
2		1
3		> 23
4		5
	Lower Sample 23-35 cm	
1		> 22
2		2
3		1
4		1

Tubes showing positive results were streaked onto Eosin Methylene Blue agar to confirm the presence of Faecal Coliforms. Of the tubes 55% gave a positive result.

Results of 7th December Sampling

Test I

The following results were obtained from soil samples taken on 7th December from the same area and using the same methods as for 23rd November. A further sample was included (Site 5), 4 m beyond site 4.

Table VIII Number of micro-organisms per g oven dry of soil. Medium TGA incubation 24° for 3 days.

Site	Depth	No of micro-organisms per g oven dry soil
	Top Sample 10-20 cm	
1		1.5×10^6
2		1.0×10^7
3		7.7×10^6
4		8.0×10^6
5		2.2×10^6
	Lower Sample 23-35 cm	
1		5.1×10^6
2		7.4×10^6
3		1.3×10^6
4		6.2×10^6
5		5.5×10^6

Test II

Table IX Number of micro-organisms per g of soil. Medium TGA incubation 37° for one day.

Site	Distance from centre of pit in m	No. of organisms per g of oven dry soil
	Top Sample 10-20 cm	
1		1.1×10^6
2		1.5×10^6
3		1.2×10^6
4		1.4×10^6
5		5.4×10^5
	Lower Sample 23-35 cm	
1		1.1×10^6
2		1.1×10^6
3		1.3×10^5
4		9.3×10^5
5		5.0×10^5

Test III

Table X Number of Coliform organisms per g oven dry weight soil using M.P.N. methods.

Site	Depth	Total Coliforms
	Top Sample 10-20 cm	
1		4
2		27
3		3
4		17
5		1
	Lower Sample 23-35 cm	
1		38
2		1
3		0
4		16
5		65

Tubes showing positive results were streaked onto EMB agar to confirm the presence of Faecal Coliforms; 46% of the tubes gave a positive result.

Site II - Perryman's Road

Introduction

A new septic tank had been installed at this property about 12 months previously. It was connected to a boulder pit. The excavations for these structures were still clearly visible and the approximate outline of the boulder pit could be discerned. It is understood that the pit had been excavated to a depth of approximately 3 m.

Because of the small amount of data available on the movement of micro-organisms in soil and the limited number of holes to be made because of the cost, some difficulty was experienced in deciding how to sample the area. It was concluded that it would be reasonable to take samples from holes designated A, B, C and D drilled 1 m, 3 m, 6 m, and 26 m from the pit. The holes were drilled down to a depth of 3.6 m. The sampling site is depicted diagramatically in Figure 2.

Drill

The drill used in the operation was a Mole Drill Rig manufactured by Mole Engineering (N.Z.) Ltd., Paremata. The method employed was to dig a small hole with a spade to about 20 cm and then use a hand auger 4" diameter to remove the soil to 60 cm. The deeper samples were taken with a percussion sampler which was driven into the ground by a weight raised on a winch.

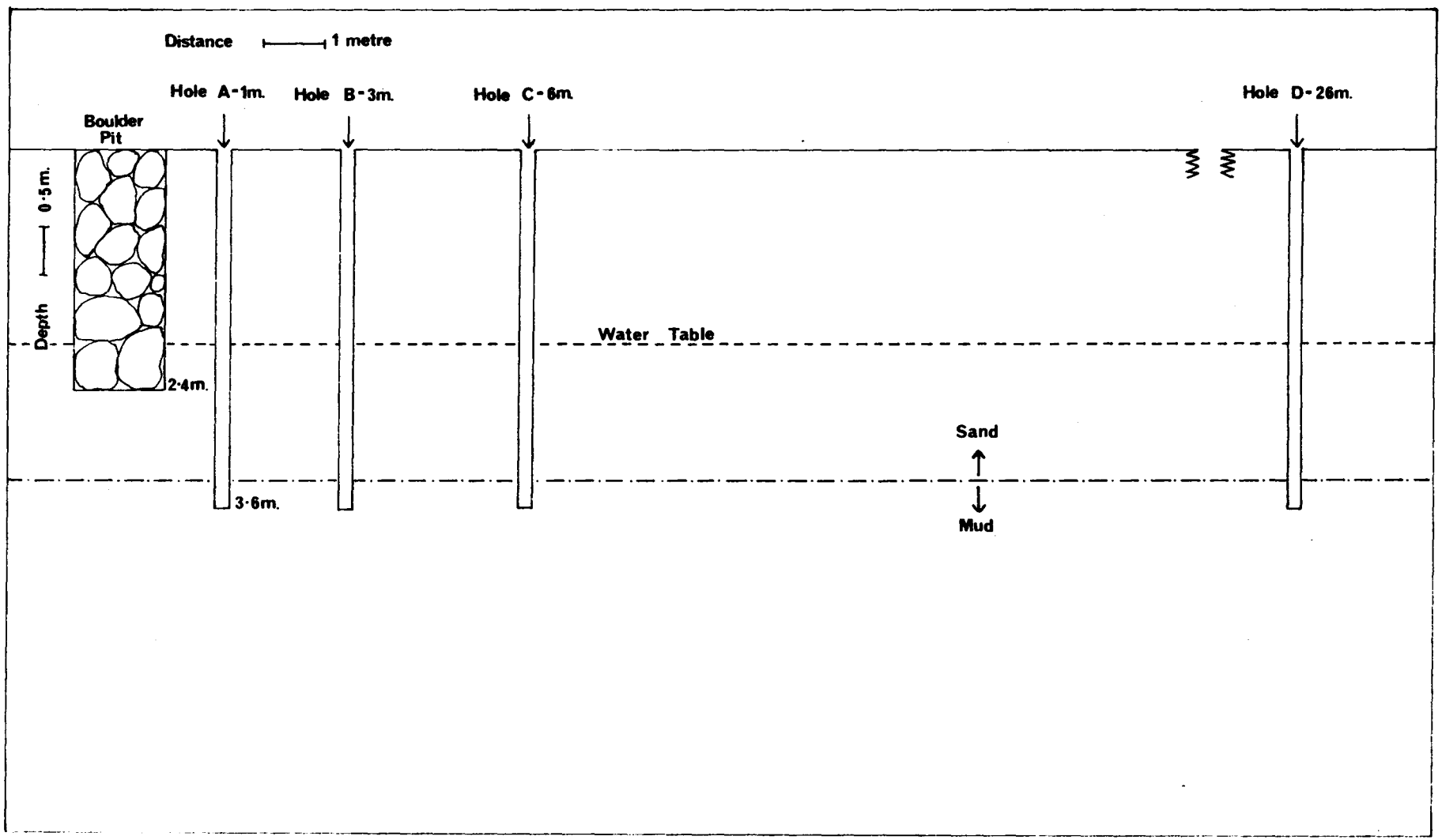
The soil was collected in a Raymond Spoon Sampler; this was a steel tube 2" outside diameter and $1\frac{3}{8}$" inside diameter and 24" long. The tube was split lengthwise. At each end there was a thread; the one at the top was used to attach it to the driving rod and the lower one to attach a short tip of hardened steel. To prevent material, driven into the sampling tube, from slipping out when the tube was withdrawn a special type of retaining spring could be inserted at the lower end. In soft soils the spring could be replaced by a short (10 cm) length of polythene tube the same diameter as the sampler. The polythene sleeve was an alternative means of retaining the sample in the tube.

At each sampling depth the Raymond Sampler was driven into the soil about 50 cm and then withdrawn by means of a winch on the rig.

Once the water-table was reached it was necessary to case the hole - this was done by driving pipe 4" in internal diameter into the soil to the depth of the next sampling point. Soil above the proposed sample was removed by means of a pump which was operated by tipping approximately 20 litres of water down the casing. The pump, a simple tube with a valve at the bottom and a plunger at the top, was lowered down the casing to soil level. The pump was then moved up and down by a rope attached to the winch.

Figure 2.

Diagram showing the relationship between boulder pit and holes at Site II.


Distance
1 metre
Hole A - 1m.
Hole B - 3m.
Hole C - 6m.
Hole D - 26m.
Boulder Pit
Depth 0·5m.
2·4m.
Water Table
Sand
3·6m.
Mud

When sufficient soil had been removed with the pump the Raymond Sampler was reinstated and hammered into the soil to obtain another sample.

After the last sample had been taken the casing pipe was withdrawn by means of the winch. The holes were refilled and the soil rammed tight.

Soil Profile

On the day prior to sampling some 30 mm of rain had fallen. Sampling revealed that it had penetrated about 50 mm and below this the soil was quite dry.

The soil profile was found to be as follows:

0	-	.8 m	Soil dry except for the top 50 mm.
.8	-	1.2 m	Brown sand loose dry above, damp below.
1.2	-	1.7 m	Blue mud wet and soft.
1.70	-	1.73 m	Layer of peat, may have been compressed during sampling.
1.73	-	1.8 m	Blue mud.
			Water Table - approximately 2.0 m
1.8	-	2.25 m	Blue sand.
2.25	-	3.3 m	Blue sandy mud.
3.3	-	4.0 m	Blue mud with peat, sand increasing with depth.
4.0	-	6.0 m	Sandy with increasing mud.
6.0	-	9.0 m	Mud with peat and wood fragments.

All the holes were very similar down to 3.6 m. One was continued to 9 m to discover the nature of the lower strata. It was found that a blue mud containing organic matter was present to this depth.

Collection of Samples

For most types of soil analyses all that is required is a knowledge of the changes in strata and a small piece of each soil type. For microbiological analysis it is necessary to obtain undisturbed samples preferably in the form of a core. In damp soil with good adhesive properties it was possible to achieve this type of sample, but in dry soil or sand the material did not hold together and in wet sand the sample tended to run out when the coring tool was brought up.

In the area examined both dry powdery soil, sand and wet sloppy sand were encountered so that a continuous core could not be obtained.

It was found that the spring device in the Raymond Sampler only worked in fairly hard soil, when it was forced open. In wet sand it did not open and the fingers of the spring went through the sand thoroughly mixing it.

The polythene liner was more satisfactory, but the pressure of driving the sampler through the soil often dislodged it; even so its presence ensured a small length of undisturbed core in each sample.

Thus it was not possible to obtain cores suitable for microbiological analysis throughout the length of the profile, but a number of relatively coherent samples were obtained from each hole. If further sampling were to be undertaken it would be desirable to have a better type of sampling device; perhaps a long polythene tube would be satisfactory, but some method of holding it in place would have to be devised.

Methods

The cores were placed in new plastic bags and transported to the laboratory. Cores from the first hole, 1 m from boulder pit, were tested the same day and those from the other holes were held in a refrigerator at approximately 4° until they could be attended to. Cores from the second hole were held for 24 hours before testing, cores from the third hole for 48 h and from the fourth hole for 72.

Enumeration of Organisms by Standard Plate Count

Ten g samples were taken from the centre of the cores using sterile techniques and dilutions prepared with sterile tap water. Duplicate plates were poured with TGA. One series of plates was incubated at 37° for 24 h and another series incubated at 24° for 72 h.

The Coliform Group

Samples were examined for the Coliform group of bacteria by the Most Probable Number Method and Faecal Coliform bacteria by the Elevated Temperature Test as described in "Standard Methods for the Examination of Water and Waste-water" (1971), and outlined in Figure 2.

The following steps led to the final identification of the Coliform bacteria:

Step 1

Tubes containing lactose broth were inoculated with aliquots of the soil suspension dilutions prepared for the plate counts and incubated at 35° for 24 h.

Depending upon the number of Coliforms expected either -

	5 tubes inoculated with	1.0 ml
	5 tubes inoculated with	0.1 ml
	5 tubes inoculated with	0.01 ml
or	5 tubes inoculated with	10.0 ml
	5 tubes inoculated with	1.0 ml
	5 tubes inoculated with	0.1 ml

were used.

The results of this test were used to compute the M.P.N. of presumptive Coliform organisms in the soil sample.

Step 2

Inoculations were made from those tubes that gave a positive reaction into

(a) Tubes of E.C. medium and incubated at 44.5° to determine the number of Faecal Coliforms present.

(b) Tubes of brilliant green lactose bile broth incubated at 35° to confirm the presence of Coliform organisms.

Step 3

Tubes of brilliant green lactose bile broth giving a positive reaction were used as inoculum for E.M.B. agar plates.

Step 4

Atypical and typical Coliform colonies appearing on the E.M.B. plates were inoculated.

(a) Into nutrient agar slopes.

(b) Into lactose broth.

Step 5

Slides were prepared for microscopical examination from slopes whose corresponding broth tube gave a positive reaction. The slides were stained by Gram's method and examined to see if the organisms present were gram negative rods without spores.

The Faecal Streptococcal Group

Tests for the Faecal Streptococcal Group were done by the tentative faecal streptococcal plate count as described in "Standard Methods for the Examination of Water and Wastewater" (1971). M-Enterococcus agar plates were incubated at 37° for 48 h. Representative colonies typical of faecal streptococci were verified by microscopic examination as gram-positive cocci.

Table XI Analysis of Variance of Numbers of Micro-organisms Growing at 24°.

Variation due to	DF	SS	MS	F
Treatments	35	38.81	1.11	33.0**
Hole	3	0.83	0.28	8.3**
Depth	8	26.41	3.30	98.3**
Hole x depth	24	11.57	0.48	14.3**
Error	34	1.14	0.03	
Total	69	34.00		
Standard error per plot	= 0.183			
Coefficient of variation	= 4.1%			

Table XII Analysis of Variance of Numbers of Micro-organisms Growing at 37°.

Variation due to	DF	SS	MS	F
Treatments	31	40.30	1.30	26.5**
Hole	3	1.20	0.40	8.2**
Depth	7	7.43	1.06	21.6**
Hole x depth	21	31.67	1.51	30.7**
Error	30	1.47	0.05	
Total	61	41.78		
Standard error per plot	= 0.221			
Coefficient of variation	= 4.5%			

RESULTS

Total Counts

The results of counting the total number of organisms in the soil at Site II are shown in Figures 3, 4 and 5 for 24° incubation and 6, 7 and 8 for 37° incubation.

At 24° incubation the numbers in each hole showed some variation. However, they all indicated a similar trend except for hole C which revealed at approximately 2.0 m a marked increase in numbers. Because this increase was present at 1.8 m as well as 2.0 m, it would appear that the rise was real and not due to an error in technique.

As shown in Figure 3 there was a drop in total numbers with depth to the 1.0 m level when the numbers levelled off and then rose slightly, before dropping again to the 3.0 m level. There was a marked rise at approximately 3.3 m, it was at this depth that the soil became less muddy and more sand occurred, probably allowing the free lateral movement of soil water.

The dotted line in Figure 4 shows a plot of figures given by Alexander (1961) (Table XIII) as typical of the numbers of bacteria in soil and the solid line indicates the results from Site II. The dramatic difference between the two lines indicates clearly that some external influence was present in the area sampled.

In Figure 5 the numbers of micro-organisms in each hole has been averaged. The graph shows that there was little difference between holes. Thus the one nearest the pit had virtually the same microbial content as the one 26 m away.

Statistical analysis was carried out on data from plate counts at 24° and 37° as only these were complete enough to be analysed. The results of the analyses are presented in Table XI and Table XII. Even in these instances the limited number of holes sampled meant that any analysis could not be rigorous. Samples were also not obtained from exactly the same depth in each hole and so for the analysis, samples were grouped into eight groups. The difference in depth between the extremes in any one group was less than 0.1 m. Some of the 24° counts from the surface soil were not available and for this reason this depth was omitted from the analysis. All data were log transformed before being subjected to analysis of variance.

As can be seen from Tables XI and XII, the treatments were highly significant although the interactions were also very significant. It was evident that there was no easily explained variation in numbers with depth or distance from the hole although the standard deviations would suggest that the surface soil of the four holes differed significantly from deeper soil. Numbers of bacteria in the soil below the surface did not decline markedly. This is in contrast to results reported by Alexander (1961) who quoted results for a typical soil profile which are set out in Table XIII and Figure 4. The decline is, however, less dramatic in profiles with a high concentration of organic matter (Alexander, 1961).

Table XIII Distribution of Aerobic Bacteria at Various Depths in the Soil Profile (Alexander, 1961)

Depth (cm)	Numbers per g soil
3 - 8	7.8×10^6
20 - 25	1.8×10^6
35 - 40	4.7×10^5
65 - 75	1.0×10^4
135 - 145	1.0×10^3

Alexander attributed most of this decline to a reduction in the quantity of available carbon and oxygen at lower levels. Since the decline in numbers with depth in the four holes was only from 10^6 to approximately 10^4 per g in 300 cm, it would seem that nutrients and/or micro-organisms could be moving from the pit into the soil.

It must, however, be remembered that numbers of micro-organisms in soil can fluctuate markedly thus Goodfellow, Hill and Gray (1968) found that in a pine forest soil numbers fluctuated from 2×10^6 to 2×10^8 per g in the space of one month. The results of one sampling can therefore sometimes be misleading.

The results obtained when plates from the soil suspensions were incubated at 37° are given in Figures 6, 7 and 8. The numbers found must be considered high for soil. After an initial drop, they remained almost constant to the 1.8 m level. There was a slight increase at the 2.0 m level and a more notable one at about 3.3 m.

Differences were evident in individual holes. A and B, and C and D, being slightly dissimilar but as shown in Figure 8 overall there was no marked difference.

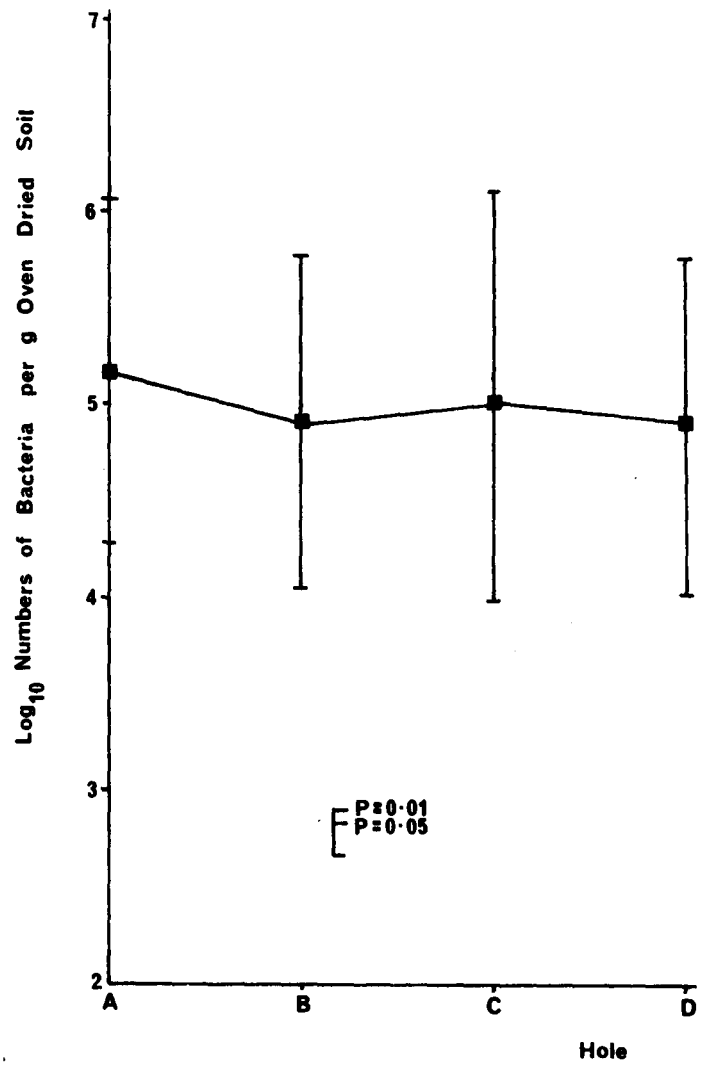

7
6
5
4
3
2
Log10 Numbers of Bacteria per g Oven Dried Soil
P=0·01
P=0·05
A
B
C
D
Hole

Figure 5.

Site II, average number of micro-organisms per g soil in holes A, B, C and D. Micro-organisms were enumerated on T.G.A. incubated for three days at 24°. Least significant difference between means and the standard deviation of the numbers in each hole are shown.

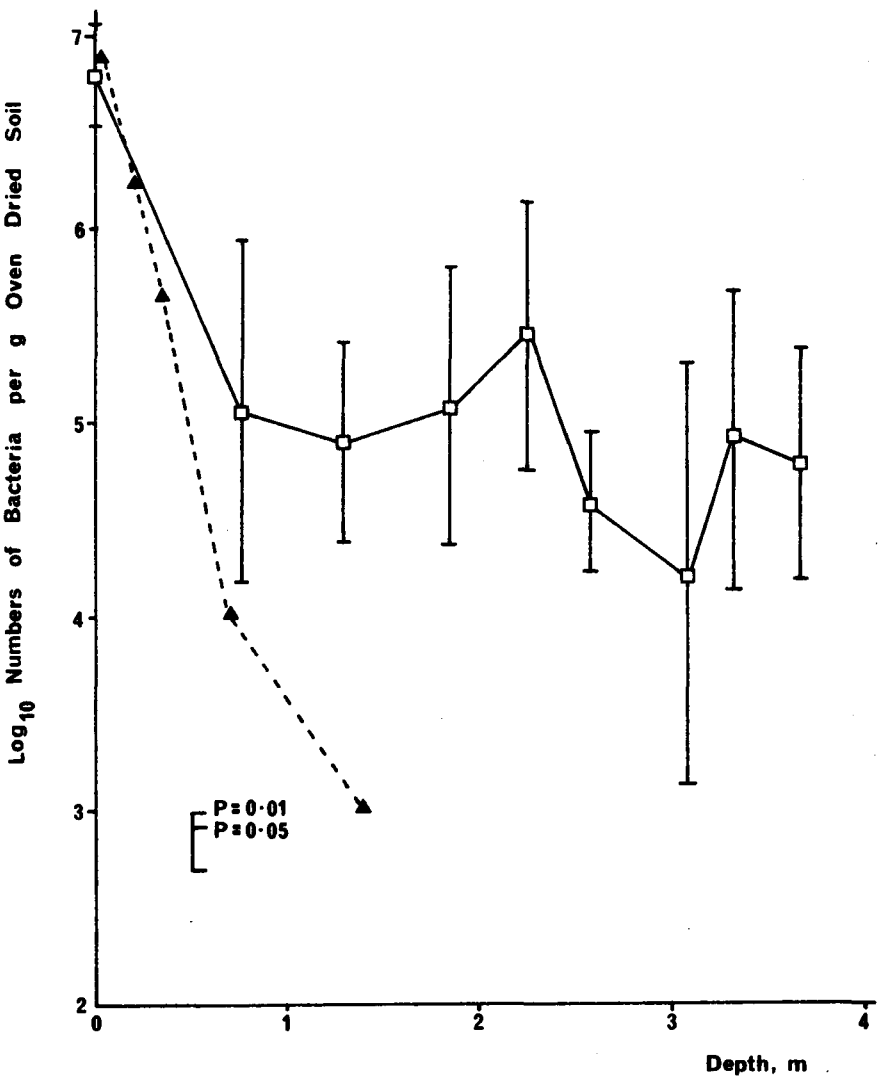

Log10 Numbers of Bacteria per g Oven Dried Soil
7
6
5
4
3
2
0
1
2
3
4
Depth, m
P=0·01
P=0·05

Figure 4.

Solid line - Site II, average number of micro-organisms per g soil in holes A, B, C and D. Micro-organisms were enumerated on T.G.A. incubated for three days at 24°. Least significant difference between means and the standard deviation at each depth are shown.

Broken line - numbers of aerobic bacteria per g soil typically found in soil horizons (Alexander, 1961).

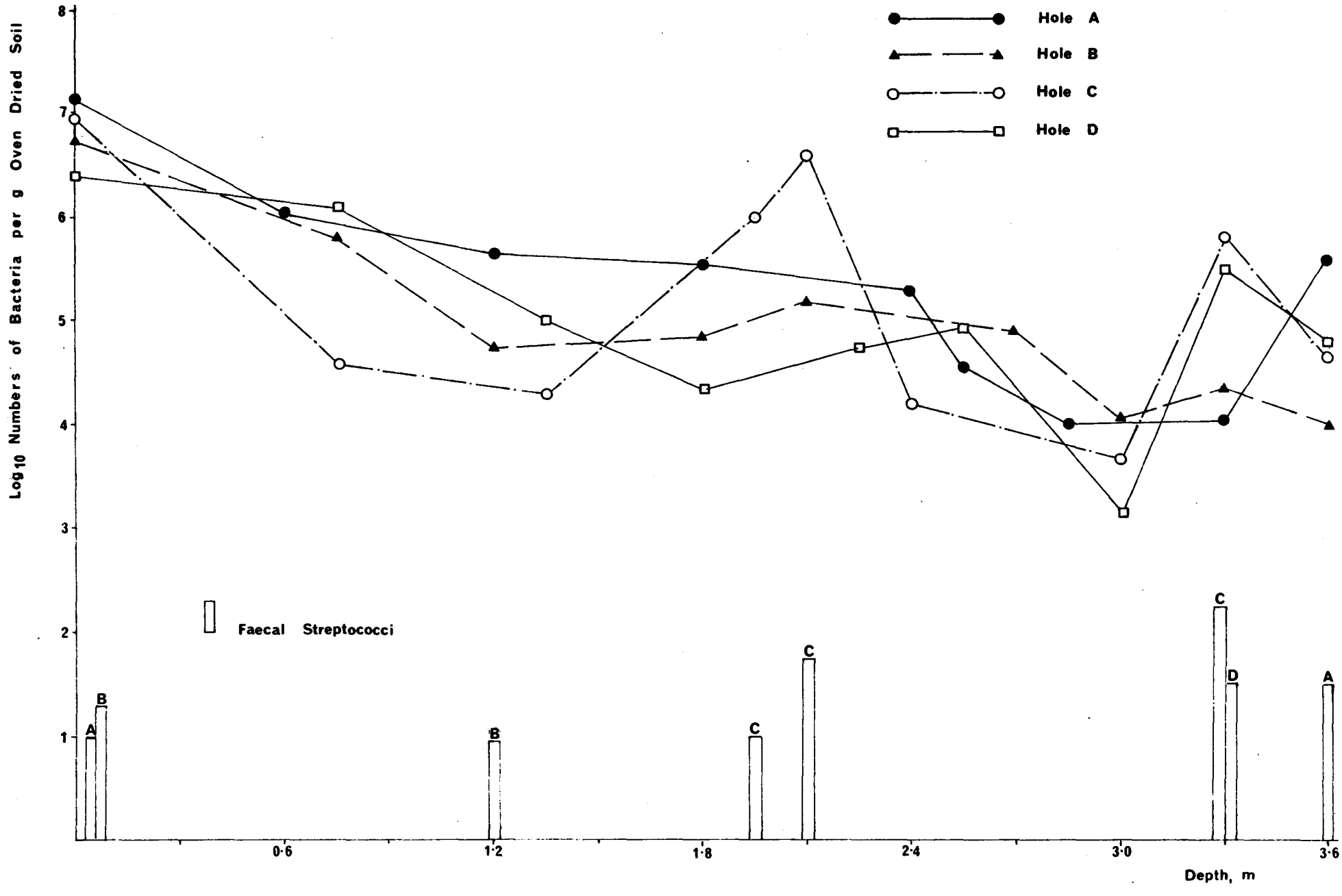

Hole A
Hole B
Hole C
Hole D
Log10 Numbers of Bacteria per g Oven Dried Soil
8
7
6
5
4
3
2
1
Faecal Streptococci
A
B
B
C
C
C
D
A
0·6
1·2
1·8
2·4
3·0
3·6
Depth, m

Figure 3.

Site II, total numbers of micro-organisms per g of soil at various depths in holes A, B, C and D. Samples were plated with TGA and incubated at 24° for 72 h. Also numbers of Faecal Streptococci enumerated on M-Enterococcus Agar. Incubation at 37° for 48 h.

Figure 6.

Total numbers of micro-organisms per g of soil at various depths in holes A, B, C and D. Samples were plated with TGA incubation 37^{o} for 24 h.

Also shown are M.P.N.'s of Total and Faecal Coliforms at various depths. Letters on top of the histograms refer to the holes in which the Coliforms were found.

Log10 Numbers of Bacteria per g Oven Dried Soil
Depth, m
Total Confirmed Coliforms
Faecal Coliforms
Hole A
Hole B
Hole C
Hole D

Figure 7.

Site II, average numbers of micro-organisms per g of soil in holes A, B, C and D. Micro-organisms were enumerated on TGA incubated for one day at 37°. Least significant difference between means and the standard deviation at each depth are shown.

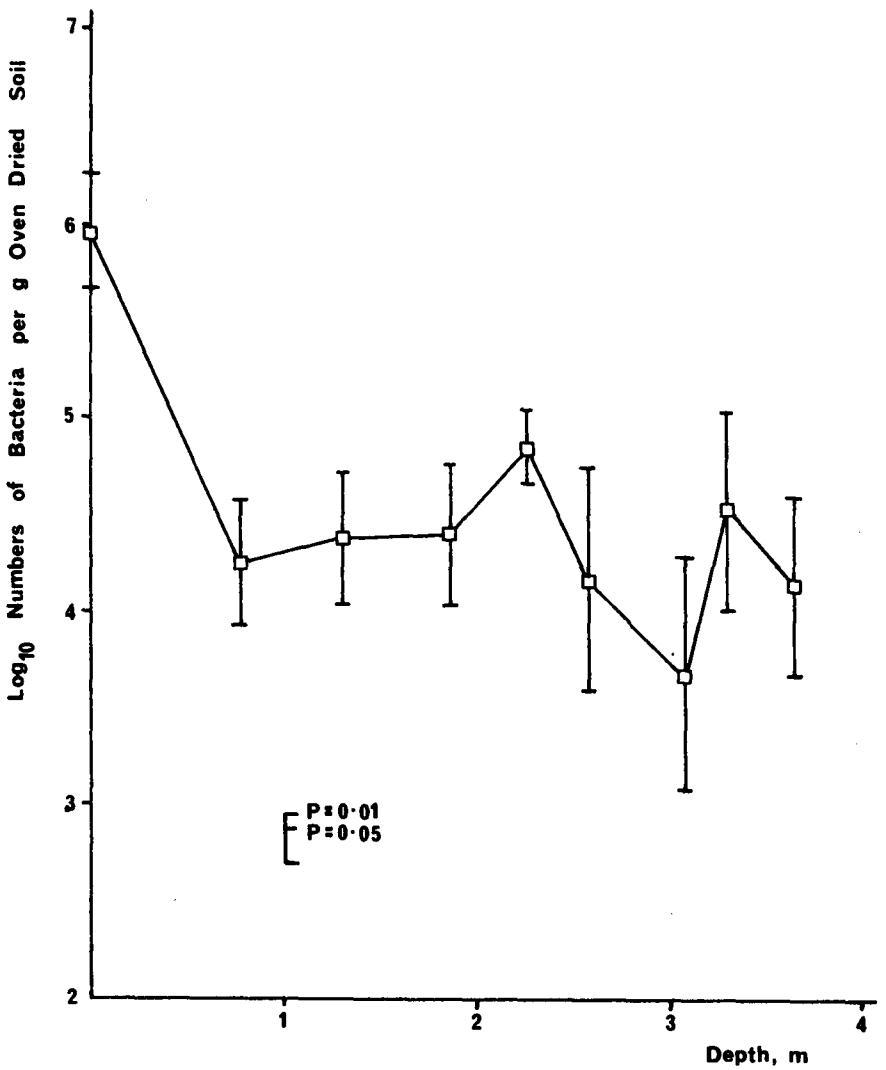
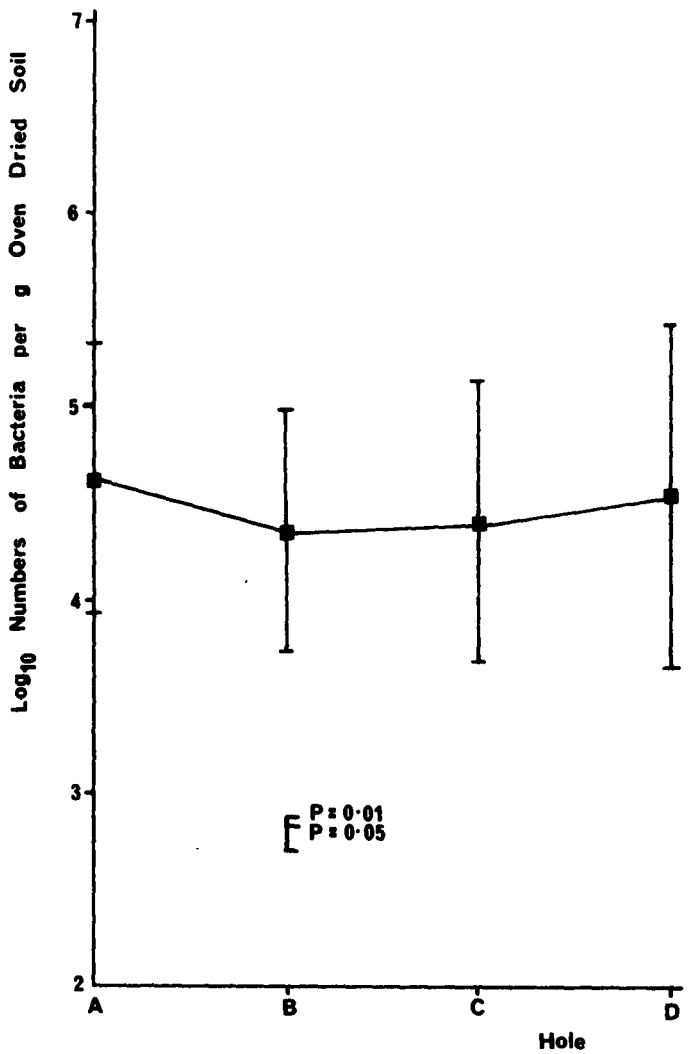

Log10 Numbers of Bacteria per g Oven Dried Soil
7
6
5
4
3
2
1
2
3
4
Depth, m
P=0·01
P=0·05

Figure 8.

Site II, average number of micro-organisms per g of soil in holes A, B, C and D. Micro-organisms were enumerated on TGA incubated for one day at 37°. Least significant difference between means and the Standard deviation of the numbers in each hole are shown.


Log10 Numbers of Bacteria per g Oven Dried Soil
7
6
5
4
3
2
P = 0·01
P = 0·05
A
B
C
D
Hole

The sustained high level of organisms growing at 37° throughout the soil profile further substantiates the results from 24° incubation, indicating the possible infiltration of nutrients and micro-organisms into the area sampled.

Faecal Streptococci and Coliforms

In Figure 3 the results of the tests for Faecal Streptococci are set out. There was fairly heavy contamination by these bacteria at the surface as would be expected because the field had horses grazing in it. However, the presence of this type of organism at the lower levels is most unusual. Since none were recorded from the sub-surface samples (0.6 m), it is unlikely that they had percolated through the soil from the surface. Of particular note was the considerable upsurge in numbers at the 3.3 m level which also coincided with the increase in total numbers at 24° and 37°.

The general pattern of occurrence of these bacteria was similar to that found for Coliform Organisms and Faecal Coliforms shown in Figure 6 where high numbers were recorded at the surface, 2.0 m and at the 3.3 to 3.6 m levels.

Tables XIV, XV, XVI and XVII set out the results of the stages in coliform testing. It is generally held (Standard Methods APHA, 13th edition) that the Presumptive test is suitable as an indicator of pollution where the fitness of the sample for drinking is not being considered. The Confirmed test is used for routine testing of drinking water and the final Completed test is to check the accuracy of the results of the Confirmed test. The Faecal test is useful in indicating whether the Coliforms have come from warm blooded animals or not.

Thus these results show, as was expected, that only a proportion of the Presumptive organisms did prove to be Coliforms. More important, however, in several instances Faecal Coliforms were present at the lower levels and not near the surface. Thus these results confirm that contamination of the lower layers had not taken place by downward percolation of contaminated water from the surface.

It is of particular note that Coliform Organisms and Faecal Streptococci were both found in Hole D at a distance of 26 m from the boulder pit, indicating that they had travelled this distance presumably from the pit without hindrance.

Coliform organisms present in the surface layers were, no doubt, due to faecal contamination by the horses but they were also present in quite large numbers at the 2 m and 3.3 m levels. What was most surprising was that Faecal Coliforms were present at these levels, indicating that there had been recent contamination by human or animal faeces.

Table XIV Hole A. Results of tests for Coliform Organisms per g soil from Soil Samples from Various Depths.

Depth of Sample Metres	Presumptive Test MPN	Confirmed Test MPN	Completed Test MPN	Faecal Test MPN
Surface	364	18	8	0
0.6	3	1	1	0
1.2	4	0	0	0
1.8	9	9	9	9
2.4	22	4	1	0
2.6	3	0	0	0
2.9	29	2	1	1
3.4	6	6	5	1
3.7	197	63	21	8

Table XV Hole B. Results of Tests for Coliform Organisms per g soil from Soil Samples from Various Depths.

Depth of Sample Metres	Presumptive Test MPN	Confirmed Test MPN	Completed Test MPN	Faecal Test MPN
Surface	⩾236	7	6	0
0.8	2	1	1	0
1.2	3	1	1	0
1.8	3	3	1	1
2.1	4	0	0	0
2.8	3	0	0	0
3.1	4	4	2	0
3.4	4	4	2	0
3.7	4	4	4	0

Table XVI Hole C. Results of Tests for Coliform Organisms per g soil from Soil Samples from Various Depths.

Depth of Sample Metres	Presumptive Test MPN	Confirmed Test MPN	Completed Test MPN	Faecal Test MPN
Surface	⩾ 233	16	1	0
0.8	13	0	0	0
1.4	2	0	0	0
2.0	⩾ 250	167	15	3
2.1	⩾ 282	⩾ 282	19	3
2.4	13	3	3	1
3.1	61	27	3	0
3.4	⩾ 282	⩾ 282	33	4
3.7	26	26	4	0

Table XVII Hole D. Results of Tests for Coliform Organisms per g soil from Soil Samples from Various Depths.

Depth of Sample Metres	Presumptive Test MPN	Confirmed Test MPN	Completed Test MPN	Faecal Test MPN
Surface	⩾ 231	3	0	0
0.7	7	1	0	0
1.4	1	0	0	0
1.8	1	0	0	0
2.3	16	3	3	0
2.6	29	1	1	0
3.1	1	0	0	0
3.4	⩾ 267	⩾ 267	102	3
3.7	20	4	4	0

GENERAL CONCLUSIONS

Discussion and Conclusions

The results of the tests made at Site I indicated that there had been considerable pollution of the surrounding soil from the boulder pit and further that over the period August to December there was no significant diminution in the level of bacteria in the soils at 24°, and in the holes furthest from the pit, at 37° only a small drop was noted. The appropriate figures are set out in Figure 9 in the form of a histogram. It is of course not possible from these results to know if the contamination was the result of a single episode or whether the numbers in the soil were being kept up by additions from the boulder pit although this would seem to be unlikely in the spring and summer when the soil had dried out and no flooding occurred.

The sanitary significance of these results is hard to gauge. Some authors would contend that the presence of Faecal Coliform organisms should be taken to mean that there had been a major breakdown in the sewage system and that immediate drastic action should be taken. For example, it is contended that no Faecal Coliform organisms should be present in swimming pool water and the upper limit for Class C water is 200 per 100 ml and none are allowed in foods.

It is easily conceivable that children could ingest some of the contaminated soil and perhaps become infected with a disease. On the other hand, the presence of Faecal Coliforms in soil, especially that of fields where animals are grazing, is common and disease among farm animals is not a serious problem from this source.

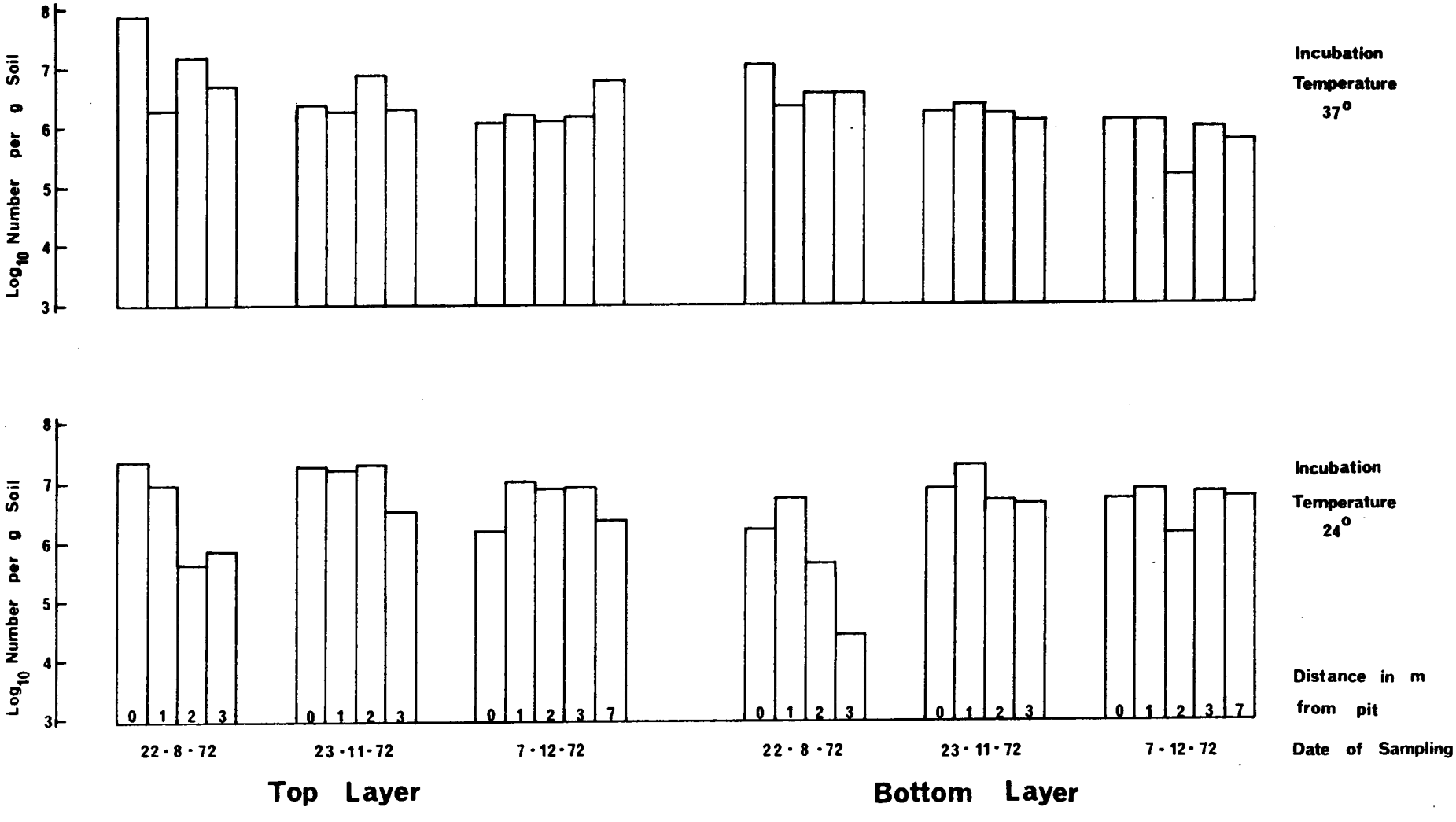
The results obtained from the samples taken in Perryman's Road are also of considerable sanitary significance. A clearer idea of the extent of the movement of the bacteria in the soil would have been possible if samples could have been taken from all around the pit but this would have been very costly so only one transect was made.

That the boulder pit was functioning well was shown by the detection of Faecal Coliforms at the most distant site. That these organisms were detected at all is, however, surprising as there are only two people in the household and unless by chance the holes were drilled in the line of the flow of ground water, the contamination would appear to be spread over a very large area.

The fact that the organisms were mainly detected at two levels, 2.0 m and 3.3 m, relates to the soil profile; thus it would appear that if micro-organisms can get into a layer in which lateral movement is possible, they may travel quickly considerable distances without vertical movement.

Figure 9.

Histogram showing the numbers of micro-organisms from soil samples taken from Site I at two depths; three sampling times; and at distances up to 7 m from the boulder pit. Micro-organisms were grown on TGA incubated at 37° and 24° for 1 and 3 days respectively.


Log10 Number per g Soil
8
7
6
5
4
3
Incubation Temperature 37°
Incubation Temperature 24°
0 1 2 3
0 1 2 3
0 1 2 3 7
0 1 2 3
0 1 2 3
0 1 2 3 7
Distance in m from pit
22 · 8 · 72
23 · 11 · 72
7 · 12 · 72
22 · 8 · 72
23 · 11 · 72
7 · 12 · 72
Date of Sampling
Top Layer
Bottom Layer

From the review of literature, it is clear that many problems remain to be solved with regard to the movement of micro-organisms through soil and shingle. Their survival, both in and on the soil, is something of an enigma. The so-called "Biological defence layer" is an interesting and possibly highly significant concept but little is known about its nature or how it is formed.

There has been increasing interest shown of late in parasitic species of bacteria that live on other species such as Escherichia coli but little is known about their ecology. Other parasites of bacteria such as protozoa and nematodes, also require further study. It may be possible by manipulating the conditions in the soil to so encourage the parasitic species that they build up to levels that will control those species it is desired to reduce or eliminate.

At a more practical level the results do not really answer the question as to whether it would be wise to allow close subdivision of land in the Tai Tapu area when sewage disposal would have to be by septic tank and boulder pit.

Two points can, however, be made from the results. First, soil can become contaminated from boulder pits, both near the surface and at depth. Second, once contamination has occurred, the micro-organisms do not die off quickly.

As pointed out above, the sanitary significance of this type of contamination is difficult to assess but it would appear that since outbreaks of enteric diseases are not notably more common in areas served by septic tanks than those with sewage disposal of effluents, this type of soil contamination cannot be of great significance in public health. Despite this, there is the nagging suspicion that a severe outbreak of an enteric disease could arise from contamination of soil or water and if it did, it would be the moral, if not the actual, responsibility of those who recommended the installation of the septic tanks.

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