MICROBIOLOGICAL STUDIES OF FARM ANIMAL WASTES, THEIR TREATMENT AND DISPOSAL ON LAND

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SUMMARY

The possibility that changes in agricultural practice, away from the production of farm-yard manure to a hydraulic system of handling animal excreta, may promote the dissemination of viable intestinal pathogenic bacteria in the environment and lead to an increased incidence of animal and human infections, are discussed. Investigations concerning the probability of salmonellae survival in pig excrement on the farm and the factors influencing the passage of enteric bacteria through soil into drainage water, are described.

In a series of experiments it was shown that some cells of Salmonella dublin can survive in pig excrement stored as a semi-liquid slurry, for at least 12 months. The rate of decline of viable cells was not affected by the suspended solids concentration of the slurry within the range expected in a storage reservoir on a farm. However the rate of decline of viable cells was affected by the anaerobic metabolic activity of micro-organisms in the environment during storage.

The rate of decline of viable S. dublin in the flocculent sludge during aerobic treatment of pig excrement was similar to that of Escherichia coli. However it was considerably higher than its rate of decline during storage when anaerobic conditions developed. Few cells of S. dublin remained viable in the liquid phase of the mixed liquor of the treatment unit, but more survived in the residual sludge which, it is argued, is the main product of aerobic treatment of animal excreta.

High numbers of enteric bacteria are shown to appear in sub-surface drainage water for short periods following the application to land of large volumes of semi-liquid animal excrement. At other times the main factors which affect the numbers of bacteria in sub-surface drainage water are (i) the rate of passage of water through soil, which is largely determined by the drainage characteristics of the soil; (ii) the amount of precipitation and evapotranspiration; and (iii) the numbers of bacteria in or on the soil and vegetation. The numbers of enteric bacteria in or on the soil and vegetation are also affected by the time since the last application of excrement to the land.

The application of these findings to better control of land treatment of animal excreta and/or the siting of new intensive animal production units is discussed.

INTRODUCTION

Treatment and disposal of animal wastes

It is almost a tradition for authors to commence a review of the problems of water pollution with a short history of sewerage and sewage treatment systems. Such histories usually finish with the proud statement that some of the major improvements in public health are almost entirely due to the development of sewage treatment methods and to the purification of water supplies. Unfortunately sewerage and sewage treatment do have some disadvantages. Marx (1887) stated that collecting the population in great centres and causing an ever increasing preponderance of town populations disturbs the circulation of matter between man and the soil. The same view was expressed recently by Davis (1971) and similarly in A Blueprint for Survival (Ecologist, 1972), arguments are put forward for a return to methods of recycling waste materials.

A situation which parallels the growth of urban human populations has been developing in agriculture in recent years. The pressures to increase productivity using less man-power and a smaller area of land have meant that more farmers have specialised in either crop or animal production. In large scale units for animal production it is now common to accumulate animal excreta in the form of a semi-liquid slurry. This slurry is usually considered to be a waste material and is disposed of as cheaply as possible. At the same time the arable farmer is using more and more artificial fertiliser to replace the minerals removed from the soil when crops are harvested for animal and human food.

The composition of agricultural slurry varies considerably. It may be composed of only faeces and urine which have been excreted by one or more types of domestic animals. The faeces from one type of animal also vary, due to differing diets, ages and physiological state of the animals. Apart from faeces and urine, slurry usually contains water from various sources, such as washing water, rain water from the roofs of buildings, and drinking water from leakage and spillage from water troughs. Sometimes other effluents such as silage liquor, or disinfectant solutions gain access to slurry tanks.

The installation of a hydraulic system makes the removal of the excreta from animal pens easier and quicker. This in turn improves the cleanliness of the house when compared with the older systems of using straw bedding.

Unlike farmyard manure, animal excreta in the form of a semi-liquid slurry is frequently thought of as a waste material to be disposed of as cheaply as possible. Methods of disposal however, produce several problems and numerous lists of these problems have been published. For example, Taiganides (1966), and Jones and Riley (1970), include problems of engineering, economics, politics, law, social nuisance, and public and animal health. Because of prosecutions under the Public Health Act 1936, against farmers for causing an unreasonable smell, and the Rivers (Prevention of Pollution) Acts 1951 and 1961, there is an urgent legal need to overcome some of the problems.

This has lead to the installation, on a few farms, of systems incorporating biological treatment in an effort to produce an innocuous product for disposal. In most cases such systems have been modifications of the cheaper methods used for the treatment of domestic sewage.

Initially it may be necessary to return all, or most, of the excreta, either directly or after primary treatment, to the land. Later however, methods of treatment which convert the material into valuable by-products, such as fertilisers, microbial protein for animal feed, or fuel, must be developed and introduced.

Whatever method is adopted the problem of animal and human health must always be taken into consideration when handling animal excreta. Enteric bacteria, many of which are potentially pathogenic, can only colonise a new host if they survive in the excrement and are then distributed into a suitable environment where they have an opportunity of acquiring a new host.

If the excreta are returned directly to the land, several new factors are introduced with the change from a farmyard manure system to a semi-liquid slurry system, as a means of handling the excrement. The microbial environment during composting of animal excrement to farmyard manure differs considerably from the environment in a slurry tank. Farmyard manure, which is a fairly dry material and valued as a fertiliser, is normally spread onto the surface of land immediately prior to ploughing. Slurry, on the other hand, is liquid and is often disposed of by spraying onto the surface of permanent pasture. Spraying of liquids produces aerosols, which are a very efficient means of bacterial distribution. The amount of aerosol produced may vary, depending on such factors as wind speed, the topography of the land and the type of spraying machine used.

Although it is recommended that animals should not be allowed to graze pastures that have been covered with slurry until a few months have elapsed, grazing often occurs after a few weeks, days

or even during spraying. Grazing animals avoid areas where dung has been deposited naturally but when slurry is evenly distributed over the land selective grazing by the animals is impossible (Venn, 1970). The application rate of slurry to a pasture at any one time may be as high as 448m^3/ha or more (Berryman, 1970). Due to the high water content of slurry, organic matter including enteric bacteria may gain access to water courses from surface run-off and land drainage.

The introduction of biological treatment systems for handling animal excreta may provide an unfavourable environment for enteric bacteria but the possibility that they survive in a treatment unit must be considered during the development of such systems. This becomes particularly important if the products of treatment of animal excreta are to be used for example as a feed additive in the form of microbial protein.

Thus it is apparent that the change from a farmyard manure system to a semi-liquid slurry system may at present be promoting the distribution of enteric bacteria in the environment.

Pathogenic bacteria in animal faeces

Numerous pathogens of domestic animals also cause disease in a range of species of other animals, including man. Many of these pathogens are excreted in, and in some cases known to be transmitted to new hosts by, animal faeces. The most significant pathogenic bacteria excreted in animal faeces include *Salmonella* sp., *Mycobacteria* sp., *Brucella* sp., *Leptospira* sp., and enteropathogenic *Escherichia coli* (Decker and Steele, 1966; Venn, 1970).

Coliform enteritis. Escherichia coli is usually the most numerous organism in the faeces of animals and man. Because it is so numerous and can be readily isolated, it is generally used as an indicator of faecal contamination, particularly in relation to water supplies (Department of Health and Social Security, 1970).

A few strains of E. coli are pathogenic and cause serious losses amongst young livestock, but although coliform enteritis is common and a considerable amount of knowledge exists about E. coli, relatively little is known about its epidemiology. Whether or not a particular animal becomes ill appears to depend more on the condition of the animal than on the pathogenicity of the organism. Reasoner (1971) suggests that E. coli receives less attention than it deserves in view of its distribution and ability to cause enteritis, but it is generally accepted that an animal becomes infected from its mother, or from other animals housed with it and it is unlikely that methods of handling faeces have any affect on the incidence of coliform enteritis.

Brucellosis. Fears that Brucella abortus can be transmitted by faeces to new hosts have been expressed by several authors (Stableforth, 1959; Kusdas and Morse, 1954; Becker and Steele, 1966). However, there is no evidence that this organism has been transmitted to animals or man from infected faeces, soil or water. Transmission is usually by animal to animal contact, contact with an aborted foetus, or infected milk. It is probable that fears of transmission via faecal material arise from the fact that aborted foetuses are often disposed of into a slurry tank.

Johnes disease. Mycobacterium johneii is excreted in the faeces of infected animals and the disease causes between 2 and 10 per cent herd mortality in the U.K. each year (Doyle, 1959). Little is known about its mode of transmission and no substantial evidence had been reported to suggest that faeces are an important vector. Nevertheless, Lovell and Francis (1944) suggest that pastures which have been grazed by infected animals should not be grazed again for twelve months.

Leptospirosis. Pigs are important hosts of Leptospira sp. and probably serve as the reservoir for disease in man (Burnstein and Baker, 1954). Bezumnova (1970) found that farm animals, particularly pigs, were the main reservoirs of infection and that transmission occurred via stagnant bodies of water. There are no other reports to suggest that animal excrement is an important vector of the disease.

Salmonellosis. Salmonellosis is probably the most economically important disease transmitted between animals and to man. Apart from S. typhi and S. paratyphi, animals are the principal hosts of salmonellae (Newell, 1967; Prost and Riemann, 1967) and animal excrement is claimed to be a vital factor in perpetuating and extending the prevalence of animal hosts (Decker and Steele, 1966). The presence of symptomless carriers of salmonellae makes the size of the animal reservoir difficult to determine. Carriers are especially important because they excrete large numbers of viable salmonellae and thereby help to maintain the level of organisms generally distributed in the environment (Chung and Frost, 1969).

Animal feeds are a major source of salmonellae. However, the lack of correlation between serotypes found in feeds and serotypes isolated from infected animals shows that other major sources of infection exist.

From the literature of recent years it is apparent that changes are occurring in the relative importance of different serotypes of salmonellae. Fifteen to twenty years ago outbreaks of salmonellosis in domestic animals were usually due to host-adapted serotypes. Reports in recent years, however, show that outbreaks of salmonellosis due to non-host-adapted serotypes in particular S. typhimurium, are increasing. This is probably due to imporved methods of treatment, changes in animal husbandry and the universal distribution of animal feed-stuffs.

Bovine salmonellosis is increasing in several parts of the world. The majority of infections are due to S. dublin but recent reviews (Kampelmacher, Guinée and Clarenburg, 1962; Hughes et al, 1971; Kahrs et al, 1972) report a marked increase in infections due to S. typhimurium.

Pigs are one of the main reservoirs of salmonellae in the animal kingdom according to Sojka and Gitter (1961). In most parts of the world S. cholera-suis is the main serotype associated with clinical disease in pigs but outbreaks due to other serotypes have increased in recent years. Morehouse (1972) suggests that non-host-adapted salmonella serotypes in pigs are more important than S. cholera-suis because of their pathogenicity for man and other animals. Skovgaard and Nielson (1972) report that in England, Wales and Denmark, of the twenty-five most common salmonellae in pigs, nine are included in the ten most common isolates from human infections.

S. typhimurium is the most important non-host-adapted serotype isolated from domestic animals. In Denmark where animal feeds are "resterilized", S. typhimurium accounts for 60 per cent of the isolates from pigs (Skovgaard and Nielson, 1972).

S. dublin is also increasing in importance for its association with animals other than cattle. In 1962 Kampelmacher, Guinée and Clarenberg reported that S. dublin was the third most common serotype isolated from pigs in the Netherlands. In England and Wales it was the seventh most common serotype before 1961 (Sojka and Gitter, 1961) but in a recent survey (Skovgaard and Nielson, 1972) it was third. S. dublin is also becoming an important pathogen of sheep and humans (Hughes et al, 1971).

Poultry are another important reservoir of salmonellae. Kraft et al (1969) provide evidence to show that changes in husbandry towards caged housing of poultry may actually promote shedding of salmonellae when compared with floored housing. If this is the case then slurry from poultry houses may be a greater hazard than the bedding and faeces from floored housing.

Survival of pathogens in animal excrement

Kraft (1969), Venn (1970) and Findlay (1972) have all suggested that the accumulation of animal excreta in the form of a semi-liquid slurry increases the risks of spread of disease. These authors support their arguments with a number of references to studies on the survival of pathogens in animal faeces. The majority of these studies are also used by their authors and in numerous press articles,

to emphasise the health hazards associated with the handling of slurry. It is useful, therefore, to consider some of these reports.

Maddock (1933), described the survival of bovine tubercule bacillus in samples of dried dung for up to six months. Lovel and Francis (1944) collected samples of faeces from an animal infected with Johnes bacillus. The faeces were stored in a porous bowl placed in the open and, therefore, subject to climatic changes. During storage it was frozen, dried, and subjected to a temperature range between -3.3 to 23.3°C. The pathogen was isolated for up to 246 days.

Henning (1939) found that Salmonella dublin survived 1069 days in infected manure which had been dried in an incubator for 48 hours and stored in bottles. Field (1949) stated that S. dublin remained viable in bovine faeces for many months. Josland (1951) isolated a culture of S. typhimurium from a case of bovine salmonellosis. He then inoculated samples of faeces with the organism. Some samples were stored in the shade but exposed to wind and rain, others were exposed to sunlight, wind and rain. The organism survived for 20 weeks in the shaded samples and between 12 to 28 weeks in the samples exposed to sunlight. Gibson (1961) collected samples of faeces from cows that were constant excretors of salmonellae and deposited them on walls. He found that the salmonellae survived for up to six months outdoors and ten months indoors.

Kusdas and Morse (1954) studied the survival of Brucella abortus inoculated into bovine excrement. They provided evidence that the initial pH value of the excrement, increases in acidity or alkalinity, the presence of other organisms, storage temperature and the presence of deleterious substances, all influenced the death of Br. abortus. Survival was enhanced by sterilising the manure prior to infection and by lower storage temperatures.

Although these reports provide some information on the survival of pathogens which are excreted in faeces, few are directly concerned with survival of pathogens in a slurry reservoir. When the excrement is accumulated as a slurry and stored in large tanks, oxygen is exhausted and anaerobic microbial degradation of the material commences. Thus, storage of slurry may be considered as a method of biological treatment of the excreta, even though the system is uncontrolled and the products of the microbial activity are more of a nuisance than the original material. The amount of degradation which occurs depends on several factors, in particular storage time and temperature. Excreta from animals such as cattle, which are only housed for the winter months, may be stored for 6 to 12 months, whereas excreta from animals housed all the year is stored for shorter periods, from a few hours to several weeks.

Survival of enteric bacteria during microbial treatment of animal excreta

Until fairly recently animal excrement was accumulated as a mixture of faeces, urine and straw bedding. Little or no water was added to the material, and aerobic microbial activity was encouraged to convert it to a valuable byproduct, in the form of farmyard manure. Microbiological studies (Gotaas, 1956) show that the treatment time and temperature during composting are usually sufficient to destroy intestinal pathogens.

On farms where a hydraulic system has been adopted, the slurry is normally sprayed onto land, because land treatment is the cheapest and probably the most effective method of disposal. When land is

not immediately available the slurry may be stored, and during storage anaerobic microbial activity occurs.

Reports on the survival of pathogens in slurries of animal excrement during storage are conflicting. Most studies have been carried out on farms under natural conditions. Few, if any, of the parameters likely to affect pathogen survival, such as temperature, pH value and suspended solids concentration have been monitored. Hahn (1967) suggested that different slurries and storage temperatures affected survival of salmonellae. He also found that survival times varied with different strains of salmonellae. Rankin and Taylor (1969) distributed a sample of slurry from a dairy farm into six tanks. The tanks were covered and left outdoors between mid-January and mid-April. Each of five tanks were seeded with one bacterial species, either Salmonella dublin, S. typhimurium, Escherichia coli, Staphylococcus aureus or Brucella abortus, leaving one tank as a control. All the organisms died out completely within twelve weeks. The pH values of the slurry samples were within the range of 7.6 to 6.4 and the dry matter content within the range of 0.35 per cent to 12.4 per cent. Findlay (1971) inoculated S. dublin into two tanks each containing 40 l of cattle slurry. One tank was covered and the other left exposed to rainfall. Some of the inoculated cells survived for at least five months. In another series of experiments Findlay (1972) found that S. dublin survived in cattle slurry for 31 to 33 weeks when slurry was artificially infected in October, but only 18 to 19 weeks when it was infected in March. Rainfall and atmospheric temperatures were recorded at the site, but the solids concentration and temperature of the slurry were not reported.

As a result of the numerous problems mentioned earlier, methods of controlled biological treatment of slurry are under investigation. Most of the treatment systems in the USA and in Great Britain have been modifications of methods used in the treatment of domestic sewage. The treatment of domestic sewage is primarily concerned with the reclamation of water. Solid wastes, arising from sedimentation, and the sludges of aerobic and anaerobic treatment systems, have been considered to be of relatively minor importance. For this reason the solids have usually been disposed of as cheaply as possible, without regard to any economic value.

The amount of water involved in slurry does not usually necessitate concern about water reclamation. The primary concern must be the treatment of the solid material (Owens, 1972). Treatment systems should be designed for the conversion of slurry to a material which can be used as a fertiliser, or alternatively to some other valuable byproduct.

During the development of biological treatment systems it is necessary to consider the fate of enteric bacteria. Kampelmacher and Noorle Jansen (1970) inoculated S. utrecht into domestic sewage and found a 90 per cent reduction in the liquid effluent. The mean residence time in the aeration reservoir was 10.5 h. The normal concentration of salmonellae in domestic sewage in the Netherlands was found to be 2 per cm^3 and they concluded that the normal expected concentration in treated effluents would be 1 per cm^3. Leclerc et al (1970) also found that only low numbers of pathogens are discharged in the liquid effluent from domestic sewage treatment plants. They found however, that the majority of pathogens, in particular salmonellae, become associated with and protected by sludge flocs.

Kampelmacher and Noorle Jansen (1971) found a 99 per cent reduction of salmonellae in the effluent of an experimental hog-excrement oxidation vat. The mean residence time of liquor in the aeration reservoir appeared to be 12 days. The aeration rotor was switched off for one hour each day and the top liquor allowed to overflow into a stream. No details of the concentrations of suspended solids or BOD of the excrement, the aerobic sludge, or the effluent are given, and the only product of this system appeared to be the top liquor. Robinson, Saxon and Baxter (1971) found that salmonellae, streptococci and staphylococci survived for more than 8 days in aerated pig urine. McCalla and Elliott (1971) stated that "salmonella added to beef manure died rapidly under aerobic conditions but survived under anaerobic conditions".

In the absence of performance details of treatment systems it is not possible to decide from such reports whether or not salmonellae survive longer in systems for treating animal excrement than those for domestic sewage. If pathogens become associated with sludge flocs and the solid material is the main product of treatment, then there is an increased risk of infection, especially if this material is to be used in animal feeds.

Survival of enteric bacteria on land and the spread of enteric diseases.

There is a considerable amount of evidence, reviewed by Rudolfs et al (1950) and van Donsel, Geldreich and Clarke (1967) that enteric bacteria, including intestinal pathogens, can survive on land for long periods. Beard (1940) and van Donsel et al (1967) conclude that soil type has very little influence on the survival of faecal bacteria, including salmonellae. Soil moisture and pH are considered to be the main parameters which affect survival.

Recently Tannock and Smith (1972) prepared suspensions of Salmonella typhimurium and S. bovismorbificans in "sterile, sietz filtered sheep faeces". These suspensions were then sprayed onto the surface of outdoor experimental plots of ryegrass-clover pasture. They found a rapid decline of viable cells in the soil. Survival was greatest where the organisms were protected from direct sunlight.

While it is relatively easy to demonstrate the presence of viable pathogenic bacteria on land or in drainage water, it is very difficult to show that their presence could result in an outbreak of disease. Mair and Ross (1960) report the case of a young girl infected with S. typhimurium. Cattle faeces had been dug into the garden a few weeks earlier and S. typhimurium was isolated from soil samples. It was not possible however to decide whether the salmonellae in the soil originated from the cattle faeces or from the child. Jack and Hepper (1969) reported an outbreak of salmonellosis in cattle grazing a pasture which had been irrigated three weeks earlier with slurry contaminated with S. typhimurium. Williams (1970) provides evidence that many clinical cases in adult cattle and sheep in Carmarthenshire arise from the grazing of recently-flooded pasture. Richardson and Watson (1971) found S. dublin in a river near a pasture where infected cattle had been grazing. They suggested that the infected water was the source of infection for seven outbreaks of disease on farms close to the same river. Orr (1972) reported an outbreak of S. typhimurium infection in cattle in Wigtownshire. The source of infection appeared to be the discharge of a septic tank following the infection of an elderly woman in the farmhouse.

Taylor and Burrows (1971) applied cattle slurry contaminated with S. dublin to a pasture. Calves became infected when allowed to graze on a pasture sprayed the previous day with slurry containing 10^6 S. dublin per cm^3.

There is little evidence to confirm the belief that the increasing practice of disposing of animal excrement, in the form of a semi-liquid slurry, onto land increases the incidence of intestinal diseases amongst domestic animals and/or man. However, if diseases such as salmonellosis are to be controlled then contamination of land and water-courses must be minimised (Newell and Williams, 1971; Hughes et al, 1971). Before this can be done, the chances of significant numbers of pathogens remaining viable in excrement during storage and/or during controlled biological treatment must be investigated. If complete elimination of viable pathogens from infected material is not practical then factors which further influence the dissemination of bacteria in the environment must also be understood.

SCOPE OF WORK

At present, animal excrement, which is accumulated and stored in the form of a semi-liquid slurry, or the residual sludge from an aerobic biological treatment system, is normally disposed of by spraying onto the surface of grassland. If appreciable numbers of viable pathogenic micro-organisms survive storage, then disposal of the slurry, or residual sludge, could increase the numbers and distribution of intestinal pathogens in the environment.

Pigs are one of the main reservoirs of salmonellae amongst domestic animals. Infected animals which may or may not develop clinical symptoms of disease excrete large numbers of the organisms in their faeces. Thus, freshly-excreted pig excrement should be considered as a potential reservoir of viable salmonellae.

During the storage of untreated excrement, large solids settle to the bottom of the slurry tank. Anaerobic conditions develop and the gases, produced by fermentation, lift some of the solids to the surface, where with the aid of pig hairs, they form a crust. This crust then acts as an air-tight seal so that most of the excrement is kept anaerobic.

On some farms aerobic biological treatment of slurry is being tried in order to reduce pollution problems, but all systems presently in use are largely experimental. The properties of residual sludges, and whether or not a liquid effluent is produced, from aerobic biological treatment systems depends not only on the type of animal and its diet, but also on the operating conditions of the treatment system to meet the requirements of the operator. Treatment systems can be operated at various biochemical oxygen demand loading rates, pH values, temperatures, mixed liquor suspended solids concentrations and dissolved oxygen concentrations. Adjustment of any of these

parameters may affect the chemical composition of the residual sludge and its dewaterability, and may also influence the chances of survival of pathogenic bacteria.

Section I of this report describes studies on some of the factors which might influence the survival of salmonellae in pig excrement during anaerobic storage, and in the mixed liquor of an aerobic biological treatment unit. Since naturally infected excrement was not readily available, it was necessary to artificially infect samples of pathogen free pig excrement with a species of salmonella.

Section II is concerned with investigations into factors which influence the passage of bacteria, through soil, into land drainage water. If appreciable numbers of viable intestinal pathogenic bacteria, such as salmonellae, are present in slurry sprayed onto the surface of grassland, then a proportion of these organisms may pass through soil into land drainage water and thence into a water course.

The concentrations of faecal bacteria in the sub-surface drainage water of a 0.7 ha pasture at the Animal Husbandry Experimental Unit, Brickrow, The West of Scotland Agricultural College, Auchincruive, Ayrshire, were first monitored in the absence of recent slurry application to the land. Pig excrement was then applied to the land and the effect on the concentrations of faecal bacteria in the water observed. A further investigation of the factors affecting the numbers of soil bacteria in drainage water was made. The numbers discharged during a winter were also compared with the numbers resident in the soil.

The drainage water of two 20 ha areas of land on a pig farm in Wigtownshire was also studied. These results were then compared with those obtained from the experimental pasture, Brickrow.

S E C T I O N I

THE SURVIVAL OF *Salmonella dublin* IN PIG EXCRETA

INTRODUCTION

Pig excrement naturally infected with salmonellae was not readily available. Because of the dangers of the spread of infection, it was not practical to artificially contaminate excrement with bacterial pathogens in a storage tank on a farm. Therefore, it was necessary to artificially contaminate samples of salmonella-free excrement with a species of salmonella and study its survival in containers in the laboratory.

Although Salmonella cholera-suis is the most common salmonella serotype associated with pigs, it is difficult to isolate and identify from infected faeces (Field, 1959) and is not commonly transmitted from pigs to other animals or man. It is also a dangerous serotype to handle in the laboratory. S. typhimurium and S. dublin are both widely distributed in pigs, and other domestic animals, and both serotypes are important for their public health significance. Because of the nature of pig excrement, it is difficult to guarantee not to spill infected material when preparing a dilution series, and equally difficult to prevent the release of aerosols from an aerobic biological treatment unit. Therefore, since S. dublin is the less virulent of the two serotypes it was chosen as the test organism.

In a series of five experiments, samples of pig excrement, diluted with tap water to a semi-liquid slurry, were inoculated with a culture of S. dublin, and stored at constant temperatures in the laboratory. In each experiment the numbers of survivors of S. dublin were estimated at intervals during storage.

The mixed liquor of a laboratory treatment unit, being used in a study on the effects of aerobic biological treatment on pig excrement, was also inoculated with a culture of S. dublin. The rate of decline of the inoculated cells was monitored and compared with the rate of decline of Escherichia coli.

MATERIALS AND METHODS

Source of pig excrement

All the samples of pig excrement used in the laboratory were collected from a pig fattening house at the Animal Husbandry Experimental Unit, Brickrow, The West of Scotland Agricultural College, Auchincruive. A plastic tray was suspended below the slatted floor of one of the pens for 24 to 48 h. The pen contained from one to ten pigs, each weighing 30 to 90 kg, and they had unlimited access to food and water. They were fed on a ration of barley meal, 62.5 per cent; fine thirds (wheatings), 25 per cent; white fish meal, 7.5 per cent; and extracted soya bean meal, 5 per cent. This was supplemented with a vitamin and mineral supplement (Elsley et al, 1969) 17.8 kg/t; copper sulphate, 0.67 kg/t; and zinc sulphate, 0.02 kg/t.

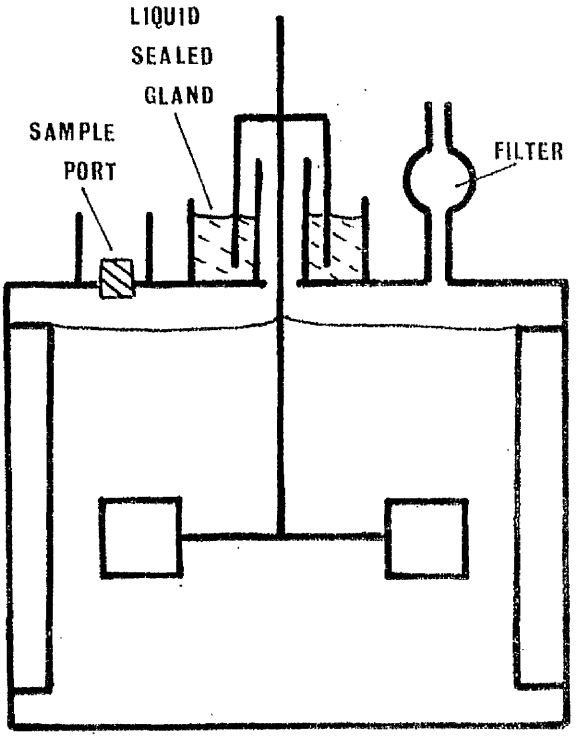
Faeces and urine, which collected in the tray, were transported to the laboratory and diluted with sufficient tap water to form an easily stirred slurry. This slurry was thoroughly mixed for 10 min and the suspended solids concentration determined. It was then further diluted with an appropriate volume of tap water to obtain a suspended solids concentration of between 9.6 and 68.3 g/l, depending on the experiment. The final suspended solids concentration was then determined.

The suspended solids concentration was determined by filtration through glass fibre filters (Whatman GF/A) and dried at 105°C. The chemical oxygen demand (COD) was also determined, using standard methods (Standard Methods 1971) and the pH value was measured using a glass electrode.

FIGURE 1

Diagram of a two litre vessel, fitted with a paddle, and baffles, used for the anaerobic storage of pig excrement in the laboratory.

FIGURE 1



Storage and sampling of pig excrement in the laboratory

Samples of pig excrement, diluted with tap water to a semi-liquid slurry, were stored in closed containers in the dark at constant temperatures. In most of the experiments the slurry was only mixed at the time of taking sub-samples for bacteriological examination and measurement of pH value. The static storage conditions allowed a crust to form on the surface of the slurry, maintaining anaerobic conditions throughout most of the sample, similar to those found in a storage tank on a farm.

Some samples were stored in 2 l polypropylene containers. These containers had a sampling port in the top sealed with a rubber bung. The port was surrounded by a short length of polypropylene tube, welded to the top of the vessel, forming a small reservoir. Any spillage of infected material, while removing samples from the vessel, was contained in this reservoir and could be treated with disinfectant after the bung was replaced. A second port in the top of the vessel was fitted with a cotton wool filter to allow fermentation gases to escape. Each vessel contained a plastic coated bar magnet. Before sampling, the contents of the vessel were mixed on a magnetic stirrer (S/MAG 30, Jencons (Scientific) Co. Ltd., Mark Road, Hemel Hempstead, Herts.) for five minutes at the fastest speed possible without losing control of the magnet. This method of mixing was only effective for slurries containing less than 55 g suspended solids/l. With thicker slurries mechanical mixing was necessary. Some vessels were therefore fitted with a paddle and internal baffles (Fig. 1). The paddle was bolted onto a stainless steel shaft which passed out of the vessel through a liquid-sealed gland. The slurry could then be mixed at 200 rpm by attaching the shaft to an electric motor. The stirring gland was filled with a mixture of sulphuric acid and glycerol.

This solution did not evaporate or splash during mixing and prevented bacteria escaping from the vessel. In each experiment the slurry was dispensed into two containers. One container was inoculated with a culture of Salmonella dublin and the other left as a control. Each vessel was removed from the incubator at intervals, the contents thoroughly mixed and samples of 10 cm^3 removed. The Most Probable Number (MPN) of salmonellae in each sample was then estimated. To avoid possible contamination of the pH probe, and release of salmonellae into the atmosphere, the pH value of the control sample only was measured.

In one experiment the pH value of the excrement was monitored continuously during storage. The slurry was stored in a three litre Biotec fermenter vessel (LKB House, 232 Addington Road, South Croydon, Surrey) fitted with a central paddle, driven at 200 rpm through a magnetic coupling by an electric motor. To achieve adequate mixing at this low speed, a larger polypropylene impeller was attached to the shaft. A fermentation lock was fitted to a port in the top of the vessel. The pH value was measured by an autoclavable glass electrode (Activion Ltd., Kinglassie, Scotland), coupled by a pH meter to a potentiometric chart recorder. The temperature of the vessel was controlled at 15°C. Samples of slurry were obtained from a port in the bottom of the vessel, through a stainless steel sampling hood. Before sampling, the tube was cleared by blowing oxygen-free nitrogen back through the tube into the vessel. A sample of 10 cm^3 of slurry was removed at the start of the experiment and the MPN of salmonellae estimated. The slurry was then inoculated with a broth culture of S. dublin. Samples of 10 cm^3 of the contaminated slurry were removed at three or four day intervals and the MPN of S. dublin estimated.

FIGURE 2

The principal components of the laboratory aerobic biological treatment unit.

FIGURE 2

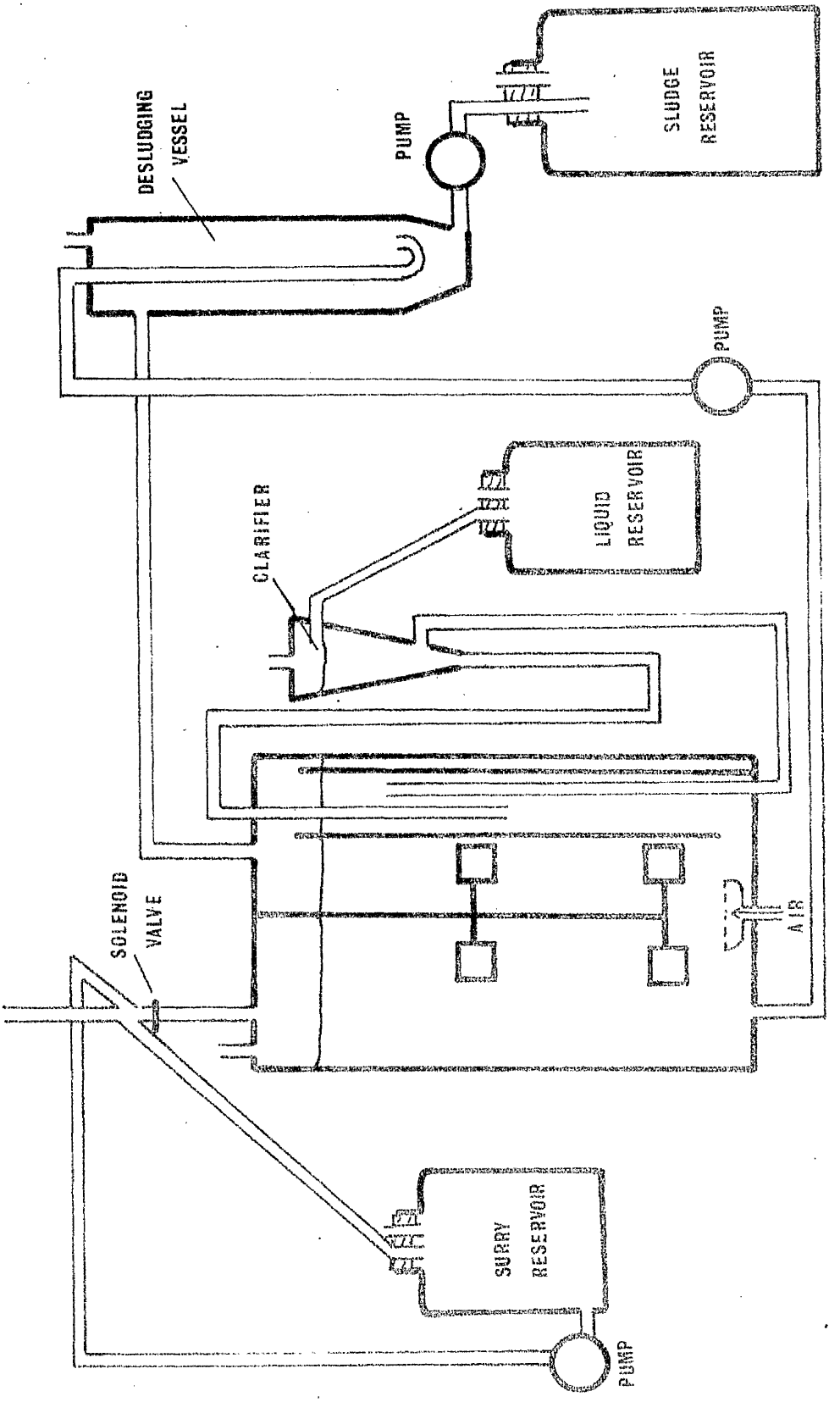
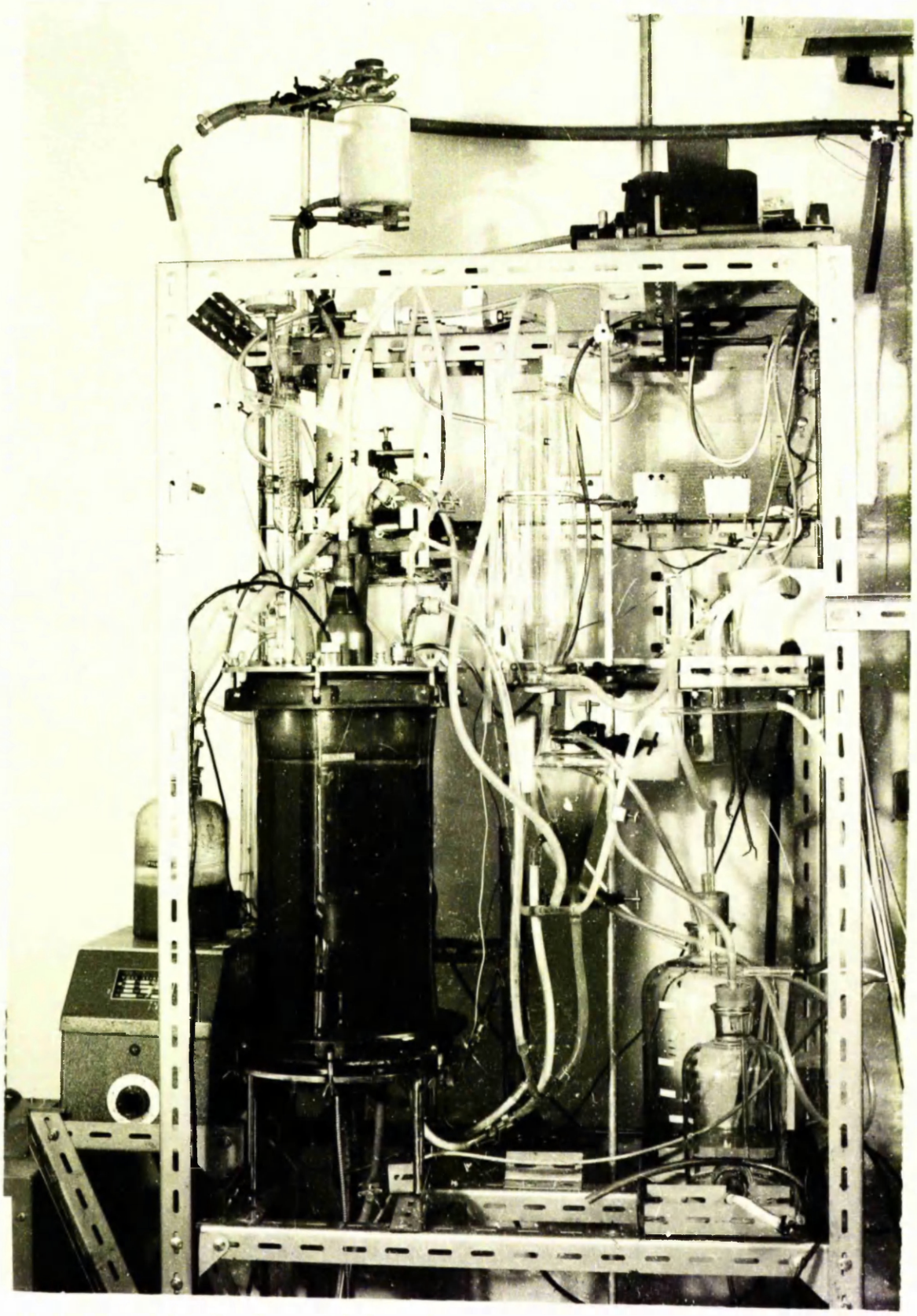

DESLUDGING VESSEL
PUMP
SLUDGE RESERVOIR
PUMP
CLARIFIER
LIQUID RESERVOIR
SOLENOID VALVE
AIR
SLURY RESERVOIR
PUMP

FIGURE 3

The laboratory aerobic biological treatment unit.

FIGURE 3



In the later experiments smaller samples of slurry, 20 cm^3 were stored in universal bottles. These bottles were sealed with plastic fermentation locks (Boots Co. Ltd.) which were filled with an iodophor disinfectant. Before sampling the slurry, the fermentation locks were replaced with screw caps, so that the contents could be thoroughly mixed. In these experiments the slurry was divided into two samples, one of which was inoculated with S. dublin. Each sample was then distributed into a 2 l container and a number of universal bottles. Two of the universal bottles containing contaminated slurry were removed at intervals of 3 or 4 days and two of the control samples at intervals of 7 days. The MPN of salmonellae in each sample was estimated and the pH value of the control samples was measured. The MPN of salmonellae and pH values of the slurry in the larger containers were estimated only at monthly intervals.

The aerobic biological treatment system

The laboratory treatment unit (Fig. 2 and Fig. 3) was an aeration vessel supplied semi-continuously with a suspension of pig excrement. The volume of mixed liquor in the aeration vessel was kept constant by the separate removal of a liquid effluent and a solid effluent.

Fresh pig excrement was collected twice weekly from the pig fattening house and transported to the laboratory. It was then diluted with an appropriate volume of tap water to a suspension containing 20 g suspended solids/l.

The dilute slurry was fed semi-continuously to the aeration vessel, using the system described by Owens and Evans (1972). The aeration vessel was a Biotec laboratory fermenter, fitted with ports in the top and bottom, and had a working volume of 15 l. The stirring rate of the fermenter contents was controlled by a variable speed

motor coupled magnetically to a central paddle inside the vessel. To allow floc formation the stirring rate was kept as low as was consistent with good mixing and aeration. This was achieved by attaching extra large impellers to the stirring shaft. The large impellers consisted of four polypropylene blades, approximately 5 cm square, welded onto a short length of polypropylene tubing at a slight angle to the vertical. Two large impellers were pushed onto the impeller shaft and clamped to the existing stainless steel impellers. Stirring rates of 70 to 100 rpm gave good mixing of the fermenter contents. The temperature of the mixed liquor was kept constant. Aeration was effected by a sintered steel sparger through which air was pumped at the rate of 1.5 l/min and this maintained an oxygen tension in excess of 150 mm Hg. The oxygen tension of the mixed liquor was continuously recorded using an oxygen electrode and a potentiometric chart recorder. The pH value of the mixed liquor was also recorded on the chart recorder and provision was made for controlling the pH value when required.

Liquid effluent was allowed to overflow from a clarifier system attached to the aeration vessel into a collection reservoir. The clarifier system consisted of an internal and external settling chamber. The internal chamber was a glass tube, of 30 mm bore, which rested on the bottom of the aeration vessel for part of its circumference. Most of the solids of the mixed liquor which entered this chamber settled to the bottom and automatically returned to the aeration vessel. The partially-clarified liquid in the upper part of the internal settling chamber overflowed into the external chamber. This consisted of an inverted Erlenmeyer flask, fitted with an inlet tube for the partially-clarified liquid just above the base of the vessel, a return tube for settled solids at the base and a tube near the top for the clarified liquid to overflow into a collection

reservoir. The settled solids were returned to the top of the internal settling chamber continuously by an air lift pump.

The solid effluent separation or desludging system consisted of a vessel into which mixed liquor was pumped at predetermined intervals. An overflow tube was fitted to the desludging vessel, at the 2 l level, to return excess mixed liquor to the aeration chamber. The mixed liquor in the desludging vessel was allowed to settle for one hour. After settling the supernatant was pumped back into the aeration vessel and the settled sludge was pumped from the bottom of the desludging vessel into a solid effluent collection reservoir. The intervals between desludging could be adjusted from 2 to 26 h. The circuitry for the process timers and pump operation was similar in principal to that described by Owens and Evans (1972), for the feeder controller.

The air spaces above the mixed liquor, the external settling chamber, the desludging vessel and the two effluent collection reservoirs were interconnected by a series of tubes. This maintained an equal pressure above all the liquid levels. A cooled condenser returned moisture from this air system to the mixed liquor, and excess gases were released to the atmosphere of the laboratory through a Biotec air filter, which had a pore size of 0.3 μm.

When Salmonella dublin was added to the mixed liquor, the desludging vessel was replaced by a 2 l measuring cylinder and the treatment unit was desludged manually. Each day 2 l of mixed liquor were pumped into the measuring cylinder. After settling for one hour, 800 cm^3 of the supernatant were returned to the aeration vessel. The remaining sample in the measuring cylinder was divided into supernatant and sludge fractions. Both fractions were then sub-sampled for microbial examination. The remainder of each sample was pasteurised at 80^{o}C for 30 min before being chemically examined.

Apart from the monitoring of the pH value and the oxygen tension of the mixed liquor, samples of feed, mixed liquor, liquid and solid effluents, were analysed chemically. The concentrations of suspended solids, total solids, volatile solids, ash, and chemical oxygen demand were determined by standard methods (Standard Methods 1971). Biochemical oxygen demand (BOD) was determined either by the standard method, using a dissolved oxygen meter to measure dissolved oxygen concentrations, or by standard manometric techniques using a Warburg respirometer. In either case the samples were seeded with settled mixed liquor from the treatment unit. From these measurements the BOD loading rate and its relationship to solids loading rate, and the solids residence time in the aeration chamber were calculated.

Inoculation of slurry and the aerobic treatment system, with Salmonella dublin

In each experiment a nutrient broth (Oxoid) culture of S. dublin was grown overnight at 37°C in an orbital incubator. Assuming a concentration of S. dublin in the broth of 10^9 viable units per cm^3, the volume of broth culture used to inoculate samples of slurry and the contents of the aeration vessel was then chosen to give a concentration of S. dublin in suspension, at the start of each experiment, of 10^7 viable units per cm^3. At the same time a surface spread plate count of S. dublin in the broth was estimated, so that the initial concentration of viable cells in the inoculated suspension could be calculated.

Estimation of the MPN of salmonellae and Escherichia coli in slurry

Salmonellae. The slurry reservoirs were removed from the incubator at intervals during storage, mixed thoroughly and 10 cm^3 transferred, using a wide bore (9 mm) pipette, to 90 cm^3 mineral salts diluent (Owens and Keddie, 1969). With sufficient practice and speed, it was possible to remove a representative sample of the suspension containing both solids and liquid. This initial dilution was shaken thoroughly and a series of dilutions prepared. In the earlier experiments a ten-fold dilution series was prepared, transferring 1 cm^3 of each preceding dilution to 9 cm^3 diluent. E-mil 1 cm^3 disposal pipettes (James A. Jobling & Co. Ltd., Stone, Staffordshire) were used for these transfers and the tubes were mixed between each stage on a Whirlimix (Fisons Scientific Apparatus Ltd., Bishop Meadow Road, Loughborough, Leicestershire). Again care was taken not to leave all the solids in the first stage of the series.

In the later experiments a two-fold dilution series of the initial decimal dilution was used. 5 cm^3 of each preceding dilution was transferred to 5 cm^3 diluent using 5 cm^3 blow-out pipettes.

The dilutions were used to inoculate tubes of tetrathionate enrichment broth (Oxoid) plus 2 per cent of an iodine solution. In the earlier estimations the iodine solution was composed of 30 g iodine, 25 g potassium iodide dissolved in 100 cm^3 distilled water. Later the iodine concentration was reduced to 20 g per 100 cm^3 of the solution. 10 cm^3 of enrichment broth was inoculated with 1 cm^3 of the dilutions. Five tubes of enrichment broth were inoculated from each dilution of the ten-fold series and four tubes of enrichment broth were inoculated from each dilution of the two-fold series. The inoculated broths were incubated at 37°C for 48 h.

After incubation the enrichment broths were examined for supporting the growth of Salmonella dublin. In the earlier experiments this was done by plating a loopful from each enrichment broth onto brilliant green agar (Oxoid) and by microscopic examination using a fluorescent antibody technique. This technique was later abandoned and a loopful of enrichment broth was plated onto modified bismuth sulphite agar (McCoy, 1969), as well as brilliant green agar.

The plates of brilliant green agar and modified bismuth sulphite agar were incubated at 37°C for 48 h. Colonies were confirmed as S. dublin by sub-culture onto triple sugar iron agar (Oxoid) and because all the control plates were negative.

The fluorescent antibody technique used was that of Silliker, Schmall and Chiu (1966). Brain heart infusion broth (Difco) was inoculated from the enrichment cultures and incubated at 37°C for 16 h. These cultures were then formalised and a loopful of each smeared on a microscope slide. The slides were air-dried, fixed in acetone for five minutes and stained with fluorescein-labelled Phase I (specific) gP-H S. dublin antiserum (Wellcome Research Laboratories, Beckenham, Kent).

The MPN of S. dublin in the contaminated slurry was calculated from the number of positive tubes of enrichment broth. For the ten-fold dilution series, this was calculated after reference to Appendix C, The Bacteriological Examination of Water Supplies (Ministry of Health and Social Security, 1969). For the two-fold dilutions the MPN was calculated after reference to table $VIII_2$, Fisher and Yates (1953).

The 95 per cent confidence limits for each count were calculated from table 6.5, Maynell and Maynell (1965), giving factors of 3.30 for the ten-fold dilution series and 2.00 for the two-fold dilution series.

Escherichia coli. To obtain the numbers of E. coli fed into the aerobic treatment unit, 10 cm^3 samples of slurry were removed from the feed reservoir. A ten-fold dilution series of the slurry was prepared in a similar manner to that already described for the S. dublin counts. Five tubes of 5 cm^3 MacConkey broth were then inoculated with 1 cm^3 of each dilution. The surfaces of violet red bile agar plates (Oxoid) were then inoculated with 0.1 cm^3 of each dilution and these plates were overlaid with a further layer of VRBA. The tubes and plates were incubated at 37°C. After 24 h the number of colonies on each plate were counted. The positive tubes of MacConkey broth were sub-cultured to fresh tubes of the same medium and were incubated at 44°C for 24 h. The cultures obtained at 44°C were later confirmed as E. coli by IMViC tests.

Estimation of the numbers of Escherichia coli and Salmonella dublin in the aerobic mixed liquor

The numbers of E. coli were estimated in either the mixed liquor, or in the settled sludge and supernatant of the mixed liquor. The latter samples were obtained by removing a sample of mixed liquor, usually 100 cm^3, from the aeration vessel and allowing it to settle for one hour. The supernatant was then decanted into a separate container. The numbers of S. dublin surviving in the aeration vessel were estimated in samples of settled sludge and supernatant.

In the earlier estimations of the numbers of E. coli in the aeration vessel, 20 cm^3 of the mixed liquor, or settled sludge, were transferred with a wide bore (9 mm) pipette to 180 cm^3 cooled mineral salts diluent. This suspension was then blended in an Atomix blender (MSE Ltd., Buckingham Gate, London SW1) at full speed for 2 min. A ten-fold dilution series of the blended suspension was prepared and used as before to estimate the numbers of E. coli.

In the later E. coli and S. dublin counts, 20 cm^3 of undiluted mixed liquor, or settled sludge, were blended on a Virtis '45' homogeniser (supplied by Cenco Instrumenten Nij n. v., P.O. Box 336, Konijnenberg, 40 Creda, Netherlands) at minimum speed for 2 min. The jar containing the sample was surrounded with iced water during blending. After blending, 10 cm^3 of the suspension was transferred with a wide bore pipette to 90 cm^3 mineral salts diluent. A ten-fold, or a two-fold, dilution series of this initial dilution was then prepared and the numbers of E. coli and S. dublin estimated as before.

TABLE 1

The concentration of Salmonella dublin in various enrichment broths after incubation at 37°C for 24 h and counted by a plate count on nutrient agar and brilliant green agar.

TABLE 1

Enrichment Broth	Number of S. dublin/cm^3	
	Nutrient agar	Brilliant Green agar
Mannitol selenite broth (Oxoid)	7.8×10^8	2.0×10^8
Tetrathionate broth (Oxoid)	5.0×10^8	2.0×10^8
Tetrathionate broth B (Rolfe, 1946)	2.0×10^7	2.0×10^7
$Mg\ CL_2$/Malachite green (Rapport, 1956)	9.0×10^3	0
Nutrient broth No. 2 (Oxoid)	1.0×10^9	6.5×10^8

EVALUATION OF METHODS

The isolation and enumeration of *Salmonella dublin*

Numerous enrichment broths and selective media are recommended for the isolation and enumeration of salmonellae from different sources. Due to the different characteristics of various salmonella serotypes, two or more enrichment broths and selective media, and a range of incubation times, are generally used to detect all the salmonellae present. In these studies only *Salmonella dublin* was being observed, therefore it was possible to select one enrichment broth and one incubation temperature. However other enteric bacteria, such as *Proteus sp.*, *Escherichia coli* and *Alcaligenes faecalis* made it necessary to use either two methods, or two selective media, for confirming the growth of *S. dublin* in the enrichment broth.

Comparison of the growth of S. dublin in different enrichment media

Tubes of 10 cm^3 of four enrichment broths and one tube of nutrient broth were each inoculated with one loopful of a nutrient broth culture of *S. dublin*. The tubes were incubated at 37°C for 24 h and the number of *S. dublin* in each broth was estimated by a surface spread plate count on nutrient agar (Oxoid) and on brilliant green agar (Oxoid).

The concentration of *S. dublin* in each broth is shown in Table 1. From the results it is clear that mannitol selenite broth (Oxoid) and tetrathionate broth base (Oxoid) plus 2 per cent iodine solution (30 g iodine, 25 g potassium iodide, 100 cm^3 distilled water) were the two most satisfactory enrichment media.

TABLE 2

The number of Salmonella dublin, Escherichia coli and Proteus sp. in pure cultures estimated by a plate count on nutrient agar and brilliant green agar, and the MPN of each culture estimated by inoculating three tubes of mannitol selenite broth and tetrathionate broth at each dilution level followed by sub-culture onto nutrient agar and brilliant green agar.

* Nutrient agar, + Brilliant green agar.

Figures in brackets indicate green colonies only on brilliant green agar.

TABLE 2

Culture	Plate Counts/cm^3		MPN counts/cm^3			
			Mannitol Selenite broth		Tetrathionate broth	
	N.A.*	B.G.+	N.A.	B.G.	N.A.	B.G.
S. dublin	3.0×10^8	1.0×10^8	1.7×10^7	3.5×10^6	4.5×10^8	4.0×10^8
E. coli	9.8×10^8	7.0×10^7	9.0×10^3	(2.3×10^4)	4.0×10^3	0
Proteus sp.	1.0×10^9	0	2.8×10^7	1.4×10^7	5.5×10^6	4.5×10^6

TABLE 3

Comparison of various selective media for the isolation of Salmonella dublin from mannitol selenite broth and tetrathionate broth. Three tubes of enrichment broth inoculated at each dilution level.

TABLE 3

Enrichment broth	Number of positive tubes					
	Mannitol selenite broth			Tetrathionate broth		
Dilution level	10^{-6}	10^{-7}	10^{-8}	10^{-6}	10^{-7}	10^{-8}
Selective agar						
Brilliant green (Oxoid)	3	3	3	3	3	3
Modified Brilliant green (Oxoid)	3	3	2	3	3	3
Brilliant green (Merck)	2	0	0	1	2	0
Brilliant green (Difco)	3	3	3	3	3	2
Bismuth sulphite (Oxoid)	1	0	0	2	1	0
Desoxycholate citrate (Oxoid)	1	0	0	1	1	1
Nutrient (Oxoid)	3	3	3	3	3	3

Selectivity of mannitol selenite broth and tetrathionate broth. The MPN of E. coli (NCTC 9001), Proteus sp. (No 15A, The West of Scotland Agricultural College stock cultures), and S. dublin in pure cultures were determined by preparing a series of dilutions of each culture and inoculating three tubes of mannitol selenite broth and three tubes of tetrathionate broth from each dilution. These enrichment broths were incubated at 37°C for 24 h and a loopful of each streaked over the surface of nutrient agar and brilliant green agar. These plates were incubated at 37°C and examined for growth after 24 and 48 h. At the same time a surface spread plate count of each culture was made on nutrient agar and brilliant green agar.

The results of these counts are shown in Table 2. In this test the MPN of S. dublin was slightly higher in tetrathionate broth than in mannitol selenite broth and was similar to the concentration estimated by the plate counts. E. coli and Proteus sp. were not completely inhibited by either of the enrichment broths, but both species produced colonies which could be distinguished from the red colonies of S. dublin.

Comparison of selective media for the isolation of S. dublin from enrichment broths. The MPN of S. dublin in a broth culture was estimated by preparing a series of dilutions and inoculating a set of tubes of tetrathionate broth and a further set of mannitol selenite broth. Three tubes of each broth were inoculated from the 10^{-6}, 10^{-7} and 10^{-8} dilutions. The enrichment broths were incubated at 37°C for 24 h and the number of positive tubes were identified by plating out onto a variety of selective media and onto nutrient agar.

The number of positive tubes in each case is shown in Table 3. From these results brilliant green agar (Oxoid) was chosen for further tests.

Methods for isolation and enumeration of bacteria in pig excrement and aerobic sludge.

Before inoculating salmonellae into pig excrement or a flocculent sludge, resulting from aerobic treatment of the waste, methods of counting total viable bacteria and E. coli were examined.

A flocculent sludge is a most difficult material to examine, hence methods of blending samples before preparing a dilution series were evaluated. Owens (Pers. Comm.) tested a variety of counting methods and obtained the highest number of viable bacteria by blending samples in an Atomix blender before preparing dilutions. 20 cm^3 of mixed liquor from a laboratory aerobic treatment unit were transferred using a wide bore (9 mm) pipette to 180 cm^3 cooled mineral salts diluent. This suspension was then blended at maximum speed for 2 min. A ten-fold dilution series of the blended suspension was prepared and 0.1 cm^3 of each dilution was spread over the surface of plates of MacConkey agar and a dilute nutrient agar (DA). This medium was composed of 2.5 g nutrient broth No. 2 (Oxoid); 1.8 g agar No. 1 (Oxoid); and 1,000 cm^3 mineral base E, pH 6.5 (Owens and Keddie, 1969), and was sterilised by autoclaving at 121°C for 15 min. The most probable number of coliforms was obtained by inoculating five tubes of 5 cm^3 MacConkey broth with 1 cm^3 suspension at each dilution. The plates of MacConkey agar and tubes of MacConkey broth were incubated at 37°C for 24 h. Cultures of coliforms were later confirmed as Escherichia coli by Eijkman and IMViC tests (Ministry of Health and Social Security, 1969). The plates of DA were incubated at 25°C and colonies counted after 7 and 14 days.

Counts of 10^9 viable bacteria per cm^3 were regularly obtained on DA and $> 10^3$ viable E. coli per cm^3 in MacConkey broth and on MacConkey agar. Later, higher counts were obtained after purchasing a Virtis '45' homogeniser. With this machine 20 cm^3 of undiluted mixed liquor, or settled sludge, were blended for 2 min at minimum speed.

The effect of blending pig excrement, and aerobic sludge, on counts of total viable bacteria and Escherichia coli

The numbers of total viable bacteria and E. coli in fresh pig excrement (20 g suspended solids/l) and in a flocculent aerobic sludge (38 g suspended solids/l) were estimated. The sludge was obtained after allowing a sample of mixed liquor from the laboratory treatment unit to settle for one hour and decanting the supernatant.

Each sample was examined by preparing a ten-fold dilution series and then inoculating plates of DA, Violet red bile agar (VRBA) and tubes of MacConkey broth. The first dilution of each series was made by transferring 10 cm^3 of sample to 90 cm^3 mineral salts diluent and further dilutions by transferring 1 cm^3 of each suspension to 9 cm^3 diluent.

Another decimal dilution of each sample was prepared by transferring 20 cm^3 of sample to 180 cm^3 cooled mineral salts diluent. This suspension was then blended in the Atomix blender and a series of further dilutions was prepared by transferring 1 cm^3 of each suspension to 9 cm^3 diluent, which were then used to inoculate plates of DA, VRBA and tubes of MacConkey broth.

A further 20 cm^3 of undiluted slurry, or sludge, were blended in the Virtis homogeniser. A decimal dilution series similar to that of the unblended samples was prepared and these dilutions were used to inoculate plates of DA, VRBA and tubes of MacConkey broth.

TABLE 4

The effect of blending pig excrement, and an aerobic sludge, prior to preparing dilutions for plate counts.

NB - Non-blended

BA - Blended on Atomix blender

BV - Blended on Virtis '45' homogeniser

TABLE 4

	Viable bacteria/gSS			
	aerobic Sludge		Pig Excrement	
	Total ($x10^9$)	*E. coli* ($x10^5$)	Total ($x10^9$)	*E. coli* ($x10^5$)
NB	6.2	7.5	39	1.1
BA	20	17	26	1.1
BV	47	23	15	1.1

The numbers of total viable bacteria and E. coli found in each case are shown in Table 4. The maximum numbers of both groups of bacteria in the sludge were obtained after blending, whereas in the raw slurry the highest counts of both groups were obtained without blending.

Recovery of Salmonella dublin from fresh pig excrement.

A sample of fresh pig excrement was divided into two parts and one part was inoculated with a broth culture of S. dublin. At the same time the number of viable S. dublin in the broth was estimated by a surface spread plate count on nutrient agar.

The two samples of pig excrement were then thoroughly mixed and examined for salmonellae. In each case 10 cm^3 of excrement were removed using a wide bore, 9 mm, pipette and transferred to 90 cm^3 mineral salts diluent. These initial dilutions were shaken vigorously and a series of decimal dilutions of each prepared. The MPN of salmonellae in each sample of excrement was then estimated by inoculating five tubes of 10 cm^3 of tetrathionate enrichment broth and five tubes of 10 cm^3 of mannitol selenite broth, with one cm^3 of each dilution. The enrichment broths were incubated at 37°C for 24 h. After incubation, they were examined for the presence of salmonellae by plating out on brilliant green agar (Oxoid). These plates were incubated at 37°C and examined after 24 and 48 h for the presence of red colonies. The suspect colonies of S. dublin were then streaked onto triple sugar iron agar (Oxoid).

Below the 10^{-3} dilution level only red colonies were present on the plates inoculated from the S. dublin infected samples, whereas no colonies developed on the plates inoculated from the control sample.

All the red colonies were confirmed as S. dublin, by streaking onto triple sugar iron agar, and by the absence of red colonies on the plates from the control sample. Serological tests were therefore considered unnecessary. At the 10^{-2} dilution level and above green colonies developed on all the plates. Consequently S. dublin could not be isolated from these dilutions of the infected samples.

The concentration of S. dublin in the infected excrement, calculated from the plate count, was 1.5×10^9 per cm^3. The MPN of S. dublin in tetrathionate broth was 1.1×10^9 per cm^3 and in mannitol selenite broth was 1.5×10^8 per cm^3.

Tetrathionate broth, brilliant green agar, and triple sugar iron agar were then chosen as the most suitable combination of media for the isolation and enumeration of S. dublin from infected pig excrement. However, because of the presence of high concentrations of coliforms at low dilution levels, a second method of detecting growth of S. dublin in the enrichment broths was necessary for the examination of excrement containing low numbers of S. dublin.

In the early experiments on survival of S. dublin, the fluorescent antibody technique of Silliker, Schmall and Chiu (1966) was adopted. This technique usually resulted in higher counts than those obtained by plating out on brilliant green agar.

Unfortunately, fresh excrement collected from the piggery after the start of experiment II contained high concentrations of Alcaligenes faecalis. This organism multiplied in both the enrichment broth and brain heart infusion broth and it stained with the fluorescent antibody. It also produced pink colonies on brilliant green agar, gave negative results in the urease and phenylalanine tests, but failed to produce H_2S on triple sugar iron agar.

Tetrathionate medium B (Rolfe, 1946) was examined as an alternative enrichment broth, but failed to inhibit the growth of Alc. faecalis. However, when the iodine solution recommended for tetrathionate medium B (20 g iodine per 100 cm^3 solution) was used with Oxoid tetrathionate broth base, instead of the solution recommended by Oxoid (30 g iodine per 100 cm^3 solution) Alc. faecalis was markedly inhibited. Modified bismuth sulphite agar (McCoy, 1962) was tested and found to inhibit Alc. faecalis while supporting the growth of S. dublin. The reduction in the iodine concentration produced higher counts of S. dublin, on brilliant green agar and modified bismuth sulphite agar than had been previously found.

PART A. THE SURVIVAL OF *Salmonella dublin* IN PIG EXCREMENT DURING STORAGE

INTRODUCTION

During storage on a farm, the temperature of animal excrement may fluctuate. In slurry channels below the slatted floors of animal houses and in large storage tanks below ground level, the temperature of the excrement will remain relatively constant for long periods, probably at temperatures similar to, or slightly above that of the soil. In slurry tanks above ground level, the temperature of the excrement may vary with short term changes in atmospheric temperature. Measurement of the contents of three slurry reservoirs, in the Auchincruive area, supports the assumption that the temperature of excrement during storage is similar to that of the soil. The slurry reservoirs examined were a slurry channel (1 m deep) below the slatted floor of the pig fattening house, a 90,000 l subsurface tank filled with beef cattle excrement, both at the Animal Husbandry Experimental Unit, Brickrow and a 45,000 l surface tank filled with dairy cattle excrement at Trabboch Mains, Trabboch, Ayrshire. The temperature in these three tanks at 1 m depth were 8.0, 8.0 and 7.5°C respectively, and the soil temperature at 1 m depth at the Auchincruive Meteorological Station on the same day was 7.8°C. In this area the soil temperature at 1 m depth varies between 4 and 14°C through the year (Plant, 1971).

The solids concentration of pig excrement depends largely on the amount of water, from various sources, entering the slurry tank. Undiluted pig excrement has a suspended solids concentration of about 100 to 130 g/l (O'Callaghan, Dodd, O'Donohue and Pollock, 1971), but is usually diluted at least 1:1 with water in slurry storage tanks (Berryman, 1970).

Since fresh pig excrement from infected animals was not readily available and it was not practical to inoculate salmonellae into a slurry tank on a farm, the survival of Salmonella dublin in pig excrement was studied in the laboratory.

It is not possible to be sure of reproducing in the laboratory all the conditions likely to prevail during the storage of slurry on a farm. However, storage conditions were chosen to simulate practical farm situations as far as possible.

The following series of laboratory experiments were designed to evaluate the chances of viable salmonellae surviving in pig excrement in a storage tank on a farm. In each of five experiments samples of pig excrement, diluted with tap water to a semi-liquid slurry, were inoculated with S. dublin. Samples of inoculated slurry, together with samples of uninoculated slurry used as controls for bacteriological methods, were then stored anaerobically at constant temperatures. At intervals during storage the pH value of the slurry was measured and the MPN of survivors of S. dublin estimated.

In experiments I and II the number of survivors of S. dublin were estimated by inoculating decimal dilutions of the slurry into enrichment broths. Brilliant green agar and the fluorescent antibody technique were used to detect the growth of S. dublin in these broths.

Experiment I was designed to determine how long cells of S. dublin could remain viable after inoculation into a sample of fresh pig excrement. The excrement was diluted to a slurry, containing 54 g suspended solids per l and 84 g chemical oxygen demand (COD) per l. The contaminated and control samples were then stored at 15°C in 2 l vessels, containing magnets for mixing.

Experiment II was designed to determine the effect of suspended solids concentration of the slurry on salmonella survival. Contaminated and control samples of a thin slurry (9.6 g suspended solids per l: 11 g COD per l) were stored at 10°C in 2 l vessels, containing magnets and of a thick slurry (68.3 g suspended solids per l: 81 g COD per l) were stored at 10°C in 2 l vessels, fitted with paddles for mechanical mixing. This experiment was stopped after 118 d when the incubator overheated.

In experiments III, IV and V a correlation between changes in the pH value of the slurry during storage and S. dublin survival was examined. Some of the technical errors of the salmonellae counting methods were reduced by the use of a two-fold dilution series in the MPN technique and by reducing the iodine concentration in the enrichment broth.

In experiment III the pH value of the slurry (60 g suspended solids/l; 70 g COD/l) was monitored continuously. The slurry was stored in a three litre fermenter and stirred constantly throughout the experiment.

Constant agitation of the slurry is not commonly practised during storage on a farm, and it also appeared to disturb the expected sequence of changes of pH value, found to occur in the unmixed samples of slurry used in the earlier experiments. Therefore the slurry in experiments IV and V was stored in several smaller volumes of 20 cm^3, as well as 2 l volumes. This allowed samples to remain unmixed from the start of the experiment up to the time of examination. The slurry in experiment IV contained 56 g suspended solids/l, 70 g COD/l and was stored at 15°C.

The slurry for experiment V (54 g suspended solids/l; 63 g COD/l) was stored anaerobically for 28 d at 15°C before being inoculated with S. dublin. The inoculated slurry and control samples were then stored at 15°C in volumes of 20 cm^3 and 2 l.

RESULTS

Since no salmonellae were isolated from the control samples of slurry in any of the experiments, it was assumed that enrichment broths, found to be positive by the fluorescent antibody technique and/or by isolation on selective media, resulted from survivors of the inoculum of Salmonella dublin. The MPN of S. dublin in the contaminated samples of slurry, at the start of experiments I and II, were between 10 and 25 per cent of the number expected, by calculation from plate counts of the broth cultures and the volume of culture inoculated into the slurry. In these experiments the MPN of survivors obtained using the fluorescent antibody technique was generally higher than the MPN obtained using brilliant green agar. However, because of the errors involved with a decimal dilution series the counts obtained by the fluorescent antibody technique and brilliant green agar were not significantly different at the 95 per cent confidence level.

In the later experiments the fluorescent antibody technique was abandoned because of the appearance of Alcaligenes faecalis in the fresh pig excrement, collected from the fattening house. The iodine concentration in the enrichment broth was reduced and positive enrichment broths detected by plating on brilliant green agar and modified bismuth sulphite agar. The MPN of S. dublin at the start of experiments III, IV and V was between 68 and 120 per cent of the number expected by calculation. The introduction of a two-fold dilution series for the MPN technique reduced the 95 per cent confidence limits for each count, and this, with the increased frequency of MPN estimations, provided a clearer indication of the shape of the survivor curve.

FIGURE 4

Experiment I

Survivor curve, with 95 per cent confidence limits, for Salmonella dublin in fresh pig excrement, stored at 15°C, in an unmixed state, under anaerobic conditions and curve of the pH value of the excrement.

Continuous line – count on brilliant green agar.

Dashed line – count using fluorescent antibody technique.

FIGURE 4

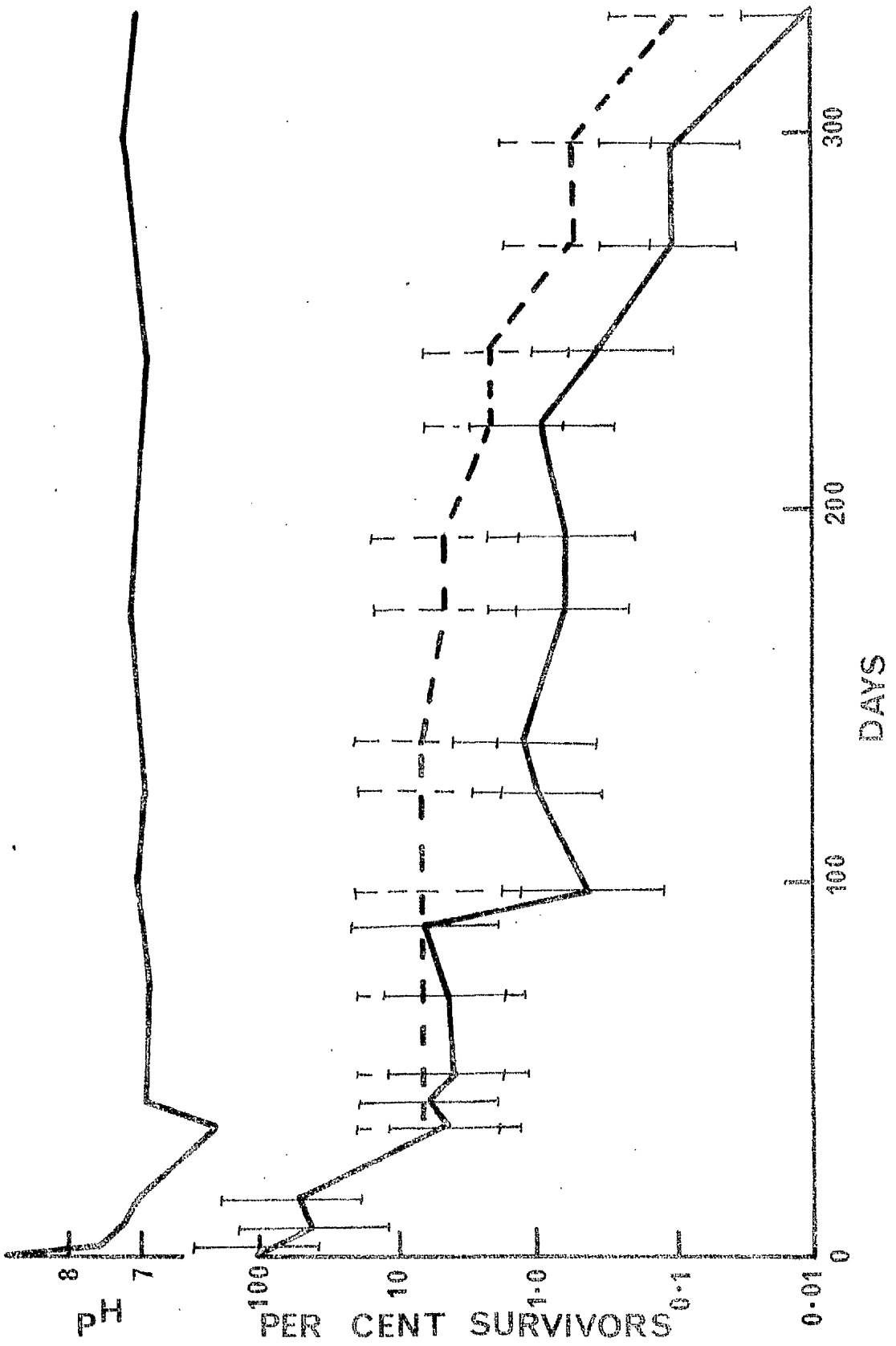


TABLE 5

The survival of S. dublin after artificial inoculation into pig excrement, and the pH value of the excrement during storage at 15°C

TABLE 5

	Viable units/cm^3
Plate count of S. dublin in broth culture	2.8×10^8
Expected concentration of S. dublin in excrement at start of experiment (day 0)	2.8×10^6

Day	pH	MPN S. dublin	
		Brilliant green agar	Fluorescent antibody
0	8.7	2.4×10^5	-
1	8.2	2.4×10^5	-
2	7.9	2.4×10^5	-
4	7.5	2.4×10^5	-
8	7.2	9.3×10^4	-
16	7.0	1.5×10^5	-
35	6.5	9.0×10^4	1.5×10^4
42	7.0	1.5×10^4	1.5×10^4
49	7.1	9.0×10^3	1.5×10^4
70	6.9	1.0×10^4	1.5×10^4
91	6.9	1.5×10^4	1.5×10^4
99	7.0	9.3×10^2	1.5×10^4
126	7.0	2.4×10^3	1.5×10^4
139	7.0	2.8×10^3	1.5×10^4
174	7.1	1.5×10^3	1.1×10^4
195	7.0	7.5×10^3	1.1×10^4
224	-	2.1×10^3	4.6×10^3
244	6.9	7.5×10^2	4.6×10^3
272	-	2.4×10^2	1.2×10^3
300	7.2	2.4×10^2	1.2×10^3
224	7.0	2.3×10^1	2.1×10^2

FIGURE 5

Experiment II

Survivor curve, with 95 per cent confidence limits, for Salmonella dublin inoculated into two samples of fresh pig excrement stored anaerobically at 10°C, and curve of the pH value of the excrement.

Continuous line - sample containing 68.3 g suspended solids per litre.

Dashed line - sample containing 5.6 g suspended solids per litre.

FIGURE 5

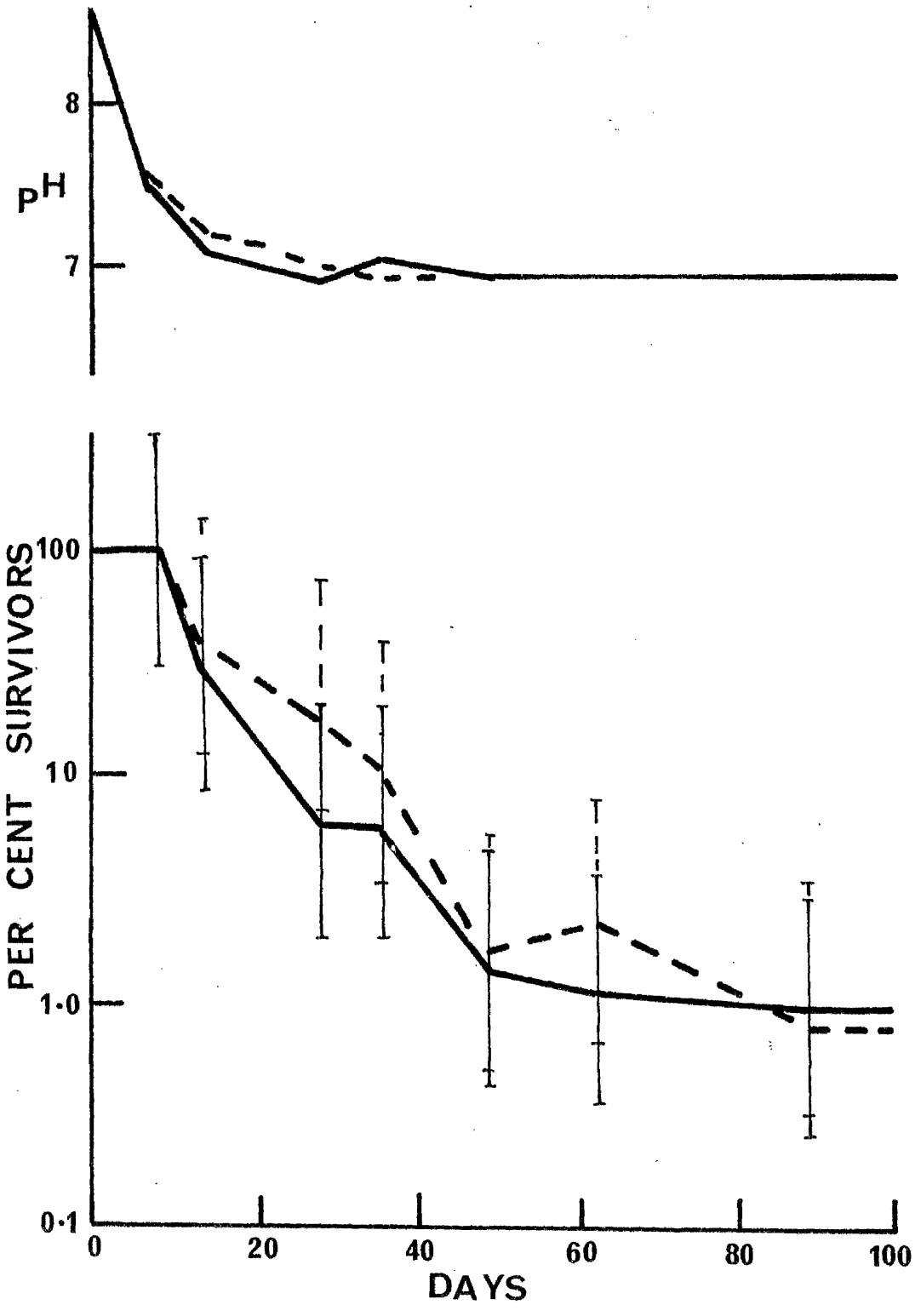


TABLE 6

The survival of S. dublin after inoculation into thick and thin suspension of pig excrement, and the pH values of the excrement samples during storage at 15°C.

TABLE 6

	Viable units/cm^3
Plate count of S. dublin in broth culture	2.6×10^8
Expected concentration of S. dublin in excrement at start of experiment (day 0)	2.6×10^6

	Thick suspension MPN S. dublin			Thin suspension MPN S. dublin		
Day	pH	Brilliant green agar	Fluorescent antibody	pH	Brilliant green agar	Fluorescent antibody
0	8.6	1.1×10^6	1.1×10^6	8.6	2.4×10^4	1.1×10^6
8	7.4	1.1×10^6	1.1×10^6	7.5	2.4×10^5	1.1×10^6
14	7.1	1.5×10^6	4.6×10^5	7.2	4.6×10^5	4.6×10^5
28	6.9	4.0×10^4	9.3×10^4	7.0	2.4×10^5	2.6×10^5
36	7.0	4.3×10^4	9.3×10^4	6.9	1.0×10^4	1.5×10^5
49	6.9	2.1×10^4	1.1×10^4	6.9	2.1×10^4	1.5×10^4
63	6.9	1.5×10^4	1.1×10^4	6.9	3.9×10^4	1.0×10^4
89	6.9	-	1.1×10^4	6.9	9.3×10^3	1.0×10^4
118	6.9	1.0×10^4	1.1×10^4	6.9	9.0×10^3	1.1×10^4

The survival of Salmonella dublin in pig excrement

Some of the cells of S. dublin inoculated into the slurry in experiment I survived as long as 334 days (Fig. 4, Table 5). The death rate, however, was not constant throughout this period. 90 per cent of the inoculated cells appeared to die during the first month, but the 90 per cent reduction time of the remaining cells was in excess of two months. The pH value of the slurry fell from 8.7 to 6.5 during the first 35 days, but returned to neutral by day 42. It then remained neutral for the next 292 days.

The two survivor curves (Fig. 5, Table 6) obtained in experiment II were similar to each other and to the survivor curve of experiment I during the first 118 days. 90 per cent of the inoculated cells, in both samples of slurry, appeared to die during the first month, whereas only 90 per cent of the remaining cells died during the following three months. The pH value of both the thick and thin slurry samples in experiment II showed similar changes to each other and to the changes in experiment I. Thus no difference in the death rate of S. dublin, or changes in the pH value of the slurry, were apparent as a result of the differences in suspended solids concentration. Similarly the slightly lower storage temperature of experiment II, when compared with experiment I, had no apparent effect on the survival of S. dublin.

The differences in the death rate of S. dublin during the first month, as compared with the remaining storage period, may have been due to insufficient data, or the errors involved using a decimal dilution series to measure a decimal reduction of viable cells, in the MPN determinations, and/or the sudden change in the environment of the S. dublin, from a pure culture on nutrient broth at 37°C to a mixed microbial habitat in slurry at 15°C.

FIGURE 6

Experiment III

Survivor curve, with 95 per cent confidence limits, for Salmonella dublin inoculated into fresh pig excrement stored anaerobically at 15°C, and continuously mixed, and curve of the pH value of the excrement.

FIGURE 6

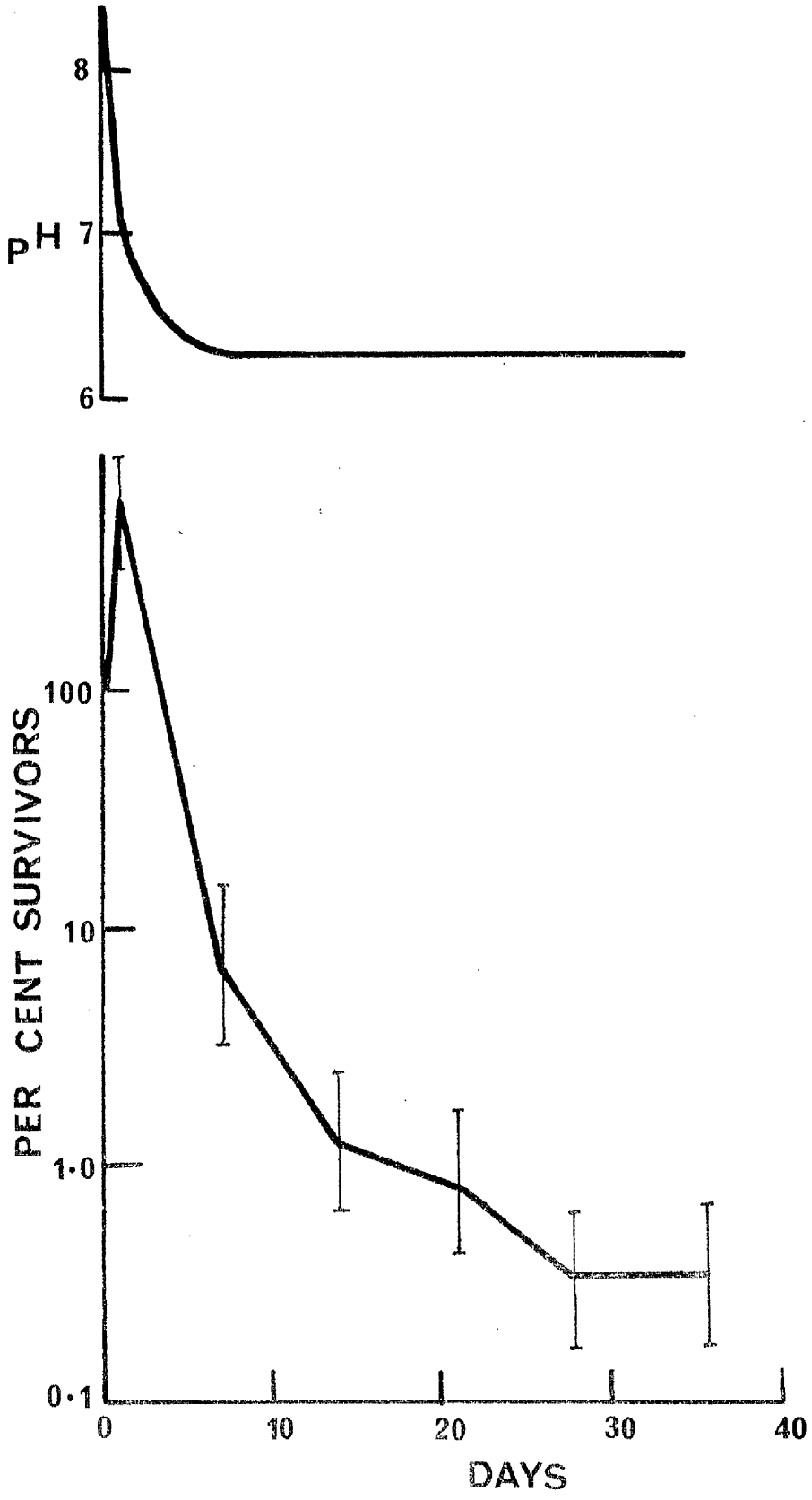


TABLE 7

The survival of S. dublin, after inoculation into pig excrement and the pH value of the excrement which was continuously mixed during storage at 10^{o}C.

TABLE 7

	Viable units/cm^3
Plate count of S. dublin in broth culture	3.0×10^9
Expected concentration of S. dublin in excrement at start of experiment (day 0)	3.0×10^7

Day	pH	MPN S. dublin	
		Brilliant green agar	Modified bismuth sulphite
0	8.6	2.1×10^7	-
1	7.1	1.4×10^8	-
7	6.3	1.5×10^6	1.2×10^6
14	6.3	2.7×10^5	2.7×10^5
21	6.3	1.8×10^5	1.8×10^5
28	6.3	7.1×10^4	7.1×10^4
35	6.3	7.6×10^4	-

FIGURE 7

Experiment IV

Survivor curve, with 95 per cent confidence limits, for Salmonella dublin inoculated into fresh pig excrement stored anaerobically at $15^{0}C$, in an unmixed state, in several volumes of 20 cm^{3}, and curve of the pH value of the excrement.

FIGURE 7

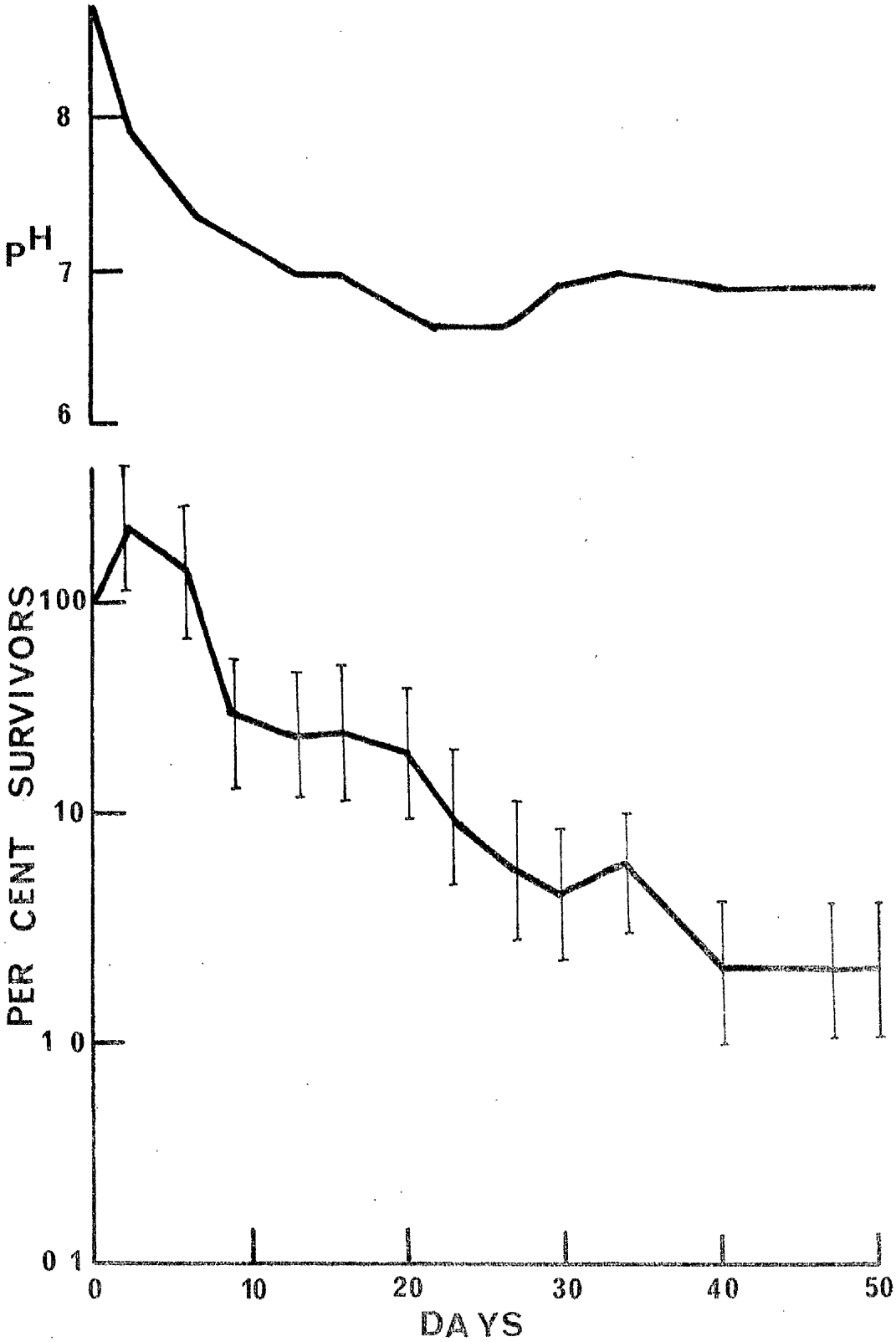


TABLE 8

The survival of S. dublin, after inoculation into pig excrement and pH value of the excrement during storage at 15°C.

* Numbers of viable S. dublin in 2 l storage vessel.

TABLE 8

	Viable units/cm^3
Plate count of S. dublin in broth culture	2.0×10^9
Expected concentration of S. dublin in excrement at start of experiment (day 0)	6.6×10^6

Day	pH	MPN S. dublin	
		Brilliant green agar	Modified bismuth sulphite
0	8.7	4.5×10^6	4.5×10^6
2	7.7	1.2×10^7	7.9×10^6
6	7.4	5.5×10^6	6.4×10^6
9	7.2	1.3×10^6	1.1×10^6
13	7.0	1.1×10^6	1.1×10^6
16	7.0	1.1×10^6	1.1×10^6
20	6.8	9.2×10^5	-
23	6.6	4.5×10^5	4.5×10^5
27	6.7	2.6×10^5	-
30	6.9	2.2×10^5	1.8×10^5
34	6.9	3.8×10^5	1.8×10^5
40	6.8	1.1×10^5	8.0×10^4
47	6.8	1.1×10^5	9.0×10^4
50	6.8	1.0×10^5	9.2×10^4
30*	7.0	1.5×10^5	1.5×10^5
50*	6.7	1.1×10^5	1.0×10^5

Alternatively, the change in death rate may have been associated, in some way, with the metabolic activity of other micro-organisms in the slurry, as reflected by changes in the pH value of the slurry. During the period of high acid production 90 per cent of the inoculated cells in experiments I and II failed to survive.

The effect of microbial fermentations during storage of pig excrement on Salmonella dublin survival

In experiment III the pH value of the slurry fell from 8.7 to 6.3 during the first 48 h and then remained at this acid level. No salmonellae were present in the fresh slurry before inoculation with S. dublin. Following inoculation the numbers of S. dublin increased slightly during the first 48 h, but then declined during the next 34 days (Fig. 6, Table 7) more rapidly than in the earlier experiments. The experiment was stopped on day 36 when 99.8 per cent of the inoculated cells appeared to have died.

Continuous agitation of the slurry provided a different environment from that in the unmixed storage vessels and this apparently disturbed the normal sequence of microbial fermentations. Therefore, in the fourth experiment, small samples of slurry were left undisturbed during storage and the 2 l samples were only mixed at monthly intervals. The results of this experiment (Fig 7, Table 8) showed a slight increase in the numbers of S. dublin during the first 48 h storage, but by day 23 the concentration of S. dublin remaining in the slurry represented only 10 per cent of the initial concentration. During the next 37 days the 90 per cent reduction time of the remaining cells was approximately 60 days. The determinations of MPN and measurement of pH values of the 2 l samples (Table 8) demonstrate that the reduced volume of slurry

FIGURE 8

Experiment V

Survivor curve, with 95 per cent confidence limits, for Salmonella dublin inoculated into 28 day old excrement, stored anaerobically at 15°C in an unmixed state, and curve of the pH value of the excrement.

FIGURE 8

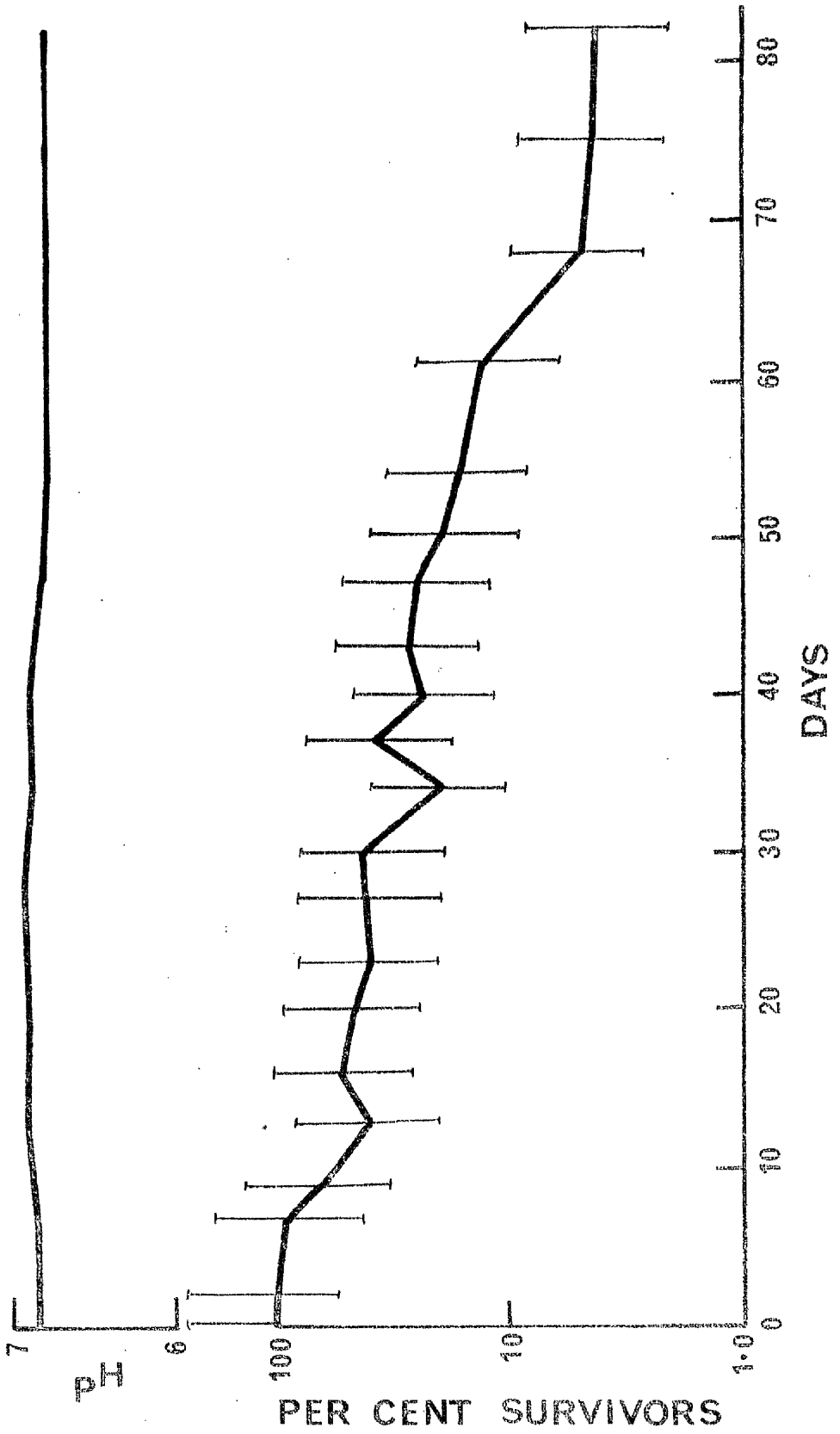


TABLE 9

The survival of S. dublin, after inoculation into pig excrement previously stored for 28 days at 15°C, and the pH value of the excrement during storage at 15°C.

* Number of viable S. dublin in 2 l storage vessel.

TABLE 9

	Viable units/cm^3
Plate count of S. dublin in broth culture	2.3×10^9
Expected concentration of S. dublin in excrement at start of experiment (day 0)	1.5×10^7

Day	pH	MPN S. dublin	
		Brilliant green agar	Modified bismuth sulphite
-28	8.8	0	0
0	6.9	1.8×10^7	1.8×10^7
2	-	1.8×10^7	2.0×10^7
7	6.9	1.8×10^7	1.3×10^7
9	-	1.1×10^7	1.3×10^7
13	7.0	9.2×10^6	5.5×10^6
16	-	1.3×10^7	6.4×10^6
20	7.0	1.1×10^7	6.4×10^6
23	-	6.5×10^6	7.4×10^6
27	7.0	7.7×10^6	6.5×10^6
30	-	7.7×10^6	6.5×10^6
34	6.9	3.5×10^6	3.5×10^6
37	-	8.0×10^6	5.5×10^6
40	6.9	3.8×10^6	4.5×10^6
43	-	3.1×10^6	6.6×10^6
47	6.9	3.2×10^6	5.5×10^6
50	-	3.1×10^6	3.7×10^6
54	6.8	2.9×10^6	2.9×10^6
61	-	2.2×10^6	2.2×10^6
68	6.8	6.4×10^5	9.2×10^5
75	-	6.4×10^5	9.2×10^5
82	6.9	6.4×10^5	7.7×10^5
82*	6.9	7.0×10^5	7.0×10^5

stored did not affect the fermentative activity of the microbial population, or the survival of S. dublin.

When the survivor and pH curves of this experiment are compared with those of the first two experiments, they are seen to be similar. The reduction of the 95 per cent confidence limits of the MPN counts and the increased frequency of examination, confirm that the rate of decline of S. dublin was greater during the first month of storage than during the following months. This result also confirmed the correlation of the pH and survivor curves.

It was still possible that the high rate of decline of viable S. dublin during the first month after their inoculation into fresh slurry could be due to the sudden change in their environment and not associated with the acid-producing fermentations of the other organisms in the slurry.

In experiment V, fresh slurry was stored for 28 days at 15°C which allowed the period of high acid production to end, and the acids to be neutralised. After 28 days, when the pH value of the slurry was 7.0, some of the slurry was contaminated with S. dublin. No initial increase in the numbers of S. dublin occurred during the first 48 h and the rate of decline of the inoculated cells during the next 82 days was fairly constant (Fig. 8, Table 9). This rate of decline of viable cells (i.e. 90 per cent reduction time of 60 days) was similar to the rate obtained in the earlier experiments after the first month of storage, when the pH value had returned to neutral.

PART B. THE SURVIVAL OF *Escherichia coli* AND *Salmonella dublin* DURING AEROBIC BIOLOGICAL TREATMENT OF PIG EXCREMENT

INTRODUCTION

Aerobic biological treatment of pig excrement was under investigation in the laboratory at The West of Scotland Agricultural College as a means of reducing problems of pollution resulting from disposal of animal excreta. While most of the studies were concerned with problems of chemical pollutants, problems of bacterial pollution, in particular the possible dissemination of pathogens, were also considered, and are described in this report.

Most of the studies on treatment were conducted in laboratory scale units. The contents of these units were normally sealed from the laboratory atmosphere. However, due to the nature of pig excrement, it was not always possible to prevent the blockage of tubes which sometimes resulted in tube couplings breaking and the consequent release of aerosols.

Because of the dangers to health of laboratory staff from infective aerosols, it was impractical to regularly feed salmonella infected slurry to the laboratory aerobic treatment unit. Therefore, preliminary studies were made on the survival of *E. coli* during aerobic treatment of pig excrement at different BOD loading rates and operating temperatures. A further advantage was obtained in that *E. coli* was naturally present in the excrement whereas there was no natural infection by salmonellae during the experimental period. In practice, the rate of loading the treatment unit was measured by the suspended solids input. For this reason the loading rate is expressed as g suspended solids/g mixed liquor suspended solids (g SS/g MLSS). The BOD loading rate was approximately 0.4 times the suspended solids

TABLE 10

Numbers of Escherichia coli in slurry fed to the aerobic treatment unit and in the aeration vessel during the operation of the unit at different loading rates.

TABLE 10

LOADING RATE gSS/gmLSS	E. coli FEED viable units/gSS	MIXED LIQUOR viable units/gSS	SOLIDS viable units/gSS	SUPERNATANT viable units/cm^3
0.10	3.0×10^6	-	2.0×10^4	-
	3.9×10^6	-	9.8×10^3	4.2×10^1
	3.0×10^6	-	1.4×10^4	4.4×10^1
	5.0×10^6	-	2.5×10^4	1.0×10^1
	6.0×10^6	-	3.1×10^4	4.8×10^1
0.25	3.3×10^6	4.6×10^5	-	-
	3.2×10^6	4.6×10^5	-	-
0.30	7.0×10^6	1.4×10^7	-	-
	4.2×10^6	1.7×10^6	-	-
	3.9×10^6	2.7×10^6	-	-
0.10	2.8×10^4	-	4.6×10^3	5.5×10^0
	2.8×10^6	-	2.1×10^5	1.1×10^1
	1.3×10^7	-	3.4×10^5	-
0.20	1.8×10^6	-	8.5×10^5	1.3×10^2
	1.6×10^7	-	6.1×10^5	1.7×10^2
	7.5×10^6	-	3.0×10^6	6.0×10^3
	1.0×10^7	-	4.4×10^6	1.6×10^3
	1.8×10^7	-	3.7×10^4	2.5×10^2
	4.5×10^6	-	3.1×10^6	5.5×10^2

loading rate. The residence times of the solids and supernatant are estimated from the solids loading rate and the dilution rate of the bacterial floc.

The concentration of E. coli in the slurry, fed to the continuous treatment unit, and in the mixed liquor, or the floc and supernatant of the mixed liquor, in the aeration chamber, was estimated on several occasions. From these results an estimate of the chances of E. coli surviving aerobic biological treatment of pig excrement was calculated.

Having determined the approximate rate of decline of E. coli during aerobic treatment, a single inoculum of a broth culture of S. dublin was added to the mixed liquor in the aeration chamber. The number of survivors of S. dublin in the sludge flocs and the supernatant of the mixed liquor was then measured daily for 10 days.

At the time of this experiment, the treatment unit was operating at $5^{o}C$ and a loading rate of 0.15 g SS/g MLSS giving a solids residence time in excess of 15 days. The pH value of the mixed liquor was controlled at 7.0 by the automatic addition of 0.1 N H_2SO_4. The MPN of salmonellae in a sample of mixed liquor was estimated before the experiment commenced.

RESULTS

The survival of Escherichia coli during aerobic biological treatment of pig excrement

The concentration of E. coli in samples of slurry fed to the treatment unit, mixed liquor, settled sludge and supernatant, examined at various times during the operation of the aerobic biological treatment unit at different loading rates are shown in Table 10. Other operating characteristics of the treatment unit and the 90 per cent reduction

TABLE 10a

Reduction time of Escherichia coli during aerobic biological treatment of pig excrement.

* pH value of mixed liquor controlled at 7.1 by automatic addition of 0.1 N H_2SO_4.

TABLE 10a

LOADING RATE gSS/gmLSS	SOLIDS RESIDENCE d	SUPERNATANT RESIDENCE d	MIXED LIQUOR TEMP. c	pH	90% REDUCTION TIME OF E. coli IN MIXED LIQUOR d
0.10	15	50	15	6.1	7
				6.1	7
				6.5	7
				5.9	7
				8.1	7
0.25	6	20	15	5.4	7
				5.5	7
0.30	4	16	15	7.5	-13
				6.9	10
				6.4	50
0.10	15	50	10	8.2	17
				8.4	13
				8.3	9
0.20	7	25	5	7.1*	21
					17
					17
					17
					10
					22

FIGURE 9

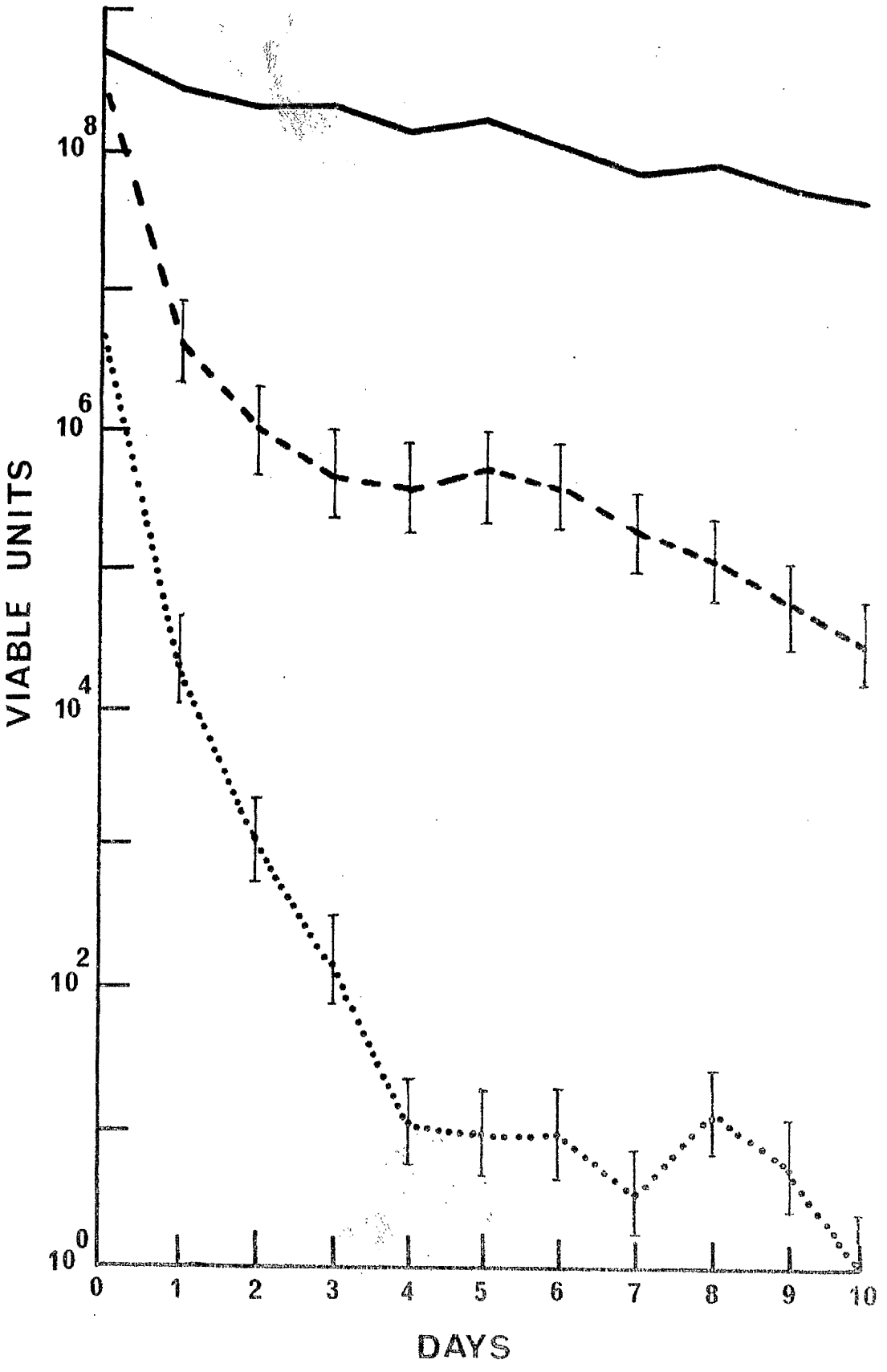
Survivor curves, with 95 per cent confidence limits, for *Salmonella dublin* in the mixed liquor of the aerobic biological treatment unit.

Continuous line – expected number of *S. dublin* per gSS, assuming no loss of viable cells other than by sludge removal.

Dashed line – observed number of *S. dublin* per gSS residual sludge.

Dotted line – observed number of *S. dublin* per cm^3 supernatant.

FIGURE 9



times for E. coli, calculated from the counts obtained, are shown in Table 10a.

The E. coli concentrations were estimated as viable units/g suspended solids, except in the supernatant where they were assumed to be free swimming and were therefore estimated as viable units/cm^3.

Most E. coli cells were found associated with the sludge flocs and only low numbers remained in the supernatant. Although the effect of pH changes, protozoal activity etc. were not investigated, the results do suggest that the concentration of E. coli in the residual sludge was largely dependent on the loading rate and on the temperature of the mixed liquor.

At high loading rates, less than 90 per cent reduction of viable E. coli occurred. The wide variation in the 90 per cent reduction time for E. coli in the mixed liquor, including an apparent increase in E. coli concentration on one occasion (90 per cent reduction time, minus 13 days), was probably due to technical errors in the operation of the treatment unit, and in the methods of sampling and counting E. coli.

At low loading rates the 90 per cent reduction times varied between 7 and 20 days. The longer times were found at the lower operating conditions but again variation may be largely due to counting errors.

The survival of Salmonella dublin during aerobic biological treatment of pig excrement

No salmonellae were present in the mixed liquor prior to inoculation of S. dublin. The survivor curves of S. dublin during the ten days following its inoculation into the mixed liquor are shown in Fig. 9.

The expected initial concentration of S. dublin in the mixed liquor immediately following the addition of the culture to the aeration vessel was 3.5×10^6 viable units per cm^3 (or 4.2×10^8 viable units per g suspended solids).

The number of S. dublin in the supernatant rapidly declined during the first four days to less than 10 viable units per cm^3. The number of S. dublin in the sludge declined during the first three days to 4.6×10^5 viable units per g suspended solids. The rate of decline in the sludge then slowed down so that by day 10 the concentration was still 3.2×10^4 viable units per g suspended solids.

After allowing for loss of S. dublin by effluent removal the 90 per cent reduction time in the sludge during the first three days was one day. During the following two days there was no apparent reduction of viable units. Between day 5 and 10 the 90 per cent reduction rate was 12 days.

DISCUSSION

The effects of anaerobic fermentation of pig excrement on the survival of Salmonella dublin

Details of the anaerobic digestion of piggery waste have been described by Hobson and Shaw (1971). Within a few hours of excretion, the action of bacterial proteases releases ammonia which increases the pH value of the excrement. This activity is followed by the fermentation of carbohydrates, to produce a mixture of volatile fatty acids, ethanol, carbon dioxide and hydrogen, which reduces the pH value of the waste. In a second stage, methane bacteria metabolise the volatile fatty acids and the pH value returns to neutral. Methane bacteria are slow growing and methanogenesis is inhibited by high concentrations of volatile fatty acids, so that if acids accumulate, the second stage is inhibited and the pH value of the waste will remain acidic.

The experiments on the survival of cells of S. dublin, inoculated into piggery waste, show that the viability of these cells was affected by the processes of anaerobic digestion. In the experiments where the excrement was stored in an undisturbed state both stages of anaerobic digestion occurred. In the third experiment the continuous agitation of the slurry appeared to inhibit methanogenesis, probably because of an increased production of volatile fatty acids.

In the first stage of anaerobic digestion the pH value of the slurry fell to about 6.5 and under these conditions the 90 per cent reduction time for the inoculated S. dublin cells was approximately 30 days. The increase in S. dublin on some occasions during the first 48 h is probably due to continued metabolism of the inoculum. Since salmonellae are not normally affected by a pH value of 6.5 in pure

culture (Prost and Reimann, 1967), it seems probable that the loss of viable cells was due either to the inhibitory effects of fermentation products, such as volatile fatty acids, or a laboratory artifact due to a sudden change in the environment of the S. dublin cells. This latter possibility may be discounted because when cells of S. dublin were inoculated into excrement after the second stage of anaerobic digestion had commenced in experiment V, and the pH value of the slurry had returned to neutral, it was found that the rate of decline of these cells was identical to the rate of decline of those cells which had survived the acidic stage of storage in the earlier experiments (i.e. 90 per cent reduction time $>$ 60 days).

Although storage temperatures and suspended solids concentrations of samples of pig excrement, stored in the laboratory, were similar to those expected on a farm, it is possible that other environmental conditions will influence the survival of salmonellae in animal excreta. In slurry channels and tanks, fresh excrement is frequently added, thus not all the excrement is of the same age. If the excrement forms layers with each addition of fresh material then a similar series of reactions to those already described will occur. In a mixed slurry methanogenesis may be inhibited resulting in continuing acid conditions, or methanogenesis will occur and neutral conditions will predominate. Therefore if S. dublin were inoculated into a slurry reservoir on a farm and its survival monitored, survivor curves similar to those obtained in experiments I or III or V may be expected. The curve obtained would probably depend on the size of the reservoir and on the amount of mixing of the contents.

The survival of Salmonella dublin during aerobic biological treatment of pig excrement

The initial rapid loss of S. dublin, after inoculation into the aeration chamber of the laboratory treatment unit, may have been a result of inoculating a high concentration of free swimming cells into the mixed liquor. It is probable that there are only a limited number of sites available on the sludge flocs, for the attachment of these cells, and that the cells remaining in the supernatant were then open to removal by protozoal grazing. If the salmonellae had been present in the slurry before its addition to the aeration vessel, then the high initial rate of loss of cells may not have occurred. Once the cells became associated with the sludge flocs they were probably protected and could survive longer.

Therefore, although S. dublin appeared to decline at a slightly faster rate than E. coli in the flocs of the aerobic mixed liquor, at a similar loading rate and operating temperature, these differences are probably not significant.

S E C T I O N II

THE BACTERIAL POLLUTION OF LAND DRAINAGE WATER

INTRODUCTION

The results obtained in Section I suggest that if viable salmonellae are excreted with pig faeces, then a significant number of these cells are likely to remain viable during anaerobic storage and/or aerobic biological treatment. Although little is known about the pathogenicity of salmonellae after prolonged anaerobic, or aerobic, storage, the excrement from infected animals must be considered a potential danger to health.

Investigations were therefore carried out to determine the factors which influence the passage of bacteria through soil into land drainage water, thus increasing the levels of bacterial pollution of water courses.

TABLE 11

Soil analysis of the experimental pasture, Brickrow.

After visual inspection of three sites of the pasture, a set of soil samples of one profile were taken from a site in the middle of the pasture.

+ mg per 100 g air dried soil.

TABLE 11

Depth, cm	Profile Description
0 - 29	Reddish brown loam
29 - 51	Light brown leached area, low clay content, few stones
51 - 57	Mottled area, higher clay content
57 - 69	Mixed material, some mottled, some grey sandy material and some clay
69 - 86	More grey sandy material

	Mechanical Analysis				
Depth, cm	0 - 29	29 - 51	51 - 57	57 - 69	69 - 86
Sand %	60.69	70.33	58.86	60.37	61.24
Silt %	12.80	8.74	11.71	12.52	11.32
Clay %	22.96	20.93	25.83	23.81	22.24
pH	6.55	6.00	6.10	6.30	6.35
P_2O_5 +	2.0	1.0	0.3	0.4	0.3
K_2O +	5.0	2.0	4.0	4.0	6.0
Moisture %	1.6	1.6	0.9	0.9	1.0
Loss on Ignition %	8.3	6.1	3.6	3.3	3.2

MATERIALS AND METHODS

The experimental pasture, Brickrow

Drainage water was obtained from the sub-surface drainage system of a pasture at the Animal Husbandry Experimental Unit, The West of Scotland Agricultural College, Brickrow, Auchincruive, Ayrshire (Grid ref. NS 3824). The pasture was believed to contain an extensive network of drains, consisting of unglazed porcelain segments resting on slate bases. They were probably installed sometime during the first half of the 19th century. The main drain was interrupted at the lowest part of the pasture to install instruments for measuring the flow rate of the discharge and for sampling. The actual location and depth of the drains throughout the pasture was unknown. At the sampling point however, the drain was found to be 90 cm below the surface of the pasture. Visual inspection of the pasture and water divining suggested that an area of land about 100 m by 65 m (0.65 ha) was drained by a series of lateral tile drains connecting into the main drain.

Analysis of the soil down to one metre depth, carried out by the Chemistry Department, The West of Scotland Agricultural College, showed the top soil to be a sandy clay loam of average fertility and the profile indicated that the soil was a member of the Bargower series. Details of the soil analysis are given in Table II. Such soils are described as imperfectly draining, this means that most of the water falling on the land, which is not lost by evaporation or transpiration, is retained in the soil. It is therefore necessary to install field drainage systems in such soils to remove the excess water.

TABLE 12

Estimation of the area of the pasture by comparison of the quantity of water discharged from the field drains with the amount precipitated, with correction for potential transpiration.

* Potential transpiration figures from (Ministry of Agriculture, Fisheries & Food, 1967.)

TABLE 12

Date	No. Days	Precipitation (R) kg/m^2	Potential Transpiration* (T) kg/m^2	Water Discharged (D) from Drain m^3	Estimated Area of Pasture ha $\frac{D}{R - T}$
1/10/68 - 29/ 2/69	151	279	127	1×10^2	0.7
1/11/69 - 8/ 1/70	69	252	57	9×10^1	0.5
14/ 1/70 - 22/ 2/70	39	103	32	5×10^1	0.7

TABLE 13

The management of the experimental pasture, Brickrow, during the summer of 1968.

TABLE 13

Date	Number of Animals Grazed	Manuring
8/ 5/68 - 16/ 5/68	11	
1/ 7/68 - 6/ 7/68	34	
10/7/68		22 m^3/ha Slurry
31/ 7/68 - 2/ 8/68	34	
2/8/68		504 kg/ha Nitro-chalk
19/ 8/68 - 21/ 8/68	34	
14/10/68 - 15/10/68	11	

During the growing season each year there was no discharge of water from the drainage system, except after particularly heavy rainfall. This was presumably due to the rate of evapotranspiration being greater than the rate of precipitation. Therefore water samples could only be collected and analysed during the winter.

The amount of precipitation, the total quantity of water discharged from the drainage system and the estimated potential transpiration (Ministry of Agriculture, Fisheries and Food, 1967) for three experimental periods are given in Table 12. From these figures the area of pasture drained were calculated, assuming that all the precipitation, or the precipitation which remained after allowing for potential transpiration, was discharged through the drains. The area obtained from the latter calculations, 0.5 to 0.7 ha, is in general agreement with the earlier estimate of 0.65 ha.

The pasture was ploughed and reseeded with grass in 1965. It was again ploughed and reseeded with red clover in 1971. Throughout the summer of 1968 until 15 October the pasture was grazed periodically by cattle and sheep (Table 13). In addition to the faeces of these animals 22 m^3/ha pig excrement were sprayed from a vacuum tanker onto the surface of the pasture. No animals were allowed onto the land and no animal excrement was applied after 15 October 1968 until the spring of 1969. The discharge of water from the drain started during the first week of September 1968 and continued until March 1969.

During the summer of 1969 there was no discharge of water from the drain. Cattle and sheep were grazed periodically throughout the summer. Water started to be discharged on 1 November 1969 and continued until April 1970. Pig excrement was sprayed from a vacuum tanker onto the surface of the pasture on four occasions during the winter of 1969/70. The first time 22 m^3/ha were applied, the second 33 m^3/ha, the third 55 m^3/ha and the fourth 55 m^3/ha. On each

occasion the excrement had accumulated during the previous three weeks, in the form of a semi-liquid slurry below the slatted floor of a piggery at the Animal Husbandry Experimental Unit, Brickrow.

The discharge of water from the drain resumed at the beginning of October 1970 and continued until the spring of 1971. 55 m^3/ha pig excrement were sprayed onto the surface of the pasture on 28 October 1970 and a similar quantity on 25 November 1970. As in the previous winter the excrement sprayed onto the pasture had accumulated during the previous three weeks.

The red clover sown during the spring of 1971 was cropped for silage during the summer. Sheep were grazed on the pasture between September and December 1971. Water started to be discharged from the drain at the beginning of September and continued until April 1972. No animal excrement other than the faeces of the sheep was deposited onto the surface of the pasture throughout the whole of this period.

The experimental farm, Culbae

Culbae farm, Wigtownshire (Grid ref. NX 3848) is situated about 9 km north east of Port William on the A 714. The farm is at about 50 m and covered about 100 ha.

About 4,500 fattening pigs were housed on the farm. They were fed a diet of whey supplemented with barley meal. The excrement from the pigs drained at the rate of 81 m^3 per day into a 380 m^3 slurry tank situated in the Midden field. The slurry in the tank was disposed of onto the surrounding fields by spraying through rain-guns. The pump was operated each day, spraying slurry at the rate of 36 m^3 per hour and the position of the rain-guns was altered every 15 minutes.

FIGURE 10

The experimental farm, Culbae, Wigtownshire.

(Grid ref. NX 3848).

 - open ditches and direction of water flow.

Shaded areas - water catchment areas, A and B.

V_1 and V_2 - V-notch weir gauges in areas A and B respectively.

a, a^1 - water sampling points, area A.

b - water sampling point, area B.

FIGURE 10

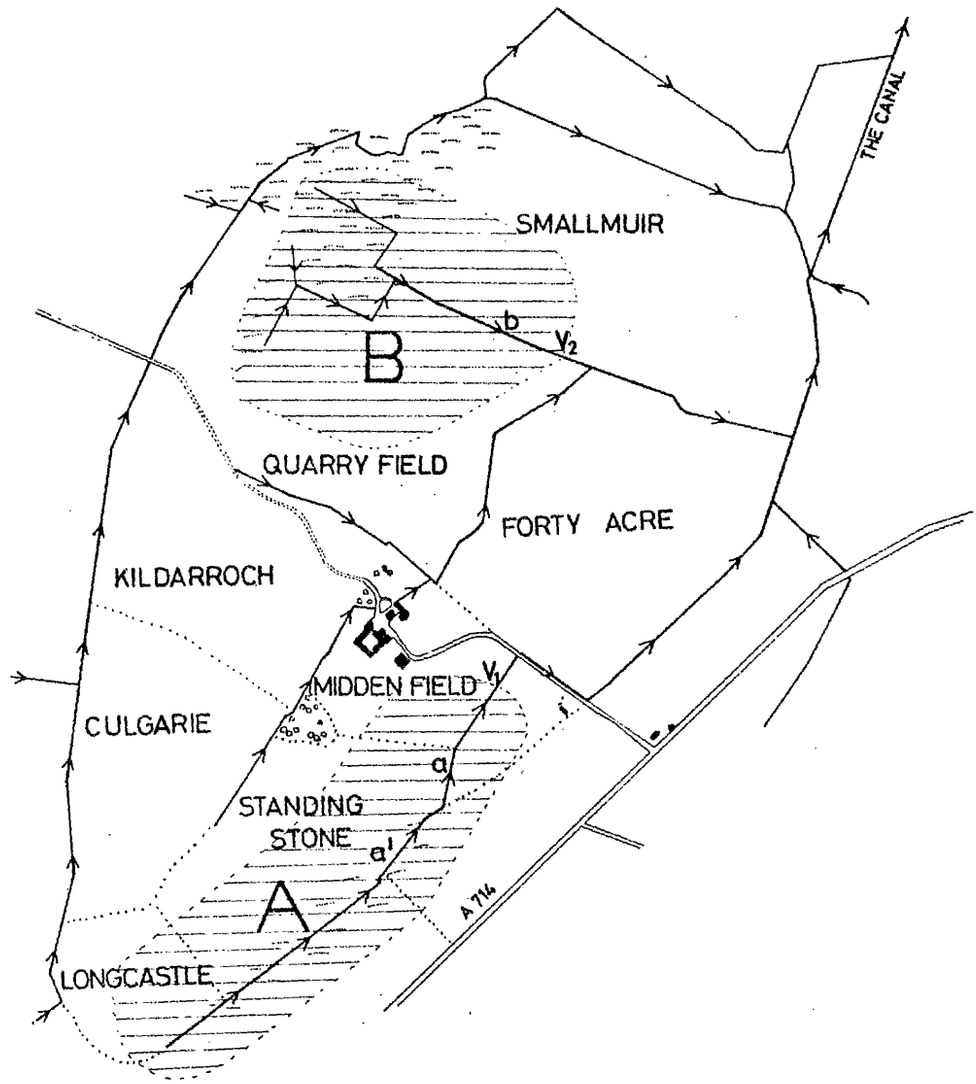

THE CANAL
SMALLMUIR
B
b
V2
QUARRY FIELD
FORTY ACRE
KILDARROCH
MIDDEN FIELD
V1
CULGARIE
a
STANDING STONE
a'
A
A 714
LONGCASTLE

TABLE 14

Soil analysis of the Standing Stone field, Culbae.

A description of the profile and mechanical analysis of top soil from the slope of the field and of basin peat from the flat area on the south east boundary of the field.

+ mg per 100 g air dried soil.

TABLE 14

Depth, cm	Profile Description
0 - 5	Grey brown loamy fine sand (surface root mat) with some small angular stones.
5 - 17	Grey brown clay loam. Many angular stones. Lower part dry no water penetration. Freely drained.
17 - 45	Reddish brown sandy loam. Many angular stones. Freely drained.
45 - 60	Reddish grey loamy coarse sand with some iron deposition. No gleying as such.

	Mechanical Analysis				
	Soil				Peat
Depth, cm	0 - 5	5 - 17	17 - 45	45 - 60	
Coarse sand (per cent)	15.65	21.18	25.66	39.04	
Fine sand (per cent)	37.62	23.39	21.19	18.32	
Silt (per cent)	16.57	24.21	23.06	17.26	
Clay (per cent)	17.70	25.23	25.10	22.44	
pH	5.30	4.90	5.20	5.70	5.70
Available P_2O_5 +	65	15	3	5	5
Available K_2O +	112	62	40	28	52
Moisture (per cent)	2.8	2.1	2.4	1.5	6.7
Loss on Ignition (per cent)	16.6	12.0	10.0	5.9	57.8

In this way slurry was sprayed onto all the fields at the rate of 150 m^3 of slurry per hectare every six months.

Neighbouring farmers were allowed to graze beef cattle and sheep on the fields. No attempt was made to keep the animals off fields while the rain-guns were in operation.

All of the fields drained into open ditches, these in turn discharged into a watercourse called The Canal, which is a tributary of the River Bladnock. Some of the open ditches received drainage water from neighbouring farms as well as from Culbae and one ditch which ran below the farmyard of Culbae received the discharge from a septic tank. For the purposes of the investigation, two drainage areas were selected, areas A and B shown in Fig. 10, which largely drained land contained within the boundaries of Culbae and did not receive septic tank discharge.

Area A. This catchment area, about 20 ha, included part of the Longcastle, Standing Stone and Midden Fields (Fig. 10) together with parts of two fields of a neighbouring farm.

The Longcastle and Midden fields were undulating, whereas the Standing Stone field had a steep gradient. The soil of the area was dominated by Silurian Greywacks and Shales and was freely drained. The top-soil on the slope of the Standing Stone field was fairly shallow with rocky outcrops. The south east side of the field was flat and consisted of basin peat. Details of the soil analysis are given in Table 14. Tile drains had been laid through the peat a week before the investigation started. These drains discharged into an open ditch which originated on the north east boundary of the Longcastle field and continued through the peat along the boundary of the other two fields. At about 10 m from the boundary wall between the Standing Stone and Midden fields the water was ducted

TABLE 15

Soil analysis of the Quarry field, Culbae.

A description of the profile and mechanical analysis of top soil.

+ mg per 100 g air dried soil.

TABLE 15

Depth, cm	Profile Description
0 - 7	Surface mat. Dark grey organic fine sandy loam with many roots.
7 - 45	Distributed mixture of grey organic sandy loam and angular stones.

	Mechanical analysis	
Depth, cm	0 - 7	7 - 45
Coarse sand (per cent)	14.87	29.81
Fine sand (per cent)	31.88	31.14
Silt (per cent)	11.64	10.13
Clay (per cent)	11.02	13.43
pH	5.20	5.05
Available P_2O_5	36	7
Available K_2O	41	41
Moisture (per cent)	3.8	3.2
Loss on Ignition (per cent)	30.6	30.4

through a 23 cm diameter pipe for 100 m. A 90° V-notch weir was installed about 50 m downstream of the pipe outlet in the Midden field (V_1, Fig. 10). After heavy rainfall the pipe could restrict the flow of water and cause flooding at the north east end of the Standing Stone field.

Area B. The second catchment area was also about 20 ha, covering most of the Quarry field and part of the Smallmuir field (Fig. 10). The north east end of these fields consisted of an area of water-logged basin peat, while the rest of the soil was similar to that of Area A. In the Quarry field the soil had been disturbed relatively recently. This disturbance had created air spaces down to 45 cm so that the profile was extremely freely drained. The high organic matter content of the entire profile indicated considerable movement of slurry down through the soil. Details of soil analysis in the Quarry field are shown in Table 15. Two open ditches originating in the peat drained from the peat into a single ditch which ran along the boundary between the two fields. A 90° V-notch weir was installed about 50 m upstream from the eastern boundary of the Quarry field (V_2, Fig. 10).

Measurement of the rate of discharge of drainage water

A continuous record of the rate of discharge of water from the sub-surface drainage system of the experimental pasture, Brickrow, was obtained using a quarter 90° V-notch. The V-notch, complying with BS 3680 part IV A, was designed and constructed to meet the following requirements: (1) there should not be any obstruction to the flow from the field drain and no surcharging which could lead to loss of water through the joints; (2) solid material (worms, etc.) entering

FIGURE 11

Diagram of the quarter 90^{0} V-notch installed in the experimental pasture, Brickrow.

FIGURE 11

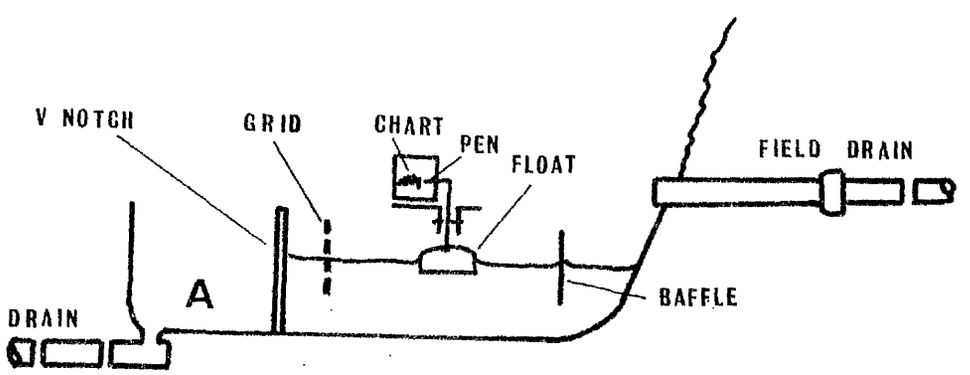


TABLE 16

Relationship between the head of water over a quarter 90^{o} V-notch and flow rate, installed in the experimental pasture, Brickrow.

* Discharge l/s = $0.3726\ (H/2.54)^{2.48}$ for heads greater than 5 cm.

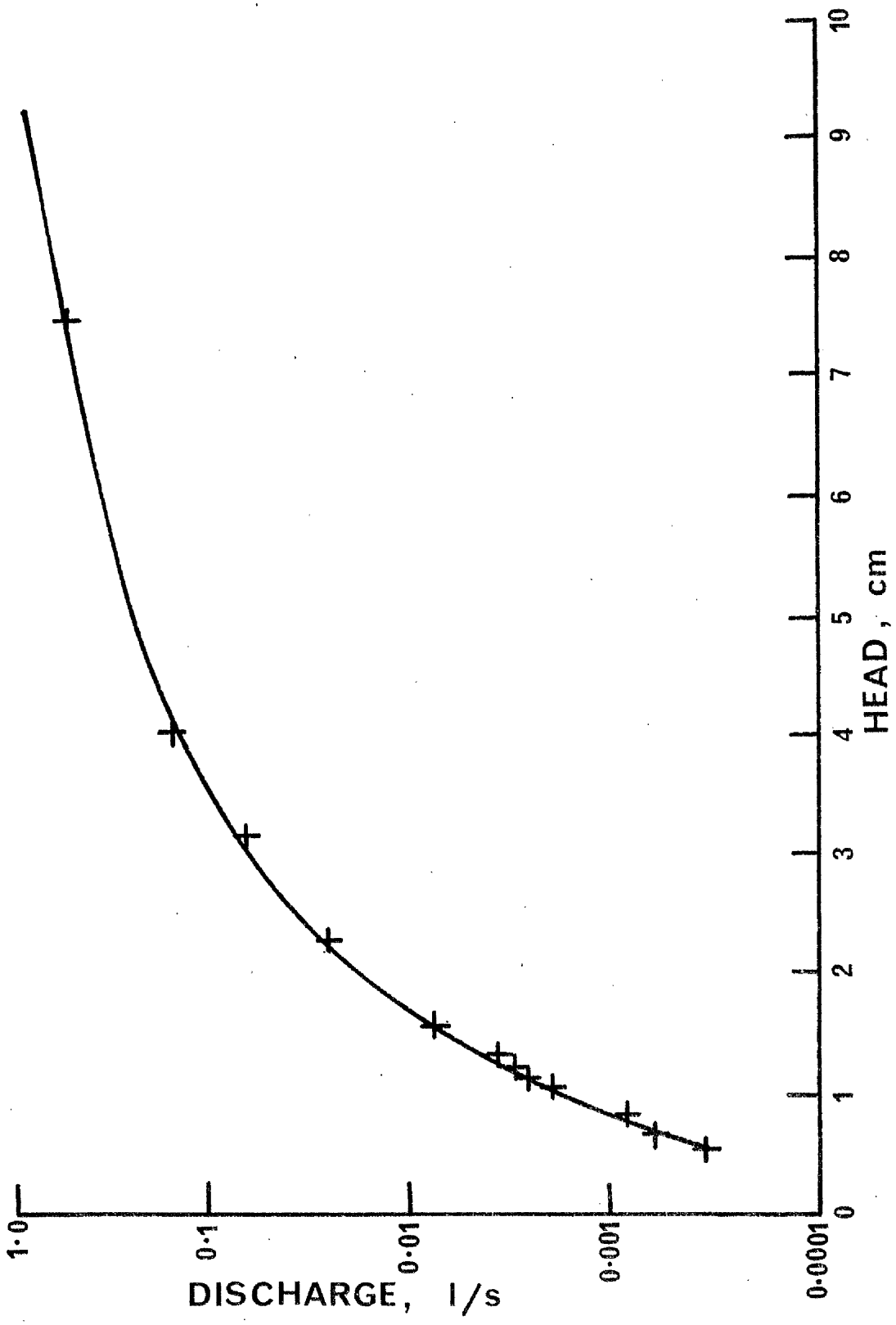
TABLE 16

Head, cm	Natural Discharge into Reservoir A l/s
0.80	0.00083
0.55	0.00033
1.07	0.00193
1.09	0.00250
1.19	0.00258
1.27	0.00318
1.52	0.00801
2.30	0.02830
3.10	0.06470
4.10	0.15500
7.40	0.53000
	Calculated Discharge*
5	0.1992
7	0.4590
9	0.8562

FIGURE 12

Calibration curve for the quarter 90^{o} V-notch installed in the experimental pasture, Brickrow.

FIGURE 12



the drainage system must not interfere in the operation of the recorder; (3) the time taken to respond to changes in the rate of flow must be relatively short; (4) all rates of flow, varying from 0 to 1.0 l/s, must be measured automatically without any alterations to the mechanism being made.

A household bath was installed below the outlet to the drain. The quarter 90^{o} V-notch (Fig. 11) was fixed across the bath in front of the drain outlet, leaving a reservoir (A) for calibration. A baffle was fixed at the top end of the bath, just in front of the drain outlet, to minimise turbulence behind the V-notch, and a mesh grid was fixed between the baffle and the V-notch to prevent debris obstructing flow over the V-notch. The head of water was recorded by a pen, attached to a domed plastic float, on a chart wrapped round a clockwork-driven drum derived from a thermograph.

For heads of water greater than 5 cm over the V-notch the rate of discharge of water over this size of V-notch (l/s) = $0.3726 (H/2.54)^{2.48}$, (Escritt, 1962), where H is the head of water over the V-notch, measured in centimetres. For heads below 5 cm the instrument had to be calibrated. A bung was placed in the outlet from the bath and reservoir A was filled with water in 0.5 l increments. After the addition of each 0.5 l the depth of the reservoir from the top of the bath to the water level was recorded on a dipstick. A steady flow of water from a hosepipe was then fed into the top end of the bath and the head of water over the V-notch noted against the rate of filling the calibrated reservoir. This was repeated for different flow rates, both from the hosepipe and the natural flow from the drain (Table 16). A calibration curve (Fig. 12) was then drawn.

During the earlier experimental periods the mean daily discharge rate from the drain was calculated by integration of the area below the line drawn by the pen on the flow recorder charts. In the later experiments the reading on the chart was noted at the time of taking samples, to obtain the actual rate of discharge. The installation was in constant operation throughout all the experimental periods. During the winter, weekly attention was required to replace the chart, rewind the clock, refill the pen with ink and remove debris from the grid. During warm weather more regular checks on the ink level were required. During the mating season a constant watch for frogs playing with the float was necessary.

The accuracy with which the rate of flow was measured depended on: (1) backlash and friction of the recording mechanism; (2) changes in density, viscosity and surface tension of the discharge water; (3) zero setting of the recorder pen; (4) the reading of the charts and calculation of the rate of flow from the calibration curve. Taking these into account, it is estimated that for a flow of 0.0002 l/s the error lies between -30 per cent and +70 per cent of the reading and for a flow of 1.0 l/s the error lies between $\pm$ 5 per cent of the reading.

Two 90^{o} V-notch weirs were installed across two open ditches on the experimental farm, Culbae (V_1 and V_2, Fig. 10). Both of these weirs complied with BS 3680 Part IV A and standard calibration curves, obtained from the formula, discharge rate, l/s = $1.4904\ (H/2.54)^{2.48}$ (Escritt, 1962), were used to measure the discharge rate. At the lower flow rates the calibration was checked on several occasions using a bucket and stop-watch. There was no provision for continuous monitoring of the head of water over the weirs, except during an

experimental period when untreated pig excrement was sprayed onto one of the fields. Usually the head of water over the weirs was only measured at the time of collecting water samples.

The sampling of drainage water

At the experimental pasture, Brickrow, samples of land drainage water were taken from a small plastic reservoir, about 60 cm^3, fixed at the outlet to the drain. This reservoir was fixed immediately above the bath, so that all the discharge had to pass through the reservoir before entering the bath. The reservoir was normally self-cleaning, but was inspected each day and any sand or soil particles removed.

Throughout the winter of 1968/9, and the first part of the winter of 1969/70, samples of land drainage water were obtained using two commercially-manufactured, automatic, interval liquid-sampling machines. Both machines incorporated a spring-driven time clock and a peristaltic pump driven by a battery operated 12 V DC motor. They both took samples at intervals of one hour. The first machine, supplied by Rock and Taylor Ltd. (Hayes Lane Trading Estate, Lye, Strainbridge) accumulated each hourly sample into one bottle to give one composite sample. The volume of each hourly sample could be set by adjusting cam-operated switches attached to the time clock. It was found that the rate of pumping, using the smallest bore tubing that would fit the peristaltic pump (i.e. 4 cm^3/s), was faster than the minimum rate of discharge from the drain to be monitored, so larger rollers were fitted to the pump. This enabled the use of 3 mm bore tubing which then gave a pumping rate of 2 cm^3/s.

The machine was set to collect a sample of approximately 120 cm^3 at the end of each hour. Twenty-four samples were collected each day for three successive days. Each day the mean volume of the individual samples was 122 cm^3. However the individual samples varied in volume between 90 cm^3 and 176 cm^3. To overcome this problem the voltage supply to the motor was halved, and later an extra reduction gear was fitted to the motor. Both of these modifications reduced the pumping rate to 1 cm^3/s and it was then possible to set the machine to take a constant sample of 125 $cm^3 \pm 1$ cm^3 each hour.

The second machine, supplied by Lea Co. Ltd. (Lea Recorder Co. Ltd., Cornbrook Park Road, Manchester, 15) was designed to take 24 separate hourly samples, but it was impossible to sterilise all the plastic funnels and bottles which came into contact with the water samples. Again larger rollers had to be fitted to the pump so that it would take small bore tubing and sample at a rate of 1 cm^3/s. On this machine the volume of each sample was controlled by cam-operated switches attached to, and operated by, the pump motor. The volumes of individual samples with this machine were reproducable. The distribution arm to the 24 sample bottles was removed and the tube outlet from the pump connected directly to the top of a glass bottle, so that as with the first machine composite samples were obtained.

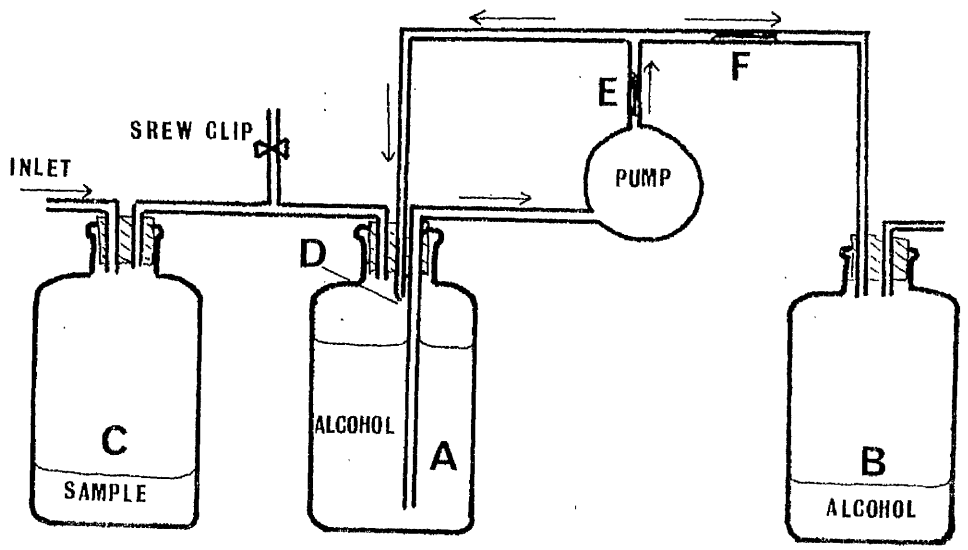
Each day during the operation of the machines in the field, one metre of sterile silicone rubber tubing was connected to each pump and the outlet inserted into the top of a sterile glass sample bottle. The other end of each tube was fixed below the water level in the small plastic reservoir at the outlet to the drain.

In the field the time clocks of both machines were adversely affected by dampness and low temperatures. During cold weather the peristaltic pumps slowed down because of tightening of bearings,

FIGURE 13

Diagram of the continuous liquid sampling machine.

FIGURE 13



thickening of lubricants and/or reduced voltage output from the battery, even when fully charged. However, a composite sample of land drainage water was obtained on most days from one or other of the machines throughout the experimental periods.

Because of the problems encountered with the machines described above, a continuous liquid sampling machine was developed (United Kingdom Patent No. 2560/71). This machine (Fig. 13) utilised a 12 V diaphragm pressure pump (S.U. Carburettor Co. Ltd.) which operated continuously to transfer alcohol from jar A to jar B. This induced a reduction in pressure in jar C causing water to flow through the sampling tube, from the sampling reservoir at the drain outlet, into the jar. The rate of sampling was controlled as follows: (1) to maintain a steady pumping rate a 1 mm bore capillary tube (E) was placed immediately after the pump outlet; (2) to obtain a low sampling rate, a recirculation system back into jar A was incorporated; (3) to maintain pressure in the system, a 1 mm capillary jet (D) was placed at the recirculation outlet into A: (4) fine control of sampling was effected by adjusting the bore of capillary F. With capillary F about 0.5 mm bore and 50 mm long the sampling rate was 0.00005 l/s. A clean sterile sampling inlet tube and sterile sampling jar were installed in the field each day. The machine was enclosed in a box lagged with expanded polystyrene and the tube between the box and the drain outlet similarly lagged.

The machine was in constant use for more than three months, exposed to various weather conditions, including air temperatures as low as -10^{o}C. Regular samples of 4.30 litres per day were obtained. The advantages of this machine, as compared with the other two machines

already described, were (1) the only moving part was the diaphragm pressure pump, which due to its particular design was unaffected by extreme temperatures and slight changes in voltage supply, (2) spare parts, including the pump, were readily available, (3) a 33 amp-hour 12 V battery only required recharging at 4 or 5 day intervals, instead of daily, (4) the sample tube was not subject to wear as with the peristaltic pump, (5) samples could be obtained at lower flow rates than was possible with the other sampling machines and (6) the sample was collected continuously.

During the winter experiments of 1970/1 and 1971/2 samples collected over short periods were required. To obtain these spot samples, clean Winchester bottles fitted with a rubber bung drilled to take a short glass tube attached to a short length of silicone rubber tubing were used. These were sterilised at 121^{o}C for 15 min and the tube clamped shut while the bottles were still hot (cf. Zobell, 1941). To collect samples the end of the tube was fixed below the water level in the small plastic reservoir at the outlet to the drain and the clamp opened. Reduced pressure caused water to flow into the bottle until it was about three-quarters full, i.e. it contained about two litres.

The water samples obtained from the experimental farm, Culbae, were all collected in evacuated winchester bottles. They were usually collected from sampling points, a in area A, and b in area B (Fig. 10). During and following slurry spraying on the Longcastle field, the samples were collected from sampling point a^{1}.

Meteorological records

Records of daily rainfall and daily maximum and minimum atmospheric temperatures were obtained for the Brickrow area from two meteorological stations, one (Auchincruive, Grid Ref. NS 3892) being one km south west of the experimental pasture and the other (Prestwick, Grid ref. NS 3852) two km due north. During the winter of 1971/2 soil temperatures at 10 cm and 100 cm depth were also obtained from Auchincruive.

Meteorological records for the Culbae area were obtained from a station at Bladnock about 8 km north east of the farm.

Bacteriological examination of drainage water

The technique of membrane filtration was adopted for counting bacteria in water samples because it has a high degree of reproducibility, it permits the use of large volumes of samples and it is more rapid to carry out than the most probable number procedure (Standard Methods, 1971). The choice of media and incubation temperature for the enumeration of Escherichia coli and enterococci was made after testing a variety of media and incubation temperatures.

Sartorious membrane filters, catalogue number 11407 (formerly MF 30) pore size 0.2 to 0.3 μm diameter 47 mm (V.A. Howe and Co. Ltd., 47 Pembridge Road, London, W. 11) were used for filtration of water samples. Before use the membranes were sterilised by autoclaving at 121^{o}C for 15 min. To keep them flat during sterilisation they were placed in a glass petri-dish and interleaved with Whatman No. 1 filter paper. The membrane filter holders used were similar to those described in "The Bacteriological Examination of Water Supplies" (Ministry of Health and Social Security, 1969).

The volumes of water filtered varied between 0.1 cm^3 and 1000 cm^3. Colonies on filters which supported between 50 and 200 colonies were counted. The filter funnels were sterilised before use. Water samples smaller than 50 cm^3 were filtered and washed through with 50 cm^3 sterile distilled water. The membranes were transferred from the funnels, with a pair of forceps previously sterilised by flaming in alcohol, onto the surface of filter pads (Whatman No. 17 filter paper, 60 mm diameter) saturated with a nutrient medium, or onto the surface of an agar based medium.

The numbers of Escherichia coli in samples of water were estimated by incubating inoculated membranes on filter pads saturated with m-endo broth MF (Difco) at 37°C for 24 h. Confirmation tests were carried out by sub-culturing presumptive F. coli colonies into peptone water. These cultures were then examined by Eijkman and IMViC tests.

The numbers of enterococci were estimated by incubating inoculated membranes on m-enterococcus agar (Difco) at 37°C for 48 h.

The numbers of bacteria capable of growth at 20°C in 48 h on m-plate count broth (Difco) were estimated by filtering 1 cm^3 of four-fold dilutions of drainage water samples. The inoculated membranes were then incubated on filter pads saturated with m-plate count broth.

Bacteriological examination of soil samples

Soil samples from the experimental pasture, Prickrow, were passed through a 2 mm sieve and 20 g of the sieved soil added to 180 cm^3 cooled mineral salts diluent. These suspensions were blended

in an Atomix blender at full speed for 2 min. Decimal dilutions of the blended suspensions were used to inoculate the surface of plates of 1/10 strength m-plate count broth solidified with 1 per cent agar (Oxoid). These plates were incubated at 20°C for 48 h and colonies counted.

Bacteriological examination of pig excrement

Samples of pig excrement were obtained from the underhouse channels at Brickrow and from the slurry tank at Culbae. In each case 10 cm^3 of the slurry were transferred, using a wide bore (9 mm) pipette, to 90 cm^3 mineral salts diluent. These initial dilutions were shaken thoroughly and series of decimal dilutions prepared. The dilutions were then used for MPN estimations and colony counts.

The MPN of Escherichia coli and enterococci were obtained by inoculating tubes of MacConkey broth and sodium azide broth respectively. Inoculated tubes were incubated at 37°C. Presumptive coliform cultures were later confirmed as E. coli by Eijkman and IMViC tests. Colony counts of E. coli were estimated by inoculating the surface of violet red bile agar. The inoculated plates were overlaid with a further layer of VRBA and incubated at 37°C for 24 h.

In the Culbae experiment a colony count of bacteria was also estimated by inoculating the surface of 1/10 strength m-plate count broth plus 1 per cent agar. These plates were incubated at 20°C for 48 h.

Chemical examination of drainage water, and pig excrement

Chemical analysis of water samples collected from the experimental pasture, Brickrow, were carried out at the laboratories of the Ayrshire River Purification Board, Ayr, using standard methods (Ministry of Housing and Local Government, 1956). The sodium azide modification of the Winkler method was used for measuring dissolved oxygen concentration. The nitrate concentration was determined by reduction by Devarda's alloy. Suspended solids concentrations were determined by filtration through glass fibre filter paper (Whatman, GF/C, 70 mm diameter) and dried at 105^{o}C.

Water samples and pig excrement collected from the experimental farm, Culbae, were chemically analysed at the laboratories of the Solway River Purification Board, Dumfries, also using standard methods. Pig excrement from Brickrow and Culbae was analysed in these laboratories by the methods described in Section 1.

EVALUATION OF METHODS

Evaluation of media for counting Escherichia coli

Comparison of four selective media incubated at 37°C. Four selective media were tested to see if they could be used to differentiate between pure cultures of Escherichia coli (NCTC 9001), Aerobacter aerogenes (NCTC 8712) and a species of Proteus (No 15A, The West of Scotland Agricultural College stock cultures), when grown on the surface of membrane filters at 37°C. A viable count of E. coli by the pour plate method on nutrient agar (Oxoid) was compared with a viable count obtained by membrane filtration.

The media used for membrane filtration were Endo membrane broth (Oxoid); and Endo membrane broth following 2 hours' incubation of the inoculated membrane on Resuscitation broth (Oxoid); Bacto-m-HD endo broth (Difco); Bacto-m-HD endo broth W/BC (Difco); and Bacto-m-HD endo broth MF (Difco).

The three test organisms were cultured overnight at 37°C in nutrient broth (Oxoid). Each broth culture was then diluted by a factor of 10^6 with $\frac{1}{4}$ strength Ringers solution to obtain suspensions containing approximately 100 organisms per cm^3. A viable count of the suspension of E. coli was determined by the pour plate method. Viable counts of each of the three suspensions were then determined by the membrane filtration method using each of the test media.

In addition, viable counts of E. coli from a mixture of equal volumes of the E. coli suspension and the Proteus sp. suspension, and from a mixture of equal volumes of the E. coli suspension and the A. aerogenes suspension, were also determined by the membrane filtration method, using each of the test media. All the inoculated membranes

TABLE 17

Comparison of the viable count of *Escherichia coli* on nutrient agar with counts obtained on m-endo broth MF after membrane filtration with and without the presence of other organisms.

TABLE 17

Counting Method	Culture	Viable count of *E. coli*/cm^3
Membrane Filtration	*E. coli*	91
	E. coli + *A. aerogenes*	90
	E. coli + *Proteus sp.*	97
Pour Plate	*E. coli*	120

were incubated at 37°C. After 24 h the membranes were examined for the presence of colonies of the test organisms and to see if the colonies of E. coli could be readily differentiated from colonies of A. aerogenes and Proteus sp.

Colonies of E. coli were counted only on those media which allowed clear differentiation of E. coli colonies from those of the other two genera. Where more than one colony type was clearly visible a selection of presumptive E. coli colonies from the membranes supporting a mixture of two genera were sub-cultured into peptone broth. After incubation at 37°C for 24 h the cultures were used for Eijkman and IMViC tests to confirm the presence of E. coli.

All the media tested using the membrane filtration method supported the growth of all three test organisms. On the Endo membrane broth, Resuscitation/Endo broth, m-HD endo broth and m-HD endo broth W/BG, the colonies of E. coli were not easily distinguishable from colonies of A. aerogenes and/or Proteus sp. On m-endo broth MF, however, a metallic sheen clearly differentiated E. coli colonies from colonies of the other two organisms.

The numbers of colonies of E. coli on the membrane filters above m-endo broth MF were counted to obtain a viable count of E. coli in the suspension. These results are shown in Table 17. Twenty-two colonies were picked from the surface of the membrane supporting a mixture of E. coli and A. aerogenes and all the colonies were confirmed as E. coli. Similarly eighteen colonies were isolated and confirmed as E. coli from the surface of the membrane supporting a mixture of E. coli and Proteus sp.

Thus m-endo broth MF was the only medium tested which was found to allow differentiation of E. coli from the Aerobacter sp. and Proteus sp. Comparison of the membrane filtration method and m-endo broth MF with the pour plate method and nutrient agar showed only a small inhibition of E. coli by the selective medium, m-endo broth MF.

Comparison of bacto MFC broth with bacto m-endo broth MF. The use of bacto MFC broth (Difco, code 0883) plus 1 per cent of a rosolic acid solution (1 g Bacto Rosolic acid + 100 cm^3 0.2N sodium hydroxide) incubated at 44°C, was compared with m-endo broth MF incubated at 37°C, for the enumeration of E. coli by membrane filtration of water samples. The isolation and enumeration of E. coli at 44°C would reduce the necessity of frequent sub-culturing from the membranes for confirmation tests.

Pure cultures of E. coli and A. aerogenes were grown overnight at 37°C in nutrient broth. Both cultures were diluted by a factor of 10^6 with $\frac{1}{4}$ strength Ringers solution to obtain suspensions containing approximately 100 organisms per cm^3. Viable counts of E. coli and A. aerogenes in the suspensions were then determined by the membrane filtration method using each of the two media. Plates containing membranes placed above m-endo broth MF were incubated at 37°C while plates containing membranes placed above MFC broth were sealed in plastic bags and the bags submerged in a water bath at 44°C. All the membrane filters were examined for the presence of colonies of E. coli after 24 h and the numbers of E. coli colonies on each membrane were counted. Counts of the numbers of E. coli in samples of land drainage water were also made using the two media.

TABLE 18

Comparison of m-endo broth MF with MFC broth for the selective isolation and enumeration of E. coli by membrane filtration.

TABLE 18

Sample	m-endo broth MF	MFC broth
E. coli culture	8.7×10^{10}	3.4×10^{10}
Land Drainage Water 1	7.2×10^{3}	2.8×10^{2}
2	1.9×10^{4}	5.2×10^{3}
3	2.8×10^{3}	8.8×10^{2}
4	6.2×10^{3}	3.8×10^{3}
5	1.8×10^{4}	7.0×10^{3}

The results of the numbers of E. coli in the suspension as determined by the two media are shown in Table 18. A. aerogenes was inhibited on MFC broth incubated at 44°C. The numbers of E. coli growing on this media were considerably lower than the numbers growing on endo broth MF. As in the previous experiment all the presumptive E. coli colonies isolated for confirmation tests were found to be E. coli. While MFC broth incubated at 44°C inhibits the growth of organisms other than E. coli, it is also more inhibitory for E. coli than endo broth MF incubated at 37°C.

Evaluation of media for counting enterococci

Bacto-m-enterococcus agar (Difco) devised by Slanetz and Bartley (1957) was claimed by the authors to be one hundred per cent selective for enterococci, even when filtering heavily-polluted water samples. They also found that the ratio of Escherichia coli to faecal streptococci in polluted water samples was higher using this medium than using other media.

This medium was compared with Bacto-m-azide broth (Difco) for the enumeration of enterococci by membrane filtration.

Broth cultures of Streptococcus faecalis (NCIB 370), Streptococcus lactis (NCDA 712), Streptococcus durans (NCIB 8587), A. aerogenes, Bacillus subtilis (NCIB 8063) were grown overnight at 37°C in nutrient broth. Each culture was then diluted by a factor of 10^6 with ¼ strength Ringers solution to obtain suspensions containing approximately 100 organisms per cm^3. Viable counts of each of the four suspensions were determined by the membrane filtration method using each of the two media. The inoculated filters were incubated at 37°C and examined

after 48 h for the presence of colonies. The numbers of Str. faecalis colonies were counted. A viable count of the suspension of Str. faecalis was determined by the pour plate method using nutrient agar. A sample of land drainage water was also filtered through membranes and the membranes incubated on broth media. Microscope slide preparations of colonies arising from drainage water were Gram stained.

Str. lactis, Str. durans, A. aerogenes and B. subtilis all failed to produce colonies on membranes incubated on m-enterococcus agar. Some growth of B. subtilis did occur on m-azide broth.

The number of Str. faecalis in the suspension as estimated by the pour plate method was 100 per cm^3, whereas the number estimated by membrane filtration using m-enterococcus agar was 60 per cm^3. Gram-stained preparations of colonies isolated from samples of land drainage water on m-enterococcus agar showed that all the colonies were comprised of Gram-positive cocci. A few colonies isolated from land drainage water on m-azide broth contained Gram-positive rods.

The numbers of Str. faecalis were in close agreement with the number obtained on nutrient agar and only Gram-positive cocci were isolated from land drainage water on m-enterococcus agar. Therefore, provided that colonies obtained on this medium are Gram-positive cocci, then it seems reasonable to assume that they are enterococci.

Examination of the reproducibility of the membrane filtration method

Depending on the flow rate of drainage water, the volume of water samples filtered for the estimation of the numbers of Escherichia coli were usually 0.5, 2, 10, 50, 250 or 1,000 cm^3.

TABLE 19

The reproducibility of the membrane filtration method.

The concentration of *E. coli* was estimated in six samples of land drainage water. The number of *E. coli* was estimated in ten sub-samples of each sample.

TABLE 19

Vol. Filtered (cm^3)	0.5	2	10	50	250	1000
Number of colonies of *E. coli*	150	140	55	194	173	210
	220	150	70	170	179	208
	134	135	81	189	176	194
	165	133	75	169	175	201
	180	144	70	177	173	197
	170	125	65	206	174	198
	193	136	77	198	179	206
	230	130	75	197	173	201
	132	150	79	171	175	199
	180	125	65	210	176	198
Standard Deviation	32.83	9.15	7.90	15.35	2.26	5.18
Coefficient of Variation, %	18.7	6.7	11.0	8.2	1.3	2.6

To examine the reproducibility of the membrane filtration method used in the laboratory, several replicate counts of E. coli in samples of drainage water from the experimental pasture, Brickrow, were made. Samples of drainage water were collected on six different occasions. These were each divided into ten sub-samples of the appropriate volume, depending on the flow rate of the drain discharge. Each sub-sample was then filtered and the concentrations of E. coli estimated after incubating the inoculated membranes on m-endo broth MF.

The numbers of colonies of E. coli obtained from each sample filtered is given in Table 19. From these results the standard deviations and coefficient of variation were calculated for each volume filtered and are also given in Table 19.

Thus for concentrations of E. coli in land drainage water below $5 \times 10^5/l$, the membrane filtration method gave very reproducible results. It was only at higher concentrations that substantial errors were apparent, but over the range of concentrations of faecal bacteria found in the drain discharge these errors are not important.

PART A. FACTORS AFFECTING THE CONCENTRATIONS OF FAECAL BACTERIA IN LAND DRAINAGE WATER

INTRODUCTION

The concentrations of faecal bacteria and chemical pollution in land drainage water from the experimental pasture, Brickrow, were first monitored in the absence of recent excrement application to the land. Pig excrement was then applied to the land and the effects on the concentrations of faecal bacteria and chemical pollutants in the water observed.

Samples of land drainage water were collected throughout three successive winters for bacteriological and chemical examination. Each sample was divided into two portions. One portion was bacteriologically examined within 30 min of collection from the pasture, while the other portion was stored at $5^{o}C$ for 1 to 120 h before being chemically analysed.

Winter 1968/9. To study the factors affecting the concentrations of faecal bacteria in land drainage water, in the absence of recent excrement application to the pasture, no animals were allowed onto the pasture and no excrement was applied during the winter. Composite samples of the discharge were collected from the sampling station, using either the Rock-and Taylor interval liquid sampling machine or the Lea liquid sampling machine. Between 17/9/68 and 18/10/68 composite samples of drainage water were collected on four days each week. After 22/10/68 composite samples were collected every day. Absence of some results was due to occasional failure of the sampling machines.

FIGURE 14

Bacterial pollution of drain discharge during September and October 1968.

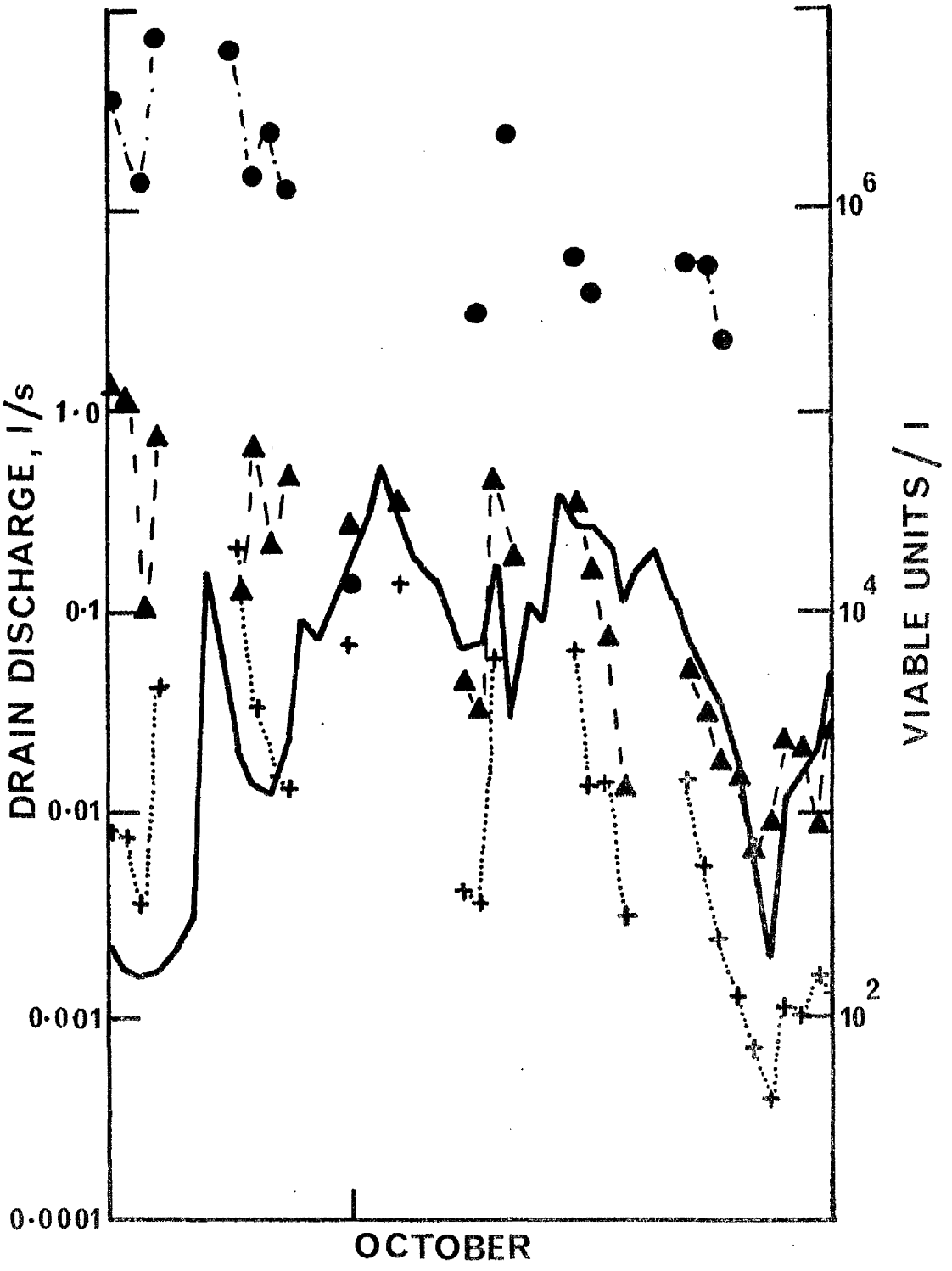
Continuous line - mean daily flow rate of the drain discharge.

-▲- -▲- - concentration of Escherichia coli.

...+.....+.. - concentration of enterococci

-·●-·-●·- - viable bacteria on m-Plate count broth at 20°C.

FIGURE 14


DRAIN DISCHARGE, l/s
1·0
0·1
0·01
0·001
0·0001
VIABLE UNITS / l
10^6
10^4
10^2
OCTOBER
1968

Winter 1969/70. The 'normal' concentrations of faecal bacteria in the drain discharge, in the absence of recent application of excrement to the pasture, were again monitored by collecting composite samples of the discharge. Pig excrement was sprayed onto the surface of the pasture on four occasions during the winter. Between November 1969 and January 1970 composite samples of drainage water were collected using the Rock and Taylor and/or Lea liquid-sampling machines. From January 1970 until April 1970 daily samples were collected using the continuous liquid-sampling machine.

Winter 1970/71. The concentrations of faecal bacteria in the discharge in the absence of recent excrement application to the pasture were estimated in composite samples of the drain discharge, collected throughout October 1970, using the continuous liquid-sampling machine. Following the start of the excrement applications in October and November 1970, spot samples of drainage water were collected at intervals of 0.5 to 4 h for about 36 h.

RESULTS

The results of analyses of composite samples of land drainage water collected in the absence of recent application of excrement to the land showed a positive relationship between the concentration of faecal bacteria in drainage water and the flow rate of the drain discharge. (Complete tables of all the results are given in the appendix.) The earlier results of examination of samples collected four days per week during September and October 1968 (Fig. 14) suggested that the concentrations of faecal bacteria in the discharge

FIGURE 15

Bacterial pollution of drain discharge during winter 1968/9.

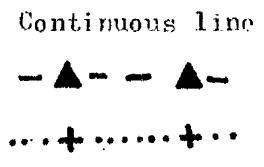


FIGURE 15

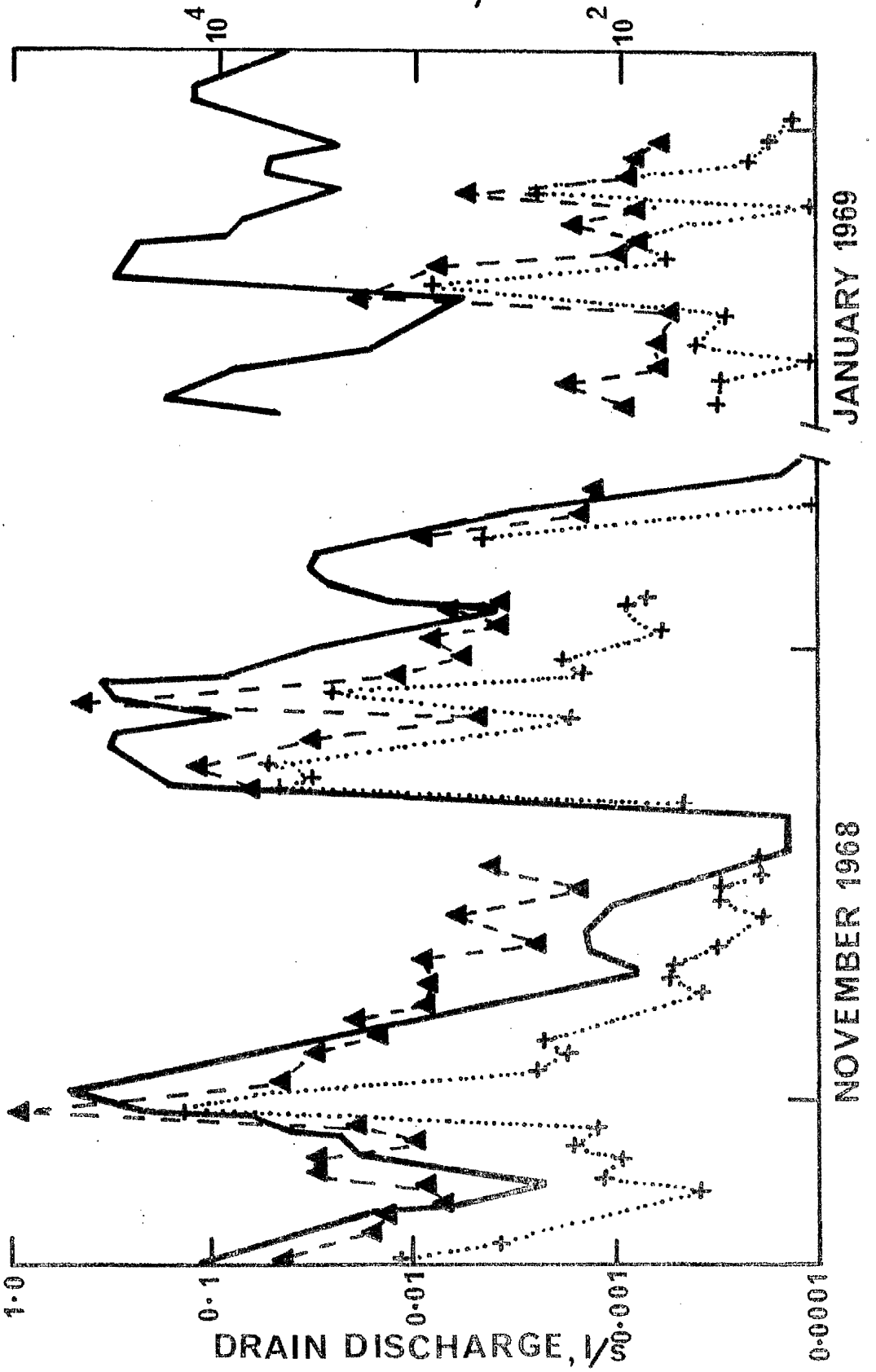

VIABLE UNITS / l
10^4
10^2
JANUARY 1969
NOVEMBER 1968
1·0
0·1
0·01
0·001
0·0001
DRAIN DISCHARGE, l/s

FIGURE 16

Bacteriol pollution of drain discharge during winter 1969/70.

Continuous line - mean daily flow of the drain discharge.

– ▲ – – ▲ – - concentration of Escherichia coli.

• • • + • • • • • + • • - concentration of enterococci.

Vertical lines - days when pig excrement was applied to the pasture.

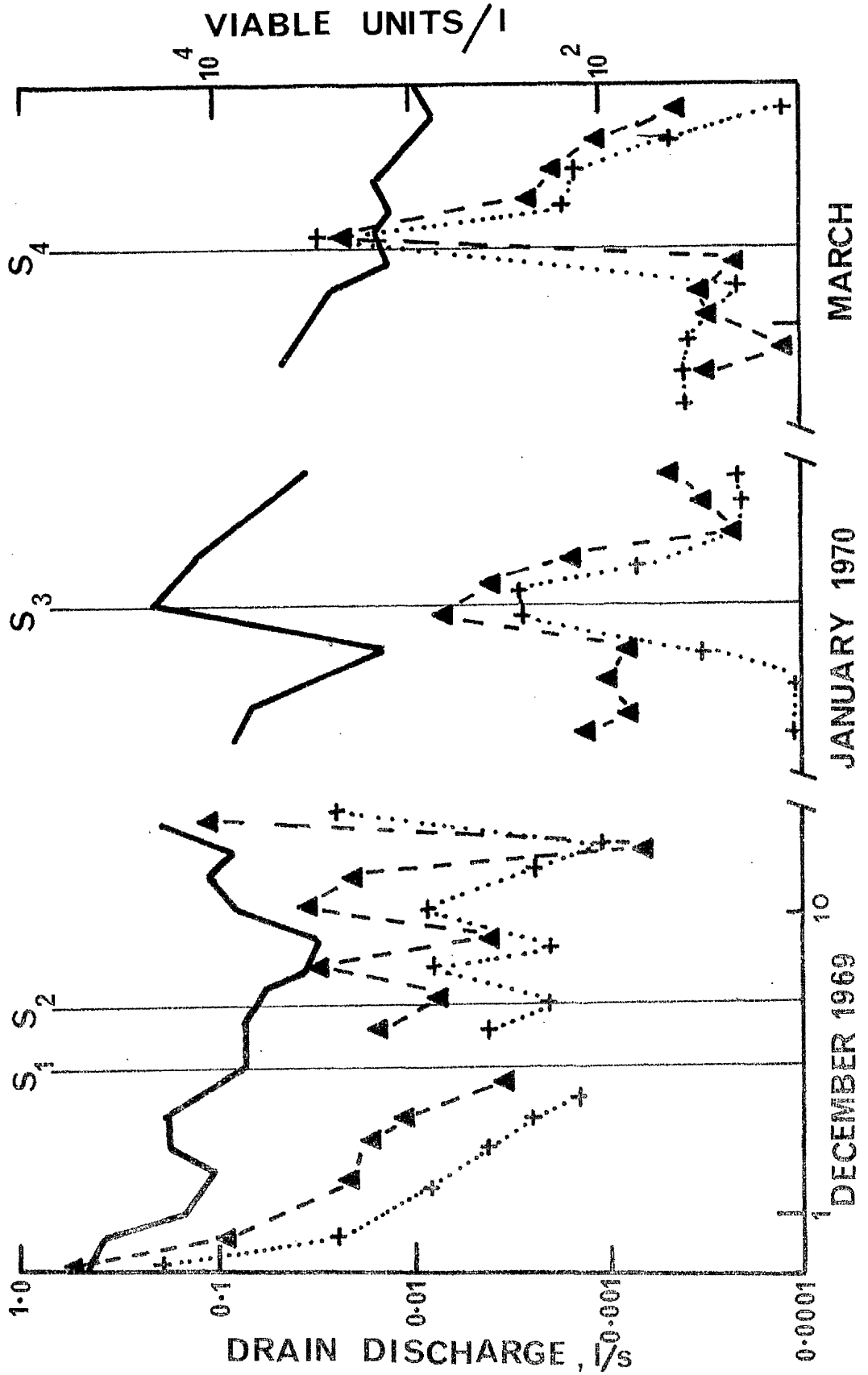
E_1 - 22 m^3/ha

E_2 - 33 m^3/ha

E_3 - 55 m^3/ha

E_4 - 55 m^3/ha

FIGURE 16


VIABLE UNITS/l
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10^2
S_4
S_3
S_2
S_1
MARCH
JANUARY 1970
DECEMBER 1969
10^0
1
1·0
0·1
0·01
0·001
0·0001
DRAIN DISCHARGE, l/s

FIGURE 17

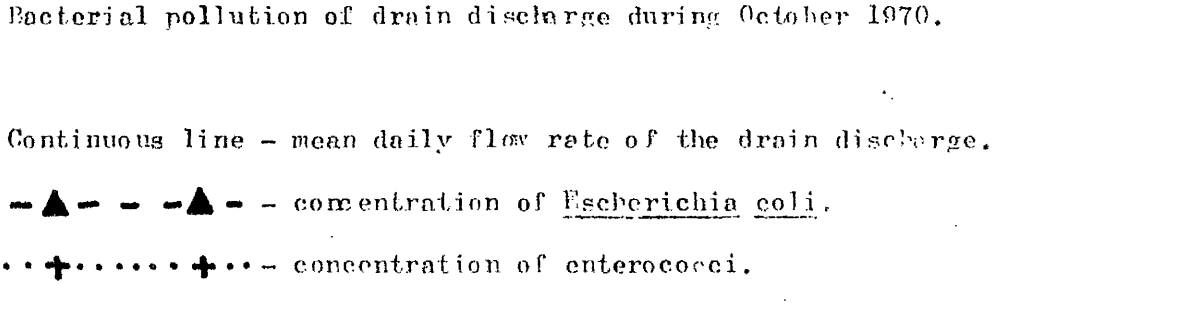
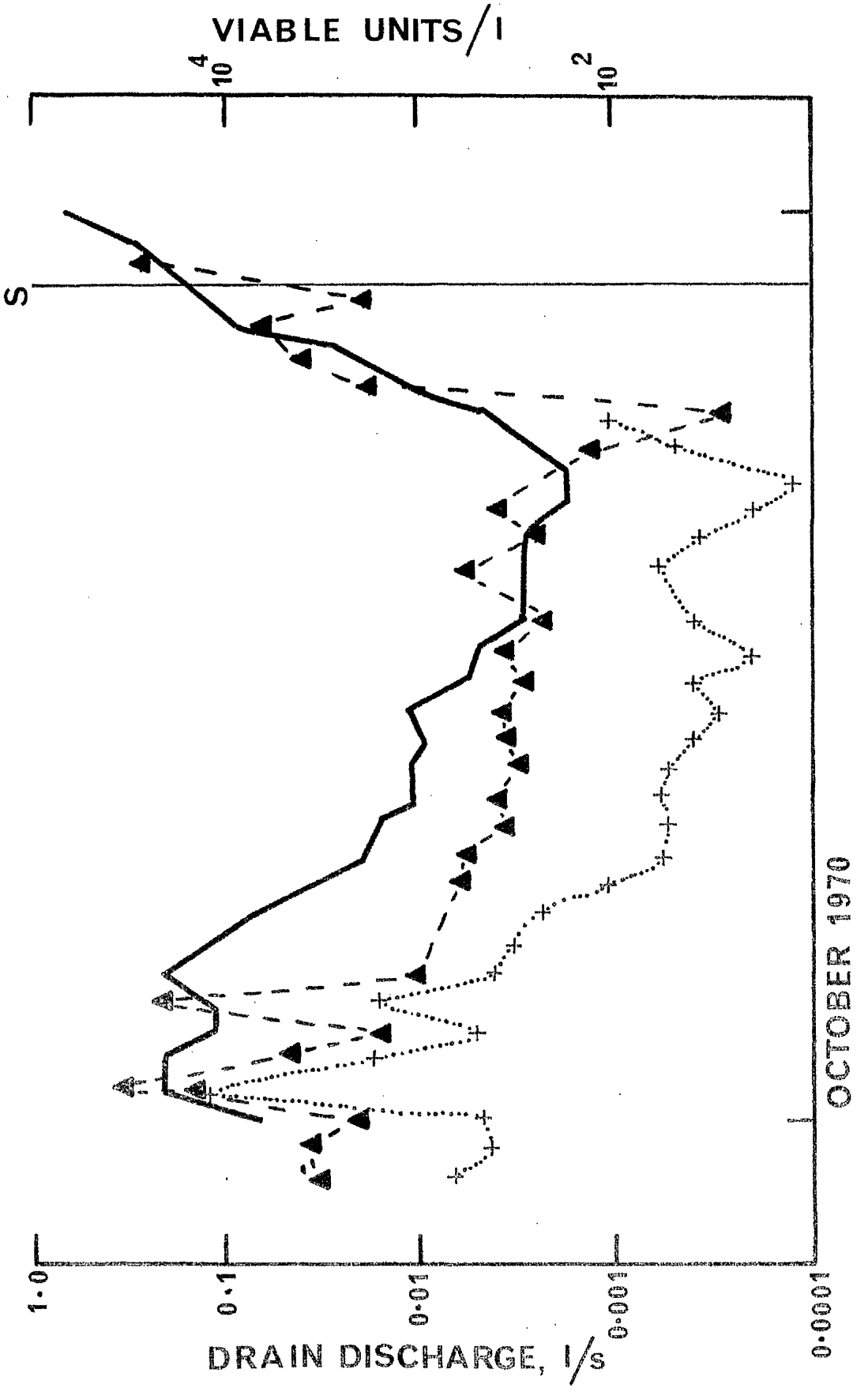

Bacterial pollution of drain discharge during October 1970.
Continuous line – mean daily flow rate of the drain discharge.
– ▲ – – – ▲ – – concentration of Escherichia coli.
· · + · · · · · · + · · · – concentration of enterococci.

FIGURE 17


VIABLE UNITS/l
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10^2
S
1
OCTOBER 1970
1·0
0·1
0·01
0·001
0·0001
DRAIN DISCHARGE, l/s

were higher at high flow rates and lower at low flow rates. These were later confirmed by statistical analysis of the samples collected 7 days each week between October 1968 and February 1969.

The relationship between the concentrations of faecal bacteria in the drainage water and the flow rate of the discharge was again demonstrated during the winter 1969/70 (Fig. 16) and during October 1970 (Fig. 17). However, because of the effects of applying pig excrement to the pasture on several occasions during the winters 1969/70 and 1970/71 there was insufficient data between slurry application to analyse the results by statistical methods.

Effect of flow rate of the drain discharge on the concentrations of faecal bacteria

The results of daily bacteriological examination of composite samples of land drainage water collected after 22 October 1968 and the mean daily discharge rate of water from the drain are illustrated in Fig. 15. Escherichia coli and enterococci were regularly isolated from samples of land drainage water throughout the winter and their concentrations ranged from 2×10^1 to 10^5 E. coli/l and 10^1 to 1.4×10^4 enterococci/l. The concentrations were higher at high flow rates and lower at low flow rates. More than 97 per cent of presumptive E. coli colonies isolated from the membrane filters proved to be E. coli. Since it is generally accepted that this organism is of exclusively faecal origin and does not multiply in soil or on vegetation (Ministry of Health and Social Security, 1969) and since no animal excrement was applied to the pasture during the winter of 1968/9, those present in the drain discharge can be presumed to have originated from faecal

material applied to the pasture during the summer. All colonies from enterococcus agar that were examined were enterococci, and also presumed to have originated from faecal material applied during the summer. It is clear, therefore, that E. coli and enterococci isolated from the drain discharge in February 1969 had survived in or on the pasture for at least four months.

Regression analysis of the results of the winter 1968/9 show that the logarithm concentrations of the two groups of bacteria in the drainage water are related to the logarithm flow rate of the discharge and the number of days after 1 October 1968 by an equation of the form

$$Y = a + bX - cZ$$

where Y = $\log_{10}$ concentration of bacteria in the discharge, viable units/l

X = $\log_{10}$ flow rate of the discharge, l/s

Z = number of days after 1 October 1968

and a, b, c, are constants.

For E. coli the equation giving the best fit is

$$Y = 4.84 + 0.521\ (\pm\ 0.055)\ X - 0.0176\ (\pm\ 0.0015)\ Z \quad \ldots\ldots \quad 1$$

and for enterococci

$$Y = 3.96 + 0.701\ (\pm\ 0.064)\ X - 0.0104\ (\pm\ 0.0017)\ Z \quad \ldots\ldots \quad 2$$

the figures in parentheses being the standard errors of the regression coefficients.

The equation for E. coli accounts for 77 per cent of the variation observed in the concentration of bacteria in the discharge and that for enterococci accounts for 70 per cent. No improvement is obtained by attempting to allow the regression coefficient, b, on flow rate to change with time. Therefore it seems reasonable to suggest that the concentrations of E. coli and enterococci in the discharge are determined mainly by those factors included in the regression equations.

The regression equation of the difference in the logarithms for the two groups of organisms is

$$Y = 0.889 + 0.180\ (\pm\ 0.044)\ X - 0.0072\ (\pm\ 0.0012)\ Z \quad \ldots\ldots \quad 3$$

Since the standard errors for the two regression coefficients in equation (3) are smaller than corresponding errors in equations (1) and (2) it can be concluded that the differences in the regression coefficients in equations (1) and (2) are highly significant.

The relationship between the time since the application of animal excrement to the land and the concentrations of faecal bacteria in the drain discharge

The time factor cZ in the regression equations indicates that the concentrations of Escherichia coli and enterococci in the land drainage water declined logarithmically with time. This result would be expected for types of bacteria believed to be incapable of multiplication in water or soil, in the absence of further inoculation of faecal bacteria onto the pasture. The values of coefficient c show that the 90 per cent reduction time for the concentration of E. coli in the drainage water was 57 days and that for enterococci was 96 days.

Effect of excrement application on the concentrations of faecal bacteria in the drain discharge

On four occasions during the winter of 1969/70 pig excrement was sprayed from a vacuum tanker onto the surface of the pasture. The concentrations of Escherichia coli and enterococci in most of the land drainage water samples collected during the winter varied with the flow rate of the discharge. However, after the application of pig excrement on three occasions, S_1, S_2 and S_4, the 'normal' relationship between the bacterial concentrations and the flow rate were disturbed (Fig. 16). The concentrations of E. coli and enterococci in the composite water samples collected throughout the day on which excrement was applied were higher than would normally be expected. On each occasion the concentrations of E. coli and enterococci in the water samples collected during the next 24 h were practically at the 'normal' value appropriate to the flow rate of the discharge in the absence of excrement applications. Following the third application (S_3, Fig. 16) of pig excrement to the pasture there were no detectable effects on the concentrations of E. coli or enterococci in the discharge.

It was clear from these results that it would be useful, after applying excrement to the pasture, to sample the drain discharge more frequently than once daily. Therefore during the winter of 1970/1 pig excrement (approximate composition 120 g suspended solids/l; biochemical oxygen demand, 35 g/l; pH value, 8.7; E. coli, 9×10^8 viable units/l) was applied to the pasture at a rate of 33 m^3/ha on 28 October (Experiment I) and a similar volume on 23 November

FIGURE 18

Experiment I

Bacterial pollution of drain discharge after application of pig excrement to the pasture.

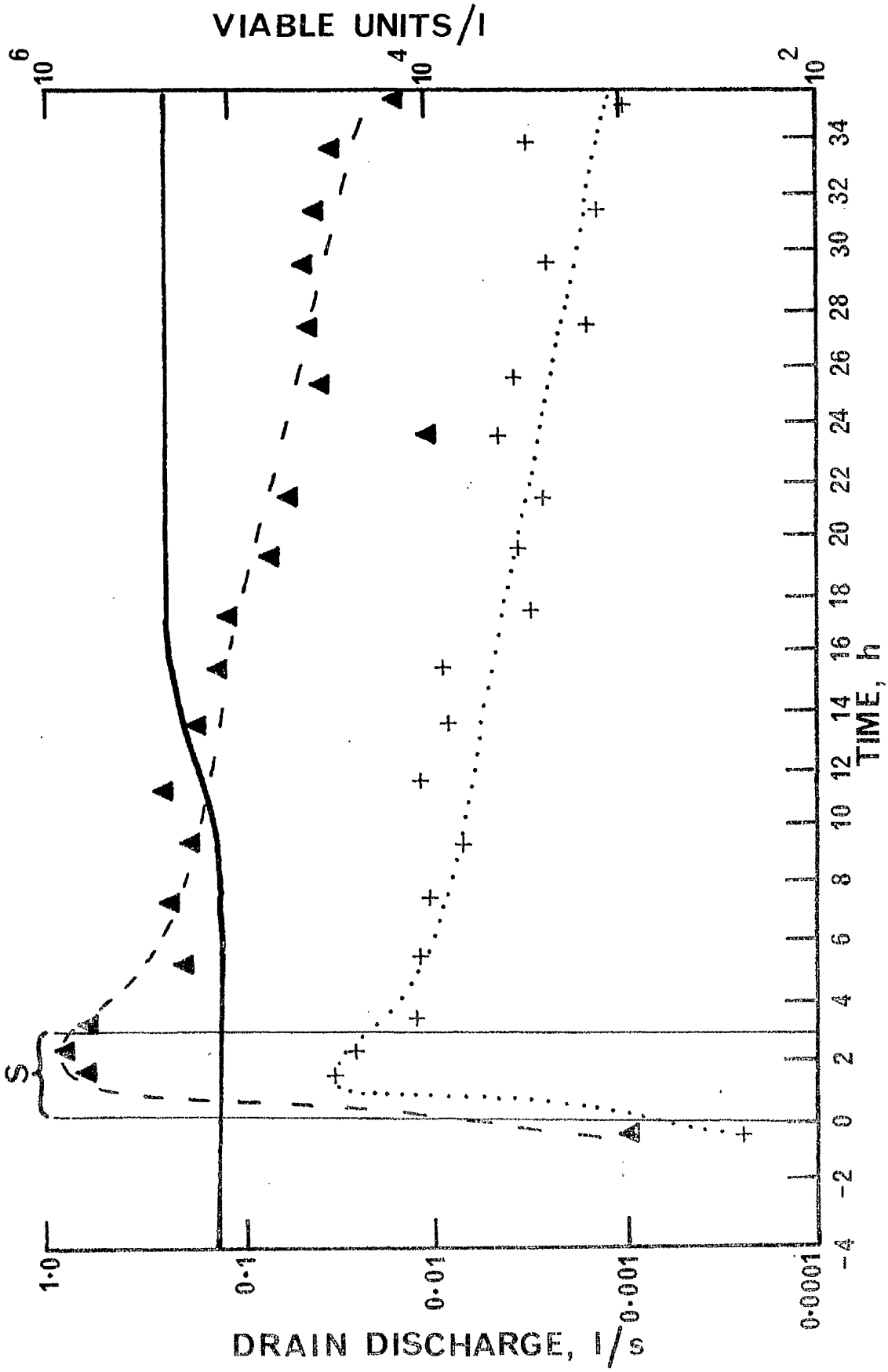
Continuous line - flow rate of the drain discharge.

-▲- - -▲- - concentration of Escherichia coli.

·· +·····+·· - concentration of enterococci.

E - period during which excrement was applied ($55\ m^3/ha$).

FIGURE 18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10^4
10^2
TIME, h
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-2
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
S
1·0
0·1
0·01
0·001
0·0001
DRAIN DISCHARGE, l/s

FIGURE 19

Experiment II

Bacterial pollution of drain discharge after application of pig excrement to the pasture.

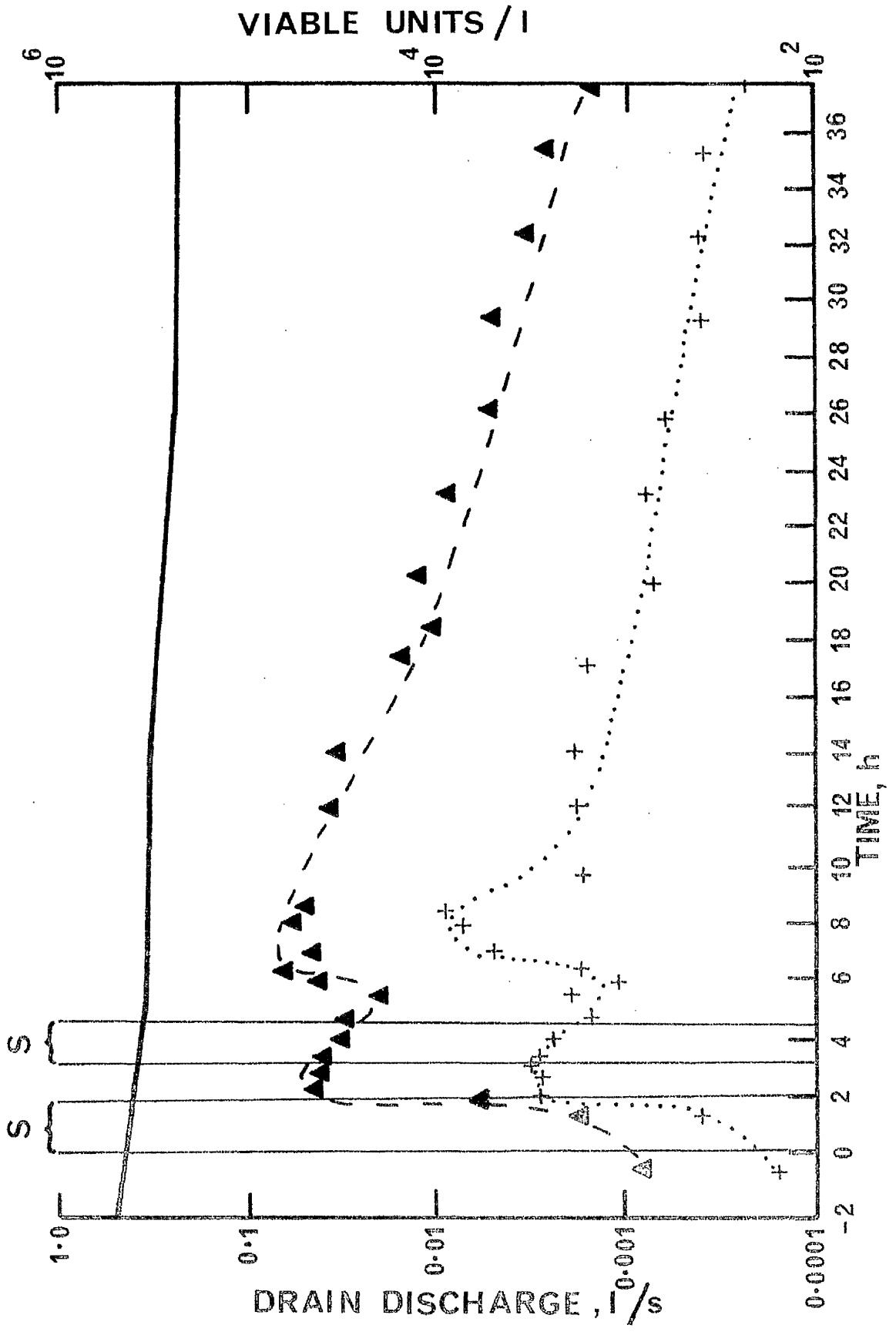
Continuous line - flow rate of the drain discharge.

–▲– – –▲– - concentration of Escherichia coli.

··+·····+·· - concentration of enterococci.

E_1 & E_2 - periods during which excrement was applied (total of 55 m^3/ha).

FIGURE 19


VIABLE UNITS / l
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10^4
10^2
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34
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30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
-2
TIME, h
S
S
1·0
0·1
0·01
0·001
0·0001
DRAIN DISCHARGE, l/s

(Experiment II) and water samples were collected at intervals of 0.5 to 4 h for about 36 h following the excrement application.

Experiment I. The flow rate of the drain discharge and the concentrations of faecal bacteria in the drainage water following the application of excrement to the pasture are shown in Fig. 18. The flow rate of the drain discharge was steady for the first 11 h following the start of excrement application and then increased slightly during the rest of the experimental period, possibly as a result of rain which fell during the experiment.

Within 90 min of starting to apply excrement the concentration of *E. coli* in the water had increased 900 fold, from 1×10^3/l to 9×10^5/l, and that of enterococci 150 fold, from 2×10^2/l to 3×10^4/l. Concentrations of both types of bacteria then steadily declined, until the experiment was stopped because the flow rate of the discharge increased markedly following heavy rain. By this time the concentrations of faecal bacteria in the discharge were only about 5 to 10 times the concentrations that would be expected in the absence of excrement application, after allowance for the increase in flow rate.

Experiment II. The results obtained in the second experiment are shown in Fig. 19. On this occasion the excrement was applied to the land in two periods separated by a 1 h interval, instead of in a single period as in Experiment I, and the flow rate of the discharge was higher. Since the flow rate of the discharge declined during the period of the experiment and was apparently unaffected by the application of excrement, it is clear that the volume of excrement applied must have been small relative to the volume of water held in the soil.

Within 2 h of the start of excrement application, the concentration of E. coli in the discharge had increased 60 fold, from 8×10^2/l to 5×10^4/l, and that of enterococci 20 fold, from 1.5×10^2/l to 3×10^3/l. The concentrations of bacteria in the drain discharge then declined before increasing again approximately 3 h after the start of the second period of excrement application. The concentrations then declined until at the end of the experiment they were practically at the levels existing before excrement was applied.

Chemical pollutants in the drain discharge

Throughout the winter of 1968/9 the samples of drain discharge water were generally clear and colourless. The pH was near neutrality, the biochemical oxygen demand was generally less than 3 mg/l, the suspended solids concentration less than 5 mg/l, and therefore not usually detectable by standard methods. The nitrate concentration was generally less than 1 mg/l throughout September, October and November 1968. During December 1968, January and February 1969, it rose slightly to between 1 and 2 mg/l.

During the winter of 1969/70 the water samples were again clear and colourless. However, the biochemical oxygen demand was slightly higher than during the previous winter, varying between 0.4 and 10 mg/l, but the suspended solids concentration was again generally less than 5 mg/l. The nitrate concentration was also slightly higher than during the previous winter. The average nitrate concentration in November 1969 was 2.8 mg/l and between December 1969 until March 1970 was 1.6 mg/l.

No significant changes in the biochemical oxygen demand, suspended solids concentration, or nitrate concentration were detected in samples of land drainage water collected after the application of pig excrement to the pasture. In Experiment I (October 1970), after the application of excrement to the pasture, the first water samples were slightly turbid and pale brown in colour but later samples were clear and colourless. The biochemical oxygen demand of the discharge increased slightly, reaching a maximum of 14 mg/l, 1 h after the start of excrement application, while the suspended solids concentration and nitrate concentration were unaffected. The pH value increased by up to half a unit for about 24 h following the excrement application.

During Experiment II (November 1970) the biochemical oxygen demand, suspended solids concentration and nitrate concentration remained at their 'normal' low levels. In this experiment all the water samples were clear and colourless and the pH value of the discharge increased by only 0.1 unit for about 8 h following the start of excrement application.

PART B. SOIL BACTERIA IN LAND DRAINAGE WATER

INTRODUCTION

In the preliminary examination of land drainage water for bacterial pollution it was noted that apart from faecal bacteria the water also contained relatively high numbers of bacteria, presumed to be normal soil inhabitants (Fig. 14). The presence of these bacteria in the drainage water raised the possibility that wash-out of natural soil bacteria in drainage water could represent a significant loss from the soil bacterial flora. In addition, it was of interest to confirm that the concentrations of normal soil bacteria in the drainage water varied with the flow rate of the drain discharge in a similar manner to that observed for faecal bacteria deposited onto the surface of the land.

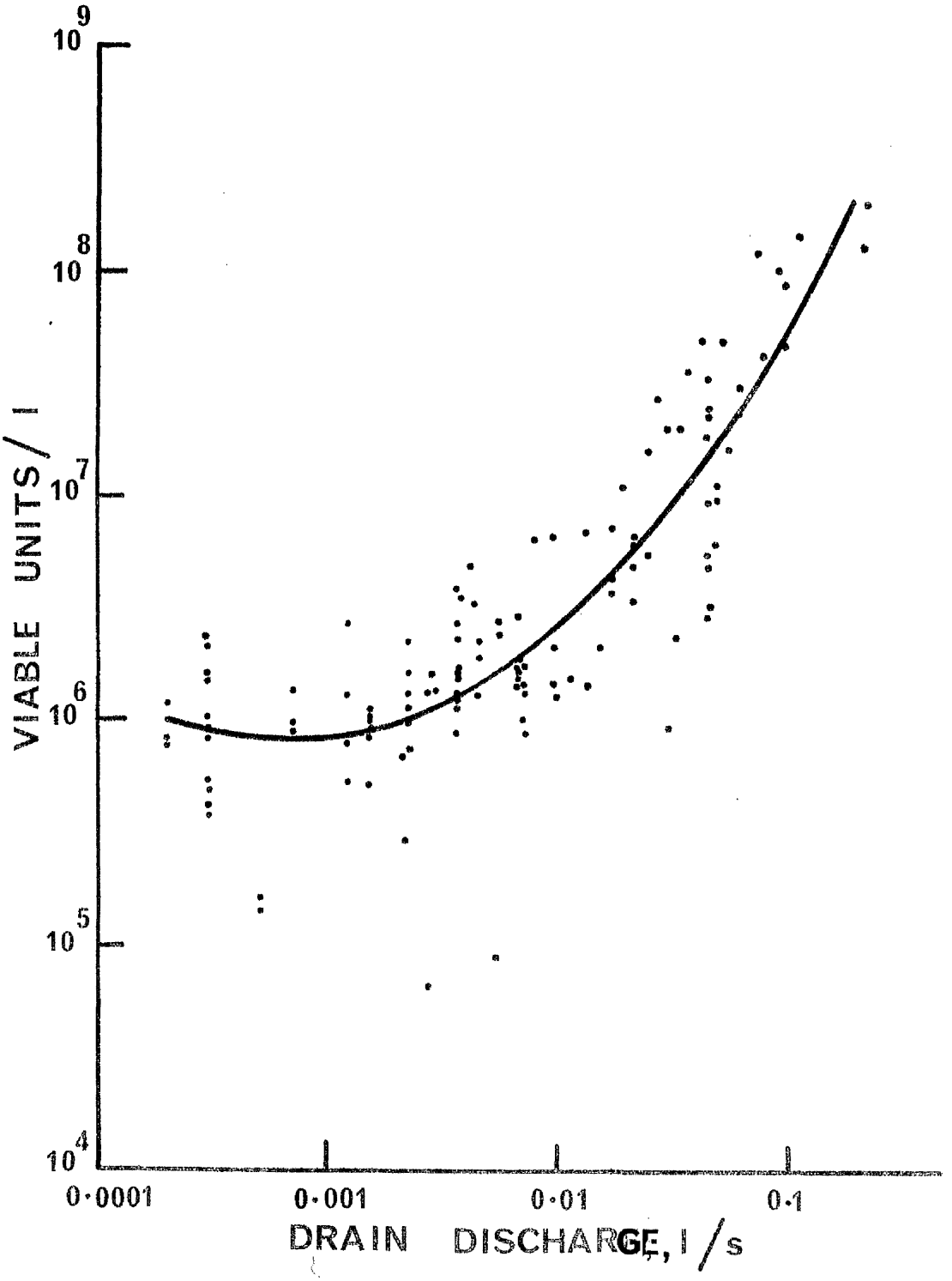
To investigate these questions a viable count of bacteria in the discharge from the drain was monitored during the winter 1971/2 and the total number of bacteria discharged was compared with an estimate of the number of bacteria in the soil of the drained area. No attempt was made to obtain maximum counts of bacteria. Only those bacteria able to produce colonies on m-plate count broth incubated at $20^{o}C$ for 48 h were counted and it was assumed that the results for this group of bacteria are representative of those that would be obtained for other groups of soil bacteria.

The experimental pasture was ploughed and reseeded with clover in March 1971. No animal excrement was applied to the pasture between the time of reseeding and the completion of this experimental period.

FIGURE 20

Relationship between the concentration of viable soil bacteria in drainage water and the flow rate of the drain discharge.

FIGURE 20


VIABLE UNITS / l
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DRAIN DISCHARGE, l / s

No viable E. coli or enterococci were detected in 1 l samples of drainage water collected in November 1971. Records of daily soil temperatures, at 10 and 100 cm depth, were obtained from the Auchincruive Meteorological Station.

Because the earlier results showed that the bacterial concentration in drainage water was greatly affected by the flow rate, spot samples of water were collected rather than composite 24 h samples. Usually two samples were collected 5 days each week and a total of 134 samples were obtained between 17 November 1971 and 24 March 1972. The flow rate of the discharge was continuously monitored and also recorded at the time of obtaining each sample.

To obtain an estimate of the number of bacteria occurring in the soil and able to grow on m-plate count broth, soil samples were collected at three different depths from each of five areas of the experimental pasture. The depths sampled were 0-10, 10-20, and 20-30 cm and each area sampled was a composite sample from five sites within the area. The number of viable bacteria capable of growing on m-plate count broth at 20°C in 48 h in each soil sample was then estimated.

RESULTS

Relationship of concentration of bacteria in the drain discharge to flow rate of the discharge

The results are summarised in Fig. 20, from which it is evident that the concentration of bacteria in the drainage water increased as the flow rate of the discharge increased. Regression analysis showed that the log concentration of bacteria could be related to the log flow rate by an equation of the form

$$Y = a + bX + dX^2,$$

where $Y = \log_{10}$ concentration of bacteria in the discharge, viable units/l

$X = \log_{10}$ flow rate of the discharge, l/s

and a, b, and d are constants. The equation giving the best fit was

$$Y = 9.70 + 2.356\ (\pm\ 0.187)X + 0.365\ (\pm\ 0.040)X^2 \quad \ldots\ldots\ 4$$

the figures in parentheses being the standard errors of the regression coefficients.

This equation explains 79 per cent of the variation observed in the concentration of viable bacteria in the discharge. No improvement was obtained by including in the equation a time factor or a third degree term. The soil temperatures showed some positive association with flow, with a slight advantage for the soil temperature at 100 cm depth, but after allowing for this association, soil temperature showed no significant association with the concentration of viable bacteria in the drain discharge.

Comparison of total number of bacteria discharged in drainage water with total number in the soil of the experimental pasture

The total number of bacteria discharged in the drainage water during the experimental period was calculated from the continuous records of the flow rate of drainage water using the regression equation given above. During the period of the experiment 3.6×10^5 l of water containing 1.2×10^{14} viable bacteria were discharged from the drain.

TABLE 20

Numbers of viable bacteria in the soil of different areas of the experimental pasture, Brickrow.

* Concentrations of bacteria growing on m-plate count agar at $20^{o}C$ in 48 h.

TABLE 20

Sampling area	Number of bacteria*, viable units $X10^6/g$ Dry weight in soil horizon		
	0–10 cm	10–20 cm	20–30 cm
A	23	23	7.2
B	22	18	5.3
C	23	14	3.8
D	22	12	4.0
E	33	16	5.3
Meau	24.6	16.6	5.1

The numbers of bacteria in the soil are shown in Table 20. It can be seen that the numbers are similar in each of the different areas sampled and that the numbers at a depth of 20–30 cm were considerably lower than those at 0–10 cm. If it is assumed that the numbers of bacteria in the soil are, in fact, uniform over the area of the experimental pasture (0.7 ha) and that the numbers of bacteria at depths greater than 30 cm can be neglected, then the total number of viable bacteria in the experimental pasture is estimated to be of the order of 10^{17}.

Thus the total number of viable bacteria in the soil able to grow on the medium used was about 1,000 times the number discharged in the drainage water over a period of four months.

PART C. THE CULBAE EXPERIMENT

INTRODUCTION

Culbae farm was situated at the top of the water catchment area, and the soil was very free draining. The disposal of 81 m^3 of pig excrement each day through rain-guns onto the fields surrounding the farmyard caused a considerable smell problem and also polluted the open ditches draining the farm.

An investigation was carried out to determine if the factors affecting the concentrations of bacteria and of chemical pollutants in the drainage water were similar to those observed for the experimental pasture at Brickrow. Bacterial and chemical pollution of the drainage water in open ditches draining two 20 ha areas of Culbae, areas A and B shown in Fig. 10, were measured. During this period, spraying of pig excrement was confined to those fields which did not drain into the two sites.

In January 1973, 160 m^3 pig excrement was sprayed onto the Longcastle field in area A, over a period of eight hours. The position of the rain-guns on the field was altered every 15 min throughout the day. The following day the rain-guns were moved away from area A. Samples of drainage water were collected from the open ditch about 300 m (a^1, Fig. 10) downstream from the Longcastle field, at various intervals during the day, and the following 40 h.

The dissolved oxygen tension and temperature of the water in the ditch at the sampling point were monitored throughout the experiment. Although a continuous flow recorder was installed at the V-notch during the experiment, flooding at the eastern end of the Standing Stone field prevented accurate measurements being obtained

FIGURE 21

Relationship between the concentration of viable bacteria in drainage water and drainage rate of area A.

FIGURE 21

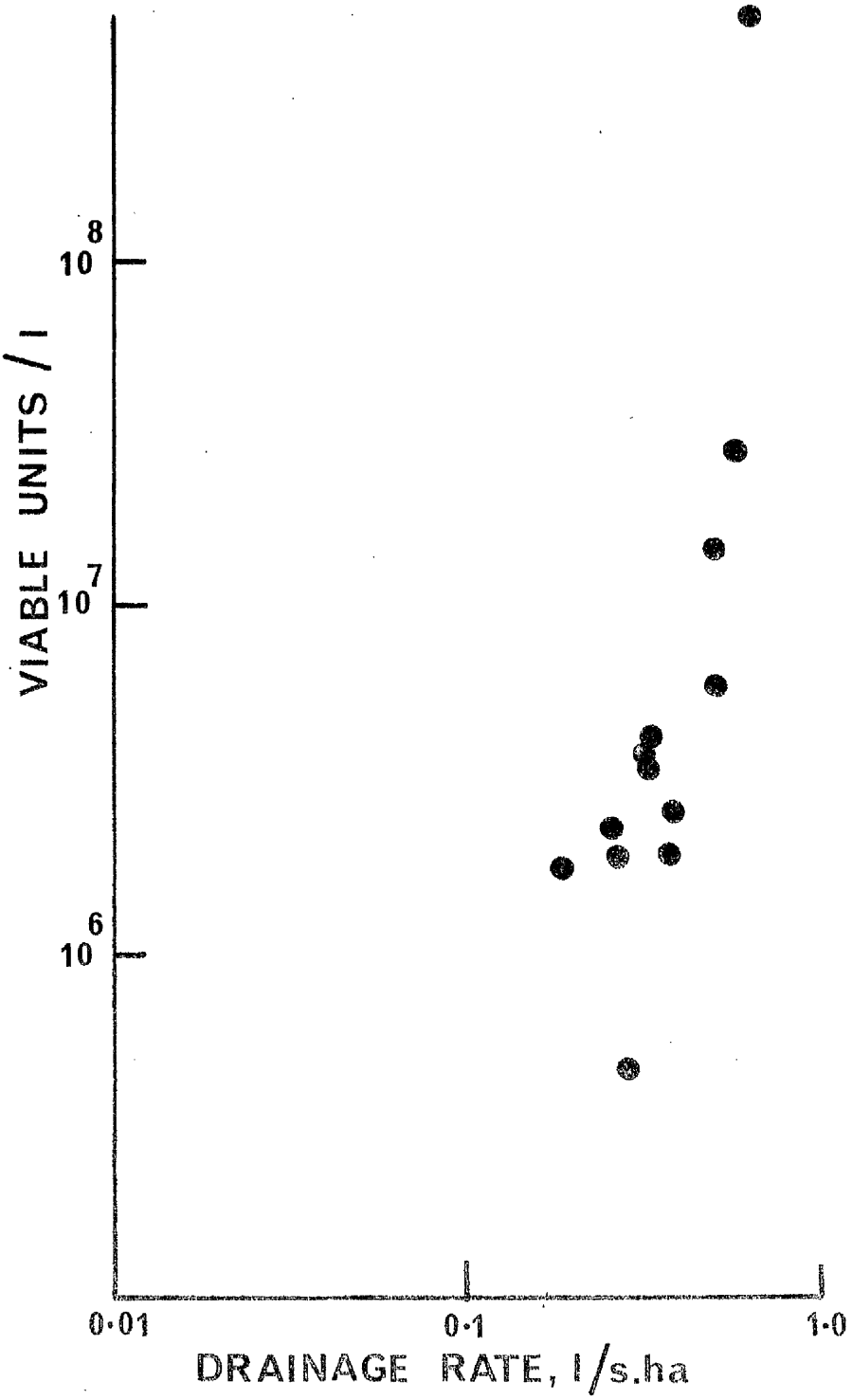


FIGURE 22

Relationship between the concentration of faecal bacteria in drainage water and drainage rate of area A.

▲ - concentration of Escherichia coli.

✚ - concentration of enterococci.

FIGURE 22

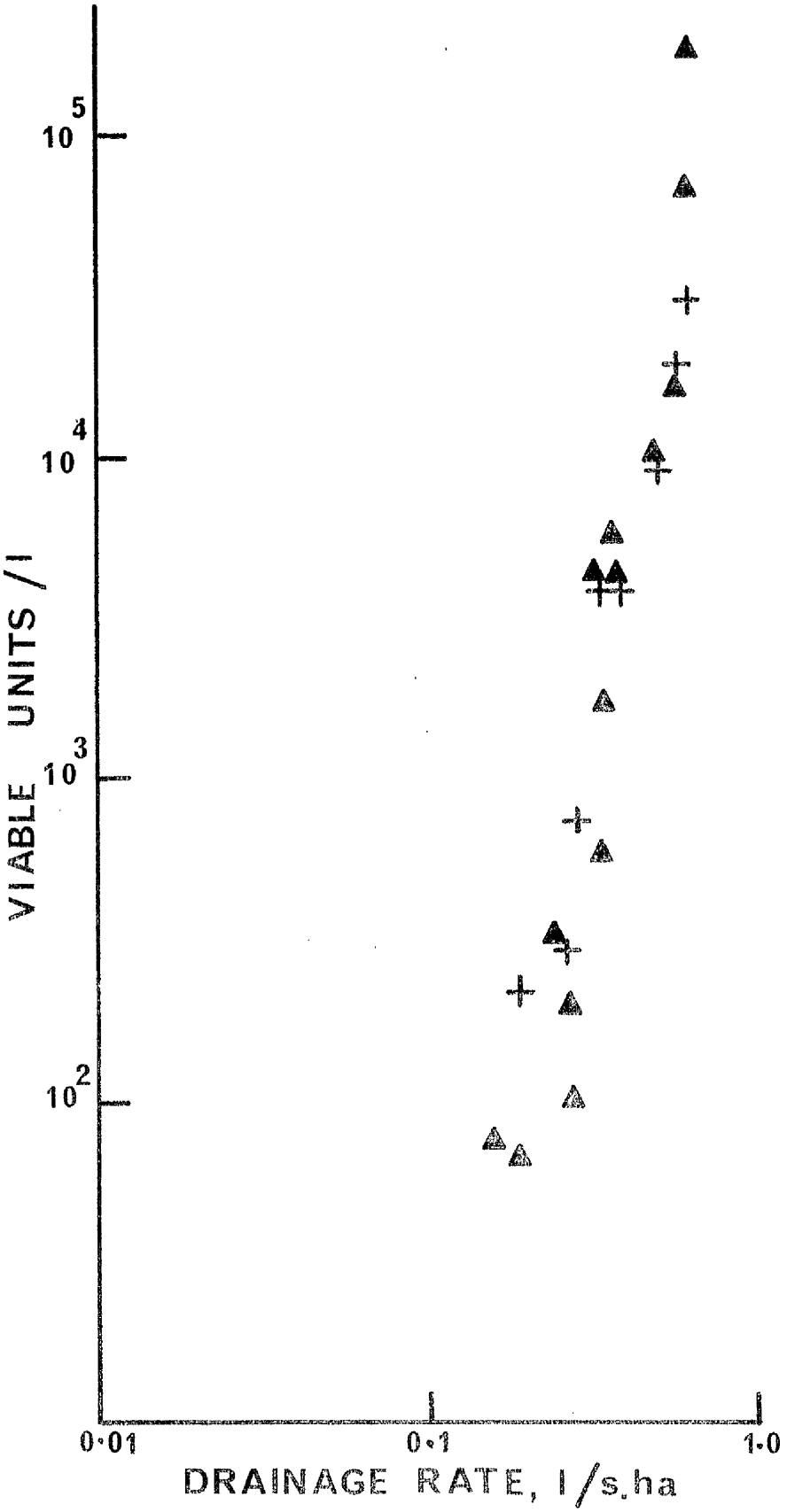


FIGURE 23

Relationship between the concentration of viable bacteria in drainage water and drainage rate of area B.

FIGURE 23

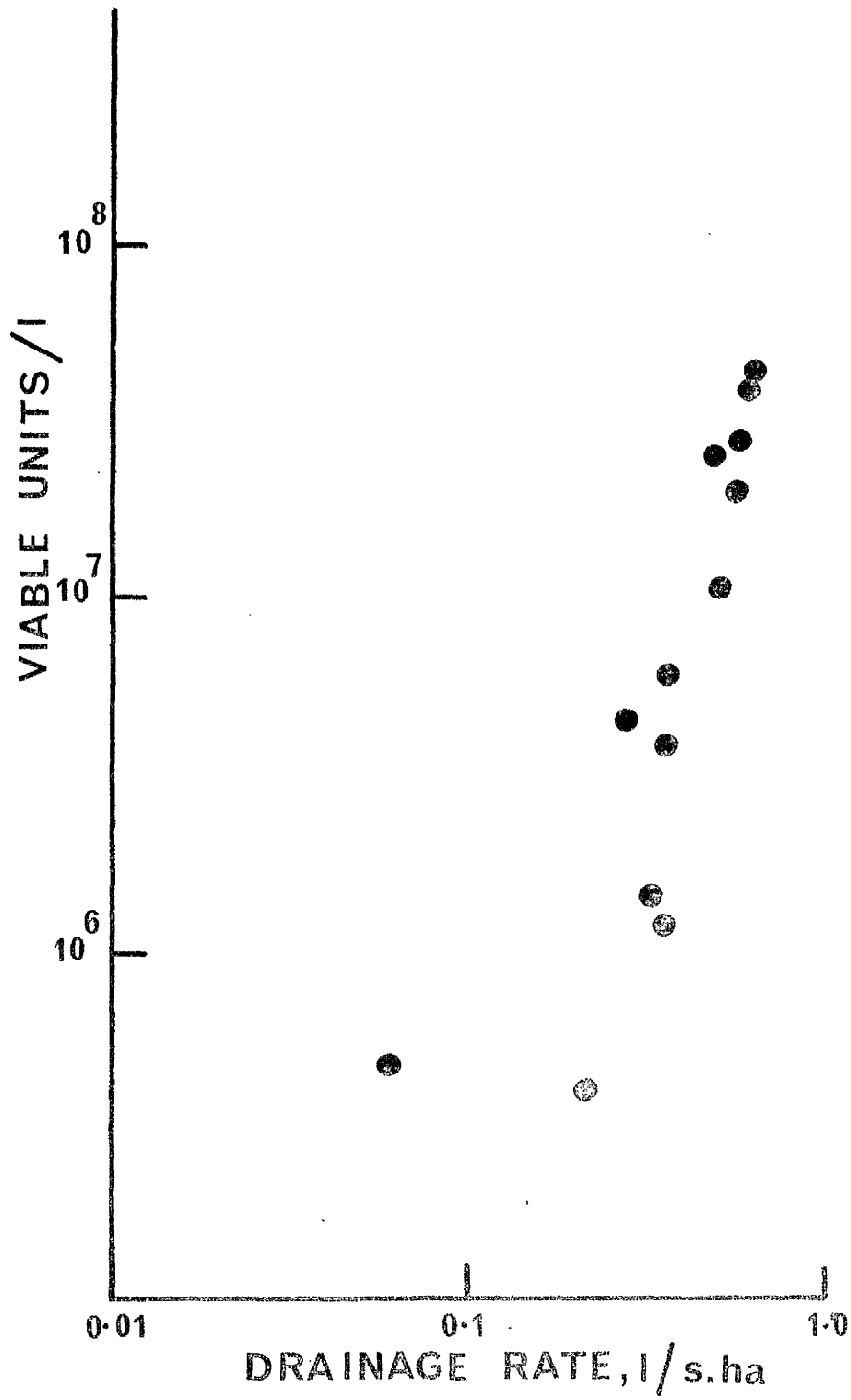


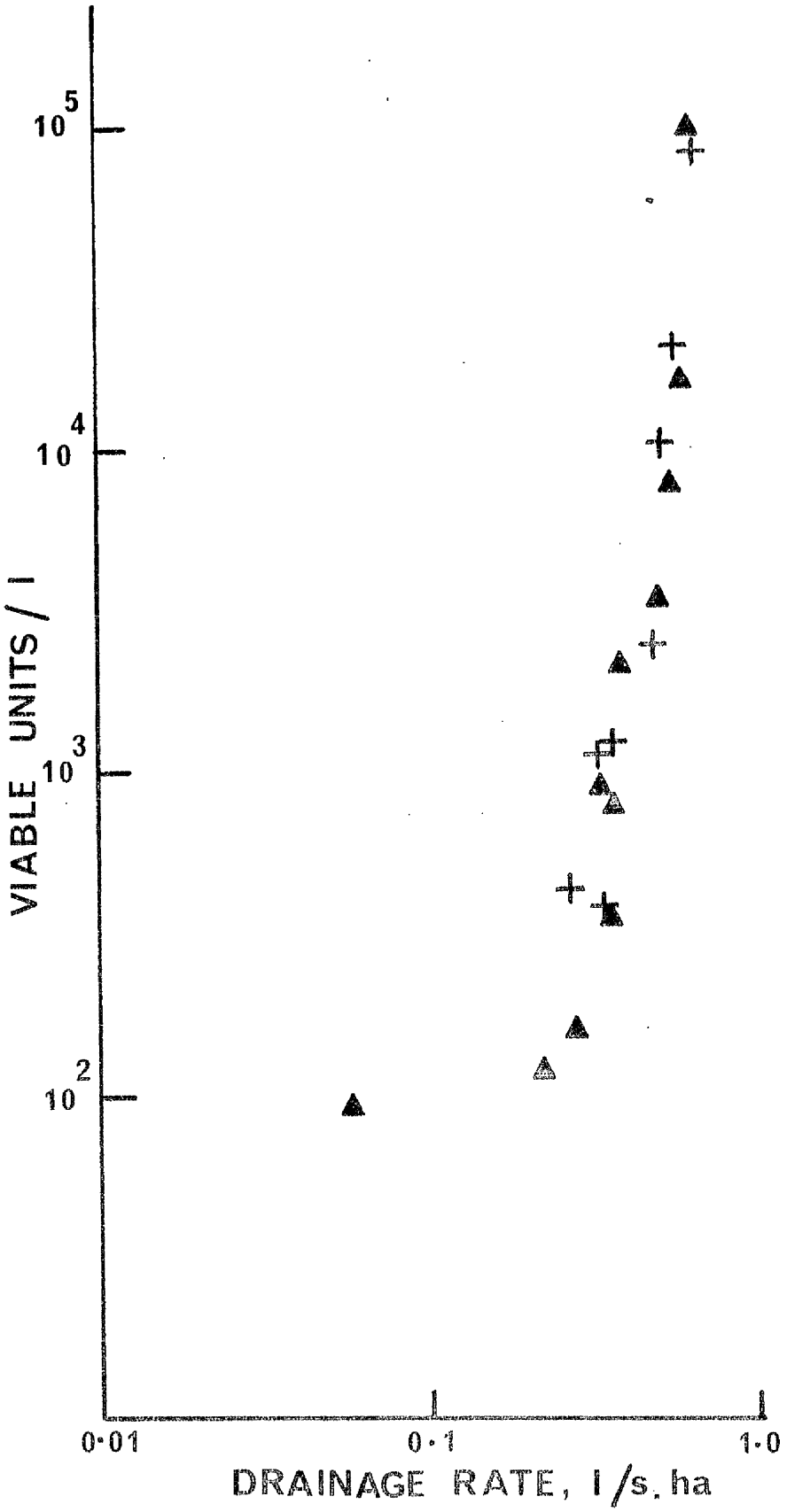
FIGURE 24

Relationship between the concentration of faecal bacteria in drainage water and drainage rate of area B.

▲ - concentration of Escherichia coli.

✚ - concentration of enterococci.

FIGURE 24



of the drainage rate for the area. The water samples were analysed bacteriologically within six hours of collection and chemically within twenty-four hours.

RESULTS

Pig excrement in the storage reservoir at Culbae contained approximately 6.1×10^7 viable Escherichia coli per l, 4.8×10^6 viable enterococci per l and 9.5×10^8 viable bacteria per l growing on m-plate count broth at 20^0C in 48 h.

E. coli and enterococci in samples of drainage water were presumed to originate mainly from the pig excrement sprayed onto the fields. Bacteria isolated on m-plate count broth from drainage water were presumed to originate from both the excrement sprayed onto the fields and from the natural bacterial population in the soil (cf. soil bacteria counts on m-plate count broth from the drainage water at Brickrow during winter 1971/2).

Relationship of concentration of bacteria in drainage water to drainage rate

A positive relationship between the concentrations of bacteria in the drainage water and the drainage rate of area A is shown in Figures 21 and 22. A similar relationship between the concentrations of bacteria and drainage rate in area B is shown in Figures 23 and 24 (actual concentrations of bacteria, chemical pollutants, drainage rates, rainfall and atmospheric temperatures are given in the Appendix Tables).

FIGURE 25

Bacterial pollution of drainage water of area A after application of pig excrement to Longcastle field (160 m^3/ha).

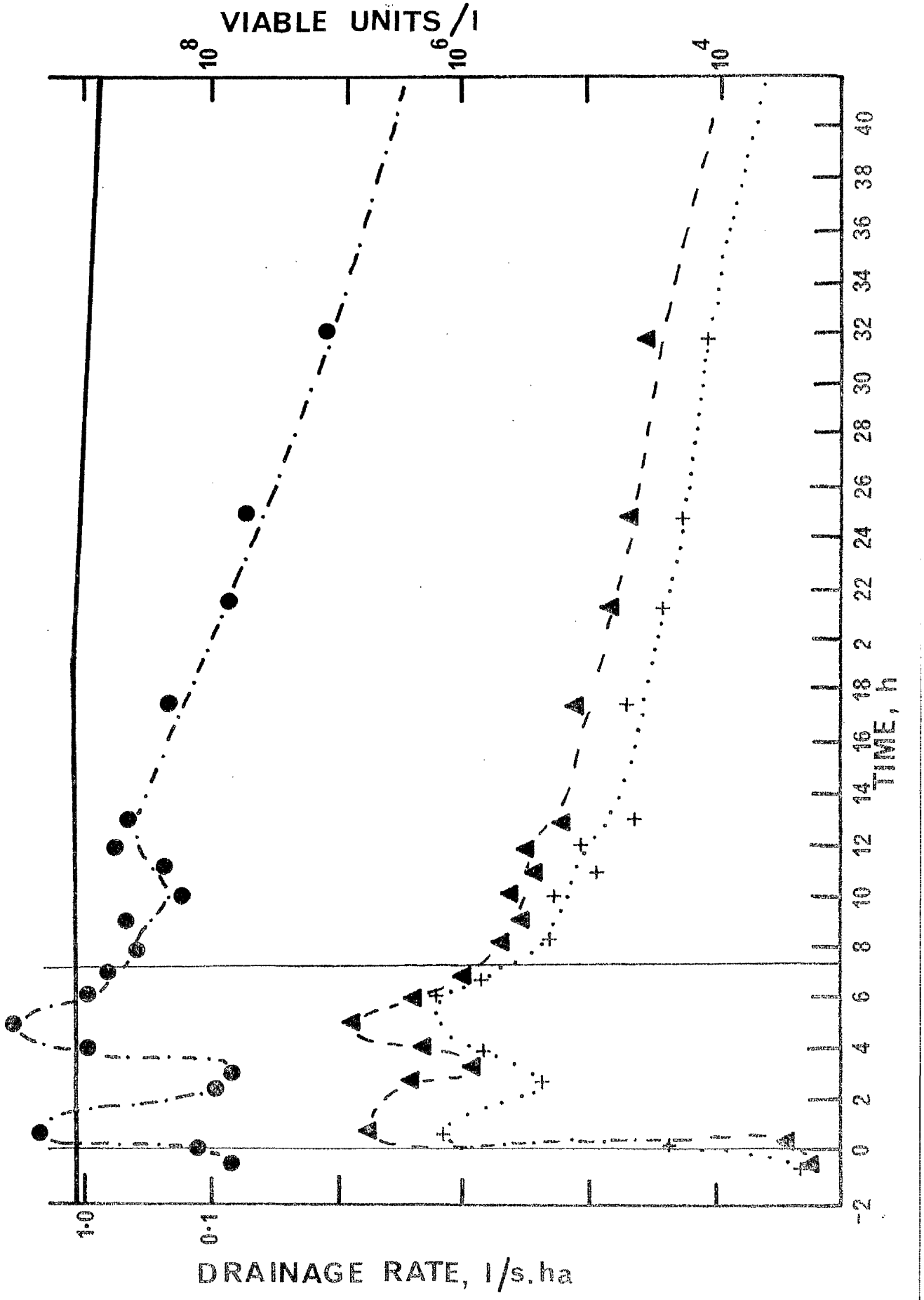
Continuous line – drainage rate

—·●·—·—·●·— – viable count on m-plate count broth at 20°C

– –▲– – –▲– – concentration of Escherichia coli.

···+·····+·· – concentration of enterococci.

FIGURE 25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4
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0
-2
TIME, h
1·0
0·1
DRAINAGE RATE, l/s.ha

Although there is insufficient data for statistical analysis, and only a few samples at low flow rates have been examined, it appears possible that the relationship between the concentrations of the three groups of bacteria, Escherichia coli, enterococci and bacteria capable of growing on m-plate count broth at 20°C in 48 h, and the drainage rate is non-linear. If this is so, then the relationship is similar to that observed between the concentrations of soil bacteria and drainage rate at the experimental pasture, Brickrow.

Effect of slurry application on the bacterial pollution of drainage water

The effects of spraying 160 m^3 of slurry from the piggery at Culbae (approximate composition: 9 g biochemical oxygen demand per litre; 9 g suspended solids per litre; pH value 7.7) onto the surface of the Longcastle field, over a period of seven hours, on the numbers of bacteria in the drainage water are shown in Fig. 25. Within 45 min of starting spraying, the concentrations of Escherichia coli in the water increased more than 300-fold, from 1.7×10^3 to 5.4×10^6 per litre, that of enterococci about 3,000-fold, from 5.2×10^2 to 1.5×10^6 per litre and that of bacteria growing on m-plate count broth at 20°C 300-fold from 6.8×10^7 to 2.3×10^9 per litre. The concentrations of all three groups of bacteria declined slightly during the next hour, but increased again to a second peak during the following two hours. After spraying was stopped, the concentrations of all three groups of bacteria steadily declined. Within 37 h of stopping spraying the concentrations of bacteria had returned to levels similar to those existing before the experiment started.

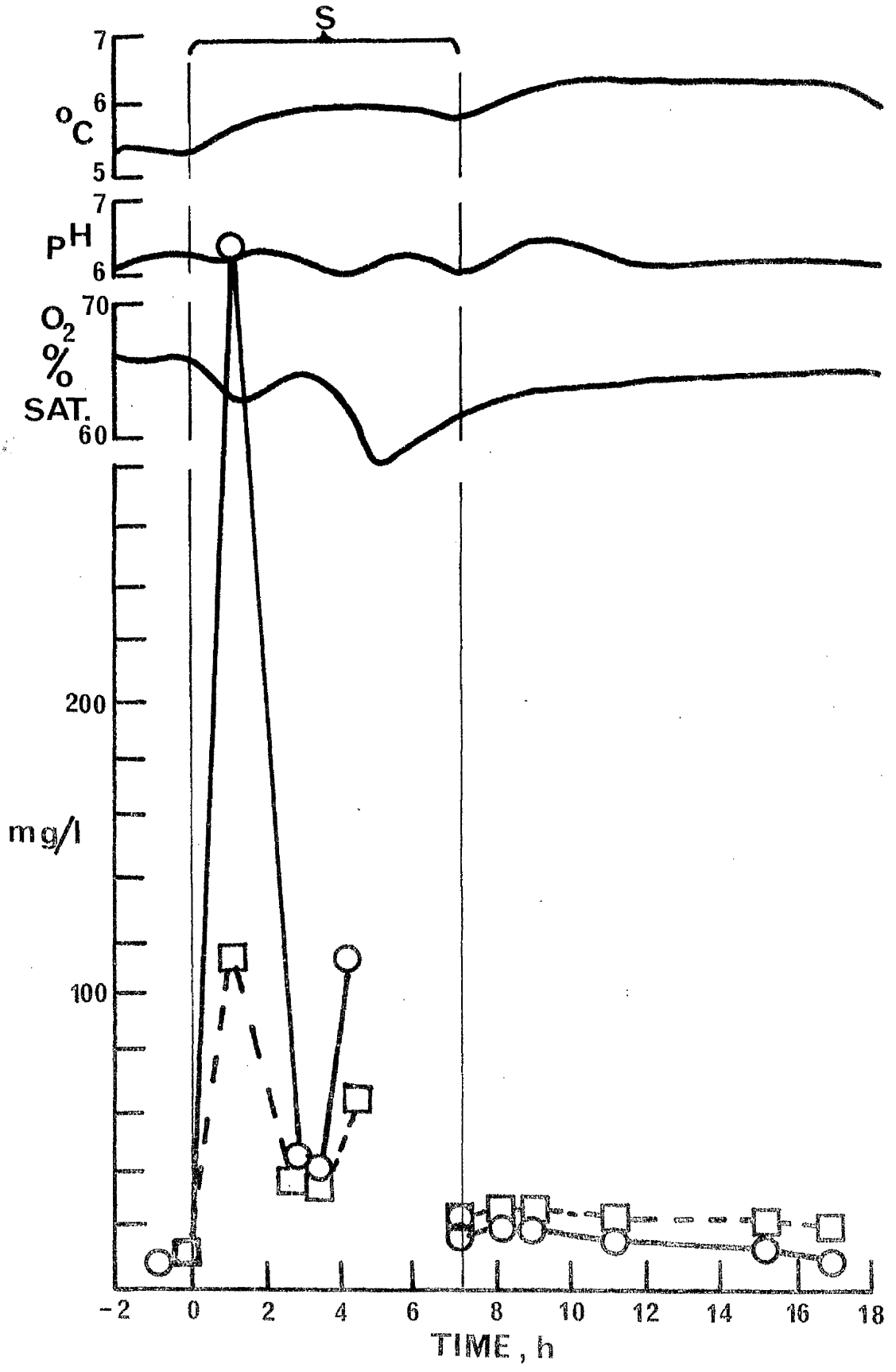
FIGURE 26

Chemical pollution of drainage water of area A after application of pig excrement to Longcastle field (160 m^3/ha).

○ - biochemical oxygen demand.

□ - suspended solids concentration.

FIGURE 26



The position of the rain-guns on the field was altered every 15 min. Drainage towards the western boundary of the field for part of the time could account for the decrease in the bacterial pollution of the drainage water in the middle of the spraying period.

The flow rate of the drainage water over the V-notch of area A (Fig. 25) during the experiment was not an accurate measurement of the drainage rate of the whole catchment area. Melting snow which had fallen two days earlier caused flooding at the north-east end of the Standing Stone field. Nevertheless the flow rate declined during the whole of the experimental period and this, together with figures for precipitation the day before, and during the experiment, suggests that the volume of slurry sprayed (160 m^3) onto the Longcastle field (2.3 ha) was not sufficient to significantly affect the drainage rate. The volume of slurry sprayed was only equivalent to 1.3 mm of precipitation over the 12 ha of catchment area above sampling point a[1].

Chemical pollutants in the drainage water

The concentrations of 5 day biochemical oxygen demand and suspended solids in the drainage water in the absence of recent slurry spraying onto either site, were generally less than 8 mg per litre and 23 mg per litre respectively.

Within 45 min of the start of slurry spraying onto the surface of the Longcastle field, there was a 360-fold increase in BOD, from 1 mg to 360 mg per litre and a 10-fold increase in suspended solids concentration, from 11 mg to 117 mg per litre (Fig. 26). These peaks were followed during the next hour by a decline of BOD to 39 mg per litre,

and suspended solids, to 35 mg per litre. Second peaks for both BOD and suspended solids concentrations probably occurred at the same time as the second peaks of bacterial concentrations, but because of the loss of three samples they were not detected. This assumption is also supported by changes in the per cent saturation of dissolved oxygen in the drainage water at the sampling site (Fig. 26).

Within 3.5 hours of stopping spraying, the per cent saturation of dissolved oxygen, the BOD and suspended solids concentrations had all returned to their original levels. There were small changes in the pH values and temperature of the drainage water as a result of slurry spraying. These are also shown in Figure 26.

DISCUSSION

These studies show that sub-surface land drainage water can contain appreciable numbers of soil and faecal bacteria, and that the concentrations of bacteria in the water are related to the flow rate of the drain discharge. The concentration of faecal bacteria are also related to the time since application of animal excrement to the land. Since the faecal bacteria in the drainage water of agricultural land usually originate either from faeces deposited on the land by grazing animals, or from excrement sprayed onto the land, it is reasonable to suppose that changes in the concentrations of faecal bacteria in the drainage water also reflect changes in the numbers or conditions of bacteria in or on the soil and vegetation. Thus the results can be interpreted as indicating that the concentration of bacteria in drainage water from farmland may be affected by two main factors: (i) the flow rate of water through the soil; and (ii) the number of bacteria in or on the soil and vegetation. The numbers of faecal bacteria are also affected by the application to the land of large volumes of semi-liquid animal excrement over short periods of time. Other factors are also important. It is evident, for instance, that the soil type exerts some influence.

Relationship of concentration of bacteria to drainage rate

The results from the experimental pasture, Brickrow, show that, in the absence of recent application of large volumes of excrement to the land, the concentrations of faecal bacteria in the drain discharge are related to the flow rate of the discharge by an equation of the form:

log bacterial concentration = a + b log flow rate - c days.

If the time factor (c days) in the equation is ignored it is seen that the relationship between bacterial concentration and flow rate is described by a linear log-log equation having a slope defined by regression coefficient b. The values of coefficient b show that for a ten-fold increase in flow rate the concentration of Escherichia coli in the drain discharge increased 3.3 fold, while that of enterococci increased 5.0 fold. Possibly, the value of this coefficient is related to the relative ease or difficulty with which bacteria attached to soil particles can be liberated into water percolating through the soil. If so, then the value of the coefficient would be expected to be related to soil type. Since the coefficient for E. coli is significantly different from that for enterococci, the intensity of the interaction between the bacterial cells and the soil particles must also depend on the nature of the particular bacterial cells.

The concentration of viable bacteria, presumed to be normal soil inhabitants, in the drainage water of the experimental pasture, Brickrow, was also closely associated with the flow rate of the drain discharge. Again, this may be interpreted as meaning that the rate at which bacteria adsorbed to soil particles become suspended in soil water is determined by the flow rate of water through the soil. Thus the higher the flow rate the higher the concentration of bacteria in suspension and the higher the concentration in sub-surface drainage water. At flow rates below about 0.001 l/s the regression curve for soil bacteria (Fig. 20) suggests that the concentration of bacteria should increase with decreasing flow rate. Future work may confirm this suggestion, or may show it to be an artefact arising out of an insufficiency of data at low flow rates.

The concentration of faecal bacteria in drainage water was related not only to the flow rate of the discharge but also to time, and the occurrence of this relationship may be attributed to the numbers of viable faecal bacteria in the soil declining with the passage of time. The numbers of viable soil bacteria in the discharge were not related to time. This suggests that the numbers of viable bacteria in the soil did not change significantly during the winter. Perhaps this is to be expected since they are presumed to be normal soil inhabitants.

At the experimental pasture, Brickrow, the concentration of soil bacteria and the flow rate of the drain discharge were best related by an equation of the form $Y = a + bX + dX^2$ (Y = log bacterial concentration: X = log flow rate) whereas the data on the concentration of faecal bacteria in the drain discharge was best related by an equation of the form $Y = a + bX$, if the time relationship was ignored. These different equations probably do not reflect any real differences in the behaviour of the two groups of bacteria. It is possible that if the water samples in the earlier studies had been collected in a few minutes instead of over 24 h and more samples examined, then the relationship of log concentration of faecal bacteria to log flow rate might also have been non-linear.

To facilitate the comparison of the results on soil bacteria with those on faecal bacteria, a linear log-log regression curve was fitted to the soil bacteria results. This equation was

$$Y = 8.01 + 0.689\ (\pm\ 0.043)X \quad \ldots\ldots\ldots\ldots \quad 5$$

and it explains 66 per cent of the variation in Y. It is noteworthy, particularly since the faecal bacteria study was done three years

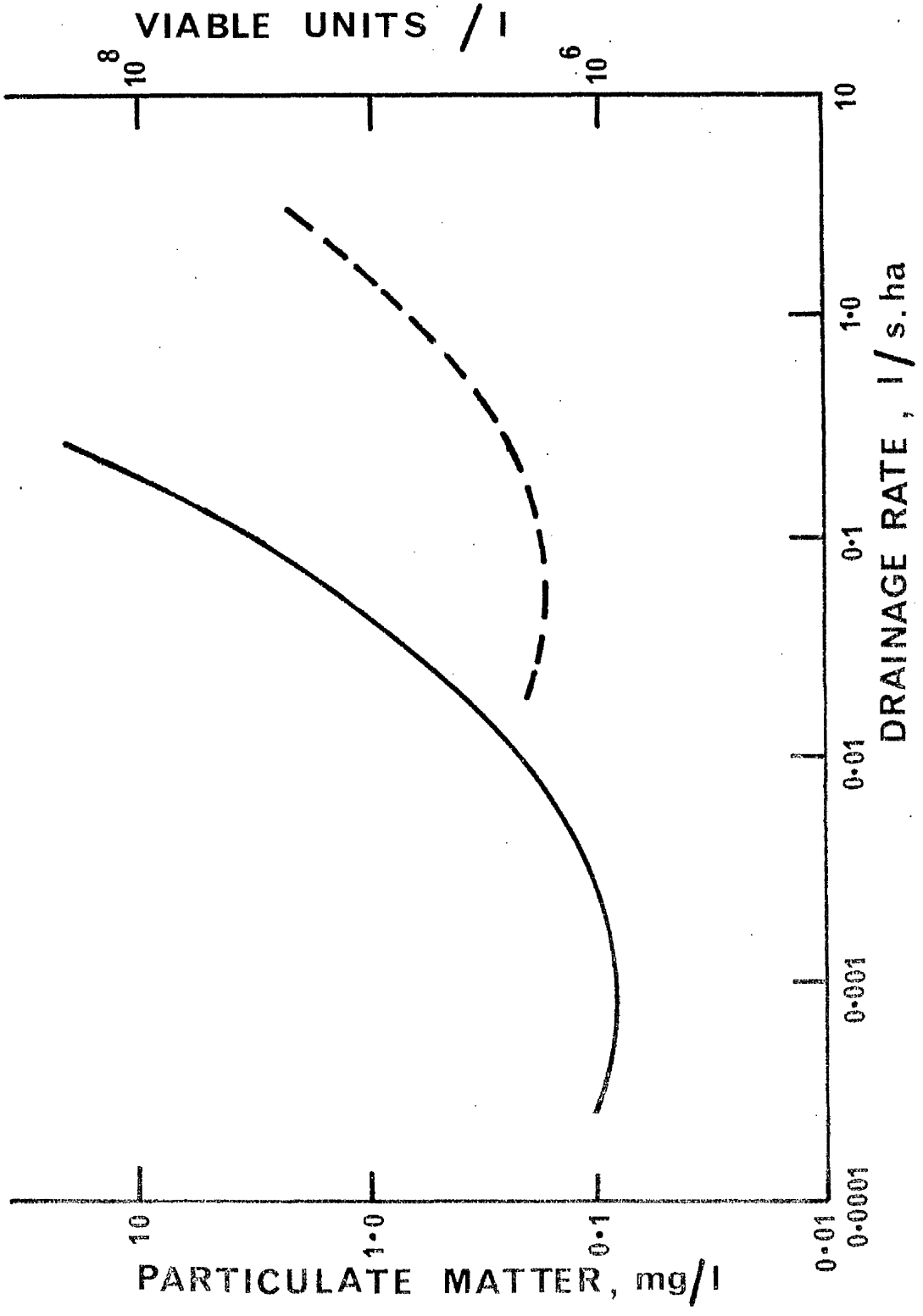
FIGURE 27

Relationship between concentration of particles in drainage water and drainage rate.

Continuous line - relationship between concentration of soil bacteria and drainage rate at the experimental pasture, Brickrow.

Dashed line - relationship between concentration of particulate matter (0.45 μm) and drainage rate of a forest in New Hampshire (data of Bormann, Likens and Eaton, 1969).

FIGURE 27


VIABLE UNITS / l
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10
1·0
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0·01
0·001
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DRAINAGE RATE, l / s.ha
10
1·0
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0·01
PARTICULATE MATTER, mg/l

earlier than the soil bacteria study, that the regression coefficient on X is not significantly different from those observed for E. coli (0.521, Equation 1) and enterococci (0.701), but it is appreciably higher than that for E. coli,(Equation 2). This would appear to support the previous suggestions that the value of the regression coefficient is dependent to some extent on the nature of particular bacterial cells and to soil type.

The fact that the soil bacteria data best fitted a curve described by a quadratic equation is interesting because Bormann, Likens and Eaton (1969) obtained a similar equation relating concentration of particulate matter (i.e. material passing through a 1 mm mesh net and retained on Millipore filters, pore size 0.45 μm) in a stream and the flow rate of the stream. To compare the two equations they were both expressed in terms of drainage rate (l/s ha) rather than flow rate (l/s), because it seems likely that it is the rate of movement of water through soil that is important, rather than the actual flow rate of the drain or stream. The equation for the relationship between the concentration of soil bacteria in drainage water and drainage rate at Brickrow was

$$Y = 9.34 + 2.243X + 0.365X^2$$

and that for the concentration of particles in a stream and drainage rate (Bormann et al) was

$$Y = -0.33 + 0.803X + 0.328X^2$$

The two curves obtained from these equations are plotted in Figure 27. As the results for viable soil bacteria refer to bacteria in drainage water from 0.7 ha of farm land and the results of Bormann et al refer to particulate matter, presumably including inanimate material and viable bacteria, in a stream draining 13.23 ha of forest in New Hampshire.

the degree of similarity in the shape of the curves is surprising. However, both curves relate mainly to sub-surface drainage water. This raises the possibility that the relationship observed between the concentration of small particles in sub-surface drainage water and flow rate may be a general one, at least over the range of drainage rates investigated. That this relationship is different from the linear log-log relationship generally observed between suspended solids load and flow rate of streams and rivers (Hoak and Bramer, 1956; Leopold and Miller, 1956; Leopold, Wolman and Miller, 1964) can possibly be attributed to the different nature of the particles examined. Suspended solids in rivers and streams particularly at high flow rates, include particles that will sediment rapidly under quiescent conditions whereas the particulate matter examined by Bormann et al and the bacterial cells studied here sediment only very slowly.

Bormann et al (1969) suggested that the shape of the curve illustrated in Fig. 27 (i.e. the values of coefficients b and d) reflects both the capacity of water to do work as its velocity increases, and the relative ease or difficulty with which moving water of a given velocity can remove material from an ecosystem. This latter factor they called the erodibility of the ecosystem, a term that could also be used to describe the relative ease or difficulty with which bacteria adsorbed to soil particles are removed by moving water. The similarity of the value of constant d in both equations suggests that the capacity for water to do work as its velocity increases is the same in both cases. The lower value for constant b in the equation of Bormann et al, when compared with constant b in the equation for the concentration of soil bacteria in drainage water, suggests that the bacteria were more easily removed from the soil at Brickrow than the particulate matter was from the soil in New Hampshire. This difference may be

due to differences in the nature of bacteria and particulate matter, or it may be due to a number of other factors. Bormann et al suggested that the erodibility of a component of an ecosystem would be affected by factors such as the type of particle, soil type, the degree of aggregation of soil particles, and the type and amount of vegetation.

Examination of the concentrations of the soil and faecal bacteria in drainage water at the experimental farm, Culbae, in the absence of recent slurry application, also shows a positive relationship between the concentrations of all three groups of bacteria and drainage rate (Figs. 21 - 24). However, the rate of increase in the concentration of bacteria with an increase in drainage rate was higher than the corresponding values obtained at Brickrow. This suggests that bacteria were more easily removed from the soil at Culbae, than from the soil at Brickrow, and may reflect the differences in soil type. At Brickrow the soil was imperfectly drained whereas at Culbae the soil was very free draining. Further estimations of bacterial concentrations in the drainage water at Culbae, in the absence of recent excrement application, particularly at low flow rates, between 0.001 and 0.1 litres per second, may confirm these suggestions.

Apart from a paper by Leninger and McClesky (1953) there is little information available on the relationship between bacterial concentration and the flow rate of rivers and streams. These authors did not observe a constant relationship between faecal bacterial concentrations and rainfall: in some instances, bacterial concentrations were higher at times of high rainfall than at times of low rainfall, and in others the reverse was true. Robbins, Howells and Kriz (1972) studied the runoff from a variety of water catchment areas. Regression analysis revealed high correlations between total organic carbon concentration

in runoff water and a variety of other pollution indices, including faecal coliform concentration. Multiple regression analyses with numbers of animals, flow rate, and temperature as independent variables and the pollution indices as dependent variables did not yield significant equations for predicting stream pollution. They stated that "high coefficients of determination for some of the land runoff data showed promise that such equations may be developed from a more detailed and longer-term study, especially if prediction equations included effects of more hydrological variables", and also "The pollution indexes for all sites except NO_3 at [one site] increased substantially, with surface runoff paralleling the hydrographs except for slower recessions than for surface runoff. Changes in magnitude of the indexes were greater for higher rates of surface runoff except for bacterial indexes. The bacterial counts were higher during the May period even though the flow rate was less because of warmer temperatures." Apart from the ambiguity of some of these statements the report does not make it clear whether or not actual or mean results of each variable were used in the regression analyses. It is not possible to decide whether or not their results confirm or contradict the results of this report, on the relationship between bacterial pollution of land drainage water and drainage rate. It is even doubtful if the report of Robbins, Howells and Kriz contributes any significant information to knowledge about factors affecting stream pollution with drainage water from agricultural land.

Soil bacterial flora and losses in drainage water

The bacteria, isolated from the drainage water of the pasture at Brickrow on m-plate count broth during the winter 1971-1972, are believed to be representatives of natural soil bacteria rather than of an intrinsic drain water flora or other habitat. The absence of Escherichia coli and enterococci in the water samples excluded the possibility that they originated from faecal material. It seems likely that some soil bacteria would find their way into drainage water, since the earlier results show that faecal bacteria deposited initially on the surface of the land appear in drainage water. Furthermore, the short time interval between the application of large volumes of animal excrement to the land and the appearance of high concentrations of faecal bacteria in the drainage water indicated that the mean residence time of water in the drain is too short to allow much growth of bacteria in the drains. However, the possibility that some of the bacteria in the water originated from the walls of the drains or from vegetation from the surface of the land cannot be excluded.

If it is accepted that most of the bacteria in the drainage water came from the soil, then the total number of viable soil bacteria in the drainage water discharged over a period of four months represented about 0.1 per cent of an estimate of the total number of viable bacteria of the same type in the soil of the experimental pasture. Since water does not usually flow from the drain for more than six months of the year, the maximum annual loss of bacteria from the soil by this route would only be 0.15 per cent of the total soil population. Even if the growth rate of bacteria in the soil is as low as the lowest estimates (Gray and Williams, 1971) this loss is an insignificant fraction of

the annual production of soil bacteria. This conclusion does not exclude the possibility that loss of bacteria by washout in drainage water could be important in some soil types or climatic conditions.

Effect of numbers of faecal bacteria in soil on concentrations in land drainage water

In the absence of further additions of faeces, the numbers of viable faecal bacteria in or on the soil and vegetation at any time must depend on: (i) the numbers originally deposited on the land; (ii) the rate at which viable organisms are lost from the land by death and/or washout. No attempt was made to determine the numbers of faecal bacteria in the soil. Therefore there is no direct information on the relationship between bacterial numbers in the soil and their concentrations in the drainage water.

The value of constant a in the regression equations of the relationship between the concentrations of faecal bacteria and the flow rate of the discharge at Brickrow, (i.e. $\log_{10}$ bacterial concentration when the flow rate is 1 l/s and z is zero days) would, in some manner, relate to the numbers of bacteria in the soil and vegetation. In this connection, it may be significant that the value of constant a is higher for Escherichia coli than for enterococci, as E. coli are generally more numerous in animal faeces than enterococci. The even higher values of constant a in the regression equation for soil bacteria also supports the assumption that constant a is related to the bacterial numbers in the soil and vegetation.

The time factor (c days) in the regression equation for faecal bacteria can then be interpreted as a correction factor for constant a, to take account of the decline in the numbers of viable faecal bacteria

in the soil with time. This decline could be due either to death and/or consumption by predators or to progressive washout from the soil, or to both. Again, since faecal bacteria in the soil were not counted, it is not possible to evaluate the relative importance of these factors.

The 90 per cent reduction times for bacteria in the drain discharge of the experimental pasture, Brickrow, (57 days for E. coli and 96 days for enterococci) are more than four times longer than those recorded by van Donsel, Geldreich and Clarke (1967) for E. coli and Streptococcus faecalis var. liquefaciens which had been inoculated into soil. These authors recorded maximum 90 per cent reduction times of 13.4 days for E. coli and 20.1 days for Str. faecalis. The different result may be due to the experimental method, or may be due to other factors, such as soil type. Further information on the concentrations of faecal bacteria in water draining from different soil types to that at Brickrow, such as Culbae, in the absence of recent excrement application, may help to evaluate this possibility.

The quantity of excrement applied annually to the land at Culbae, about 300 m^3 per hectare, was more than double the quantity applied annually to the experimental pasture, at Brickrow, between April 1968 and April 1971. The range of concentrations of E. coli was from 1.2×10^1 to $1.9 \times 10^5/l$, and enterococci from 8.0×10^1 to $1.5 \times 10^6/l$ at Culbae. It has already been suggested that, at Culbae, bacteria were more easily removed from the soil by moving water than at Brickrow. Unless the numbers of faecal bacteria lost from the soil by washout represent a large percentage of the numbers deposited on the soil, the range of concentrations of faecal bacteria in drainage water at Culbae, in the absence of recent excrement application, may be expected

to be higher than that at Brickrow. The fact that they are similar suggests that the rate of decline of viable cells in the soil is considerably greater at Culbae than at Brickrow. This may be due to increased washout of cells or other factors. Further examinations of drainage water samples and continuous measurement of drainage rate are necessary in order to estimate the annual loss of faecal bacteria from the soil at Culbae.

The mean annual precipitation between 1916 and 1950 in both Wigtownshire and the Auchincruive area was about 1,000 mm (Meteorological Office, 1971). Because of the free drainage properties of the soil at Culbae, the rate of discharge of precipitation through the soil into the drains is greater than that at Brickrow. Therefore, although the annual precipitation is similar in both areas, the washout of bacteria into drainage water may be greater at Culbae than at Brickrow.

It is also possible that the survival time for faecal bacteria in the soil at Culbae is shorter than that at Brickrow. Beard (1940), and van Donsel, Geldreich and Clarke (1967), suggested that the main factors which influence the survival of bacteria in different soils were pH values and moisture levels, rather than soil type. The drainage properties of the soil at Culbae make it likely that the bacteria remaining in the soil are more frequently subjected to low moisture levels than the bacteria in the soil at Brickrow and the pH value of the soil at Culbae (Table 14) was lower than that at Brickrow (Table 11). Since the numbers of faecal bacteria in the soils were not monitored these possibilities cannot be evaluated.

Effect of excrement application on the concentrations of faecal bacteria in drainage water

The application of large volumes of semi-liquid pig excrement to two different areas of grassland over short periods of time caused large increases in the concentrations of faecal bacteria in drainage water. In each experiment the volume of slurry applied to the land was insufficient to cause any detectable increase in drainage rate.

The different maximum concentrations of faecal bacteria obtained in each experiment were largely due to variation in the dilution by ground water. At Brickrow, the water samples examined comprised only water draining the area of pasture sprayed with excrement, but the drainage rate during experiment II was twice the drainage rate during experiment I. At Culbae, the water samples examined comprised water draining an area about six times the area of land sprayed with excrement, and the drainage rate during the experiment was about one to two times the drainage rate of that at the pasture at Brickrow during experiment II. Despite the increase in dilution, the maximum numbers of E. coli found in the drainage water at Culbae were about 117 times the maximum concentration reached in experiment II and 12 times that in experiment I. The time interval between starting to spray excrement onto the land and the increase in the numbers of faecal bacteria in the drainage water was also shorter at Culbae than at Brickrow. This shorter time interval, and the increased maximum concentrations of faecal bacteria, are probably due to the better drainage characteristics of the soil at Culbae.

The total numbers of E. coli discharged in the drainage water at Brickrow in the 36 h following the start of excrement application (experiments I and II) and a rough estimate of the total number applied

TABLE 21

Estimation of the fraction of Escherichia coli, applied to the pasture in pig excrement, that was discharged in drainage water within 36 h of the start of application to the pasture.

TABLE 21

	Experiment	
	I	II
Volume pig excrement applied to pasture (litres)	2×10^4	2×10^4
Number of *E. coli* in pig excrement	2×10^{13}	2×10^{13}
Water discharged during 36 h (litres)	2×10^4	4×10^4
E. coli discharged during 36 h	7×10^9	1×10^{10}
E. coli discharged/applied	1/3000	1/2000

to the land in the excrement, are shown in Table 21. Comparison of these figures suggests that the numbers of viable E. coli discharged in the drain represented about 1 in 3,000 in experiment I and 1 in 2,000 in experiment II, of those applied. Similar calculations of the numbers of faecal bacteria discharged into drainage water at Culbae were not possible, because of inaccuracies in the measurement of drainage rate. However, if the drainage rate is only assumed to be equal to that in experiment II and the concentration of faecal bacteria in the drainage water before spraying started also supports this assumption, then the numbers of viable E. coli discharged in the 36 h following the start of excrement application represented about 1 in 160 of those applied. This again may be due to the better drainage characteristics of the soil at Culbae, but because of the large volumes of excrement applied annually to the land, the numbers of viable organisms remaining in the soil would still be expected to be higher than the numbers in the soil at Brickrow.

Chemical pollutants in drainage water

In the absence of recent excrement application to the land, the concentrations of chemical pollutants in drainage water at Culbae were higher than those at Brickrow, but they were all usually below levels considered to be a pollution hazard.

When excrement was applied to the pasture at Brickrow, only small changes in the levels of chemical pollutants in the drainage water were detected. This relative lack of chemical pollutants is attributed to the combined effect of filtration and of adsorption of solid material

to the soil particles. At Culbae however, large increases in the levels of chemical pollutants, in particular the concentration of biochemical oxygen demand, occurred during the spraying period, but returned very rapidly to their normally low levels when the spraying stopped.

The differences in the results obtained from Culbae and Brickrow are attributed to poorer adsorption and the free-draining properties of the soil at Culbae, and to the different composition of the excrement. As a result of the whey diet of the pigs at Culbae, the volume of excrement per pig was approximately twice that per pig at Brickrow. The excrement at Culbae had a lower suspended solids concentration and higher biochemical oxygen demand/suspended solids ratio. It was therefore more likely to cause an increase in the levels of biochemical oxygen demand of drainage water.

CONCLUSIONS

CONCLUSIONS

The survival of salmonellae in pig excrement on farms

The excrement from intensive units for pig breeding and/or fattening is frequently sprayed onto adjacent grassland through rain-guns, or by vacuum tanker. Since pigs are often housed throughout the year, and not just in the winter, it is unusual to store pig excrement as long as cattle excrement. It may be sprayed onto land within a few hours of excretion, or after storage for a few days or weeks.

If high concentrations of salmonellae are excreted by a number of pigs in an intensive unit, and the slurry is sprayed immediately onto grassland, then numbers of the pathogens may be expected in the aerosols produced during spraying and high numbers will be deposited onto the land surface.

When the excrement is stored as a slurry, the numbers of salmonellae would be expected to decline with increasing storage time. The results of this report provide evidence that the activity of anaerobic bacteria influences the rate of decline of viable Salmonella dublin. Ninety per cent reduction of inoculated cells occurred in 30 days when acid fermentations reduced the pH value of the slurry below 7.0. Later, when methanogenesis reduced the acid concentrations, the 90 per cent reduction time increased to more than 60 days. However, if large numbers of salmonellae are excreted, then large numbers will still be present in the slurry even after a 90 or 99 per cent reduction. Further experiments are necessary to determine if the reduction times for other salmonellae are

similar to those for S. dublin, but due to the large number of serotypes found in the intestines of pigs, and the dangers of handling some of these in the laboratory, it was thought that the use of a variety of serotypes would not have provided sufficient additional data to merit the risks involved.

The increasing distribution of serotypes such as S. dublin and S. typhimurium may be due to these serotypes being more tolerant to a wider range of environmental factors, than host-specific serotypes such as S. cholera-suis. Newell (1967) and Morehouse (1972) suggest that only those serotypes which are of significance to animal and public health need be considered in the control of salmonellosis. When pigs become infected with S. cholera-suis the development of clinical infections usually neccessitates control of the pathogens within the herd. However, infection with other serotypes of salmonellae are not often detected or controlled. Therefore precautions against dissemination of salmonellae via infected excrement may be necessary.

The survival of salmonellae during aerobic biological treatment of pig excrement

Aerobic biological treatment of pig excrement is being developed as a method of reducing smell and pollution of water-courses. In most cases the products of treatment are spread over the surface of land. The studies on the survival of Escherichia coli and Salmonella dublin in the aeration vessel of a laboratory treatment unit support the idea that these organisms become associated with the sludge flocs and only low numbers remain in the supernatant.

Although there was a reduction in the numbers of E. coli in the sludge, particularly at low loading rates, a solids residence

time of 15 days only resulted in a 99 per cent reduction from the initial concentration. When S. dublin was inoculated into the mixed liquor of the aeration vessel, there was an initial rapid reduction of their numbers in the supernatant and it was thought that this may have been largely due to such factors as protozoal grazing. By the third day, however, 0.1 per cent of the inoculated cells had become associated with the sludge flocs. These cells then survived at least as well as E. coli in the flocs. If the salmonella had been present in the slurry fed to the aeration vessel, then it seems likely that their survival would have been similar to E. coli.

Unless storm water from roofs and yards is piped into slurry tanks, only small volumes of liquid effluent will be produced for discharge. The main product of any biological system for treating animal excrement will be a residual sludge. If, in the future, this material is to be considered as a source of microbial protein to supplement animal feeds, then the possibility that pathogens could still be present becomes an even greater hazard to animal health.

Bacterial pollution of water-courses following land treatment of animal excreta.

Since it is likely that some pathogens excreted in animal faeces will survive storage on a farm, then spraying infective material onto grassland could produce a health hazard to grazing animals and to animals drinking water from drainage ditches.

The results reported here show that, from both free-draining and imperfectly draining soils, high concentrations of faecal bacteria can be detected in the drainage water for approximately

two days following the spraying of slurry onto land. There is no reason to suppose that pathogens, such as salmonellae, would behave any differently. The short time interval between spraying and the appearance of faecal bacteria in the drainage water makes it unlikely that die-off of less resistant pathogens in the soil would occur.

In the intervals between slurry applications, the numbers of bacteria in drainage water are mainly affected by the flow rate of water through the soil and by the numbers of bacteria in the soil. The numbers of bacteria washed out of the soil of both catchment areas studied appeared to be insufficient to significantly decrease the numbers remaining in the soil between slurry applications. It is unlikely that the numbers of enteric bacteria washed out of clay soils would be as high as the numbers washed out of the two soils studied here. Therefore the number of viable pathogens in the soil largely depends on the number in the slurry, their survival time in the soil and the time between slurry applications. Although soil type or the pH value of the soil may influence survival, there are reports (Beard, 1940; Nottingham and Urselman, 1961; Taylor and Burrows, 1971) that salmonellae can survive in soil for as long as E. coli.

Further studies (now in progress) on the numbers of bacteria in drainage water from clay soils may show a similar relationship between their concentration, drainage rate and numbers in the soil, unless the increased chances of fissuring disturbs this relationship. If similar relationships do exist, it is likely that the rate of increase in concentration of bacteria in drainage water with increasing drainage rate would be less for clay soil, than the rates observed for the two soils described.

Aerobic treatment of animal excrement may reduce the numbers of viable enteric bacteria, particularly in the liquid phase. This should reduce the numbers which may be discharged to a water-course immediately following spraying of a residual sludge onto land. So far it has not been possible to investigate this hypothesis, because of the non-availability of a residual sludge from an aerobic treatment unit at a suitable time.

It is evident, under present farming practice, that relatively high numbers of salmonellae can be discharged to a water-course for a short time following the application of contaminated slurry to land. If contaminated slurry is applied to land at frequent intervals then low numbers of salmonellae are likely to be continually discharged to water-courses between applications. The numbers discharged would depend largely on the amount of precipitation, evapotranspiration and the drainage properties of the soil.

Health hazards

Little is known about the pathogenicity of salmonellae after prolonged survival outside of its host, in excrement, in anaerobic or aerobic conditions, or in soil, or water. The numbers of organisms that need to be ingested before infection occurs varies with different serotypes and hosts. Newell (1967) suggested that it would be impossible to reduce all salmonellae in the environment to insignificant levels, in a similar way to other pathogens, such as Mycobacterium tuberculosis in Great Britain.

Since only a few serotypes are important in animal and human health, it may be desirable to consider reducing their numbers only. The "food-poisoning" salmonellae are carried and excreted by domestic animals, especially pigs. The main sources of infection are animal feeds, infected animals, abattoirs and food processing plants. However, because of the lack of correlation between serotypes found in clinical cases and the serotypes in the environment of the diseased animal or human, other major sources exist (Kampelmacher, Guinée and Clarenberg, 1962; Skovgaard and Nielson, 1972). Several authors (Hughes et al, 1971; Newell, 1967; Newell and Williams, 1971; Morehouse, 1972; and Skovgaard and Nielson, 1972) suggest that stricter controls must be maintained on the farm. This includes improved husbandry techniques, detection and elimination of infected animals, and greater care in handling infected excrement.

It is evident from the results reported here that salmonellae may survive anaerobic storage and aerobic biological treatment of pig excrement. Prolonged storage will reduce the numbers of viable salmonellae present but reduction to insignificant levels may require storage times in excess of 12 months. The solids residence time in aerobic treatment units designed to reduce smell and soluble BOD prior to land treatment of the material, or for the production of microbial protein, is also unlikely to be sufficient for the reduction of viable salmonellae to insignificant levels. Disinfection of residual sludges and supernatants by appropriate methods such as extended aerobic treatment, pasteurization or chlorination, may be desirable in some cases.

When excrement containing viable salmonellae is sprayed onto land some of the organisms will pass through soil into drainage water and thence to a water-course. In some cases it may be

desirable to apply small volumes of slurry frequently to land rather than large volumes occasionally, or it may be better to apply slurry during periods of low rainfall and/or high evapotranspiration. The studies at Culhae suggest that greater attention to the drainage properties of the soil and the expected annual precipitation of particular areas may be advisable in the siting of new intensive animal production units. Where this is impractical, alteration of the properties of the material, by methods such as aerobic treatment, may provide a solution. At present it appears that the change from the production of farmyard manure to a hydraulic system of handling excrement increases the chances of pathogen survival, and the probability of their dissemination in the environment. This hazard exists even in areas where increased chemical pollution of water-courses does not occur and where the problem of smell is not important.

APPENDIX I

Data from studies of land drainage water.

Table i. Data from the experimental pasture, Brickrow, 17/9/68 - 21/10/68 (Fig. 14).

Date	Atmospheric Temp °C Min.	Max.	Rainfall mm	Flow l/s	E. coli/l	Enterococci/l	BOD mg/l	Nitrate mg/l	20C Viable Units/l
17/ 9/68	10.6	16.1	-	0.0023	1.4×10^5	8.2×10^2	0.8	1.2	4.1×10^5
18	4.4	16.7	-	0.0017	1.2×10^5	8.0×10^2	-	-	-
19	5.0	23.9	-	0.0016	1.1×10^4	3.5×10^2	0.9	0.4	1.4×10^6
20			6.2	0.0017	7.1×10^4	4.4×10^3	1.3	0.6	7.3×10^6
21			0.9	0.0022	-	-	-	-	-
22			0.1	0.0030	-	-	-	-	-
23			13.5	0.1620	-	-	-	-	-
24	16.7	16.7	-	0.0671	1.3×10^4	2.2×10^4	1.0	0.7	6.2×10^6
25	7.2	16.1	-	0.0208	6.0×10^4	3.6×10^4	1.4	0.4	1.5×10^6
26	10.6	10.6	2.1	0.0139	2.3×10^4	1.5×10^3	0.8	0.5	2.5×10^6
27	10.6	16.1	1.0	0.0127	5.0×10^3	1.3×10^3	0.3	0.3	1.3×10^6
28			7.2	0.0243	-	-	-	-	-
29			8.5	0.0984	-	-	-	-	-
30			4.5	0.0694	-	-	-	-	-
31			6.3	0.1270	2.7×10^4	7.0×10^3	0.7	0.4	1.4×10^4
1/10/68			-		-	-	-	-	-
2	7.8	14.4	29.7	0.3010	-	-	-	-	-
3	12.2	16.1	4.1	0.5900	-	-	-	-	-
4	12.2	15.0	7.4	0.5090	3.5×10^4	1.4×10^4	-	-	

Table i (continued)

Date	Atmospheric Temp °C Min.	Max.	Rainfall mm	Flow l/s	E. coli/l	Enterococci/l	BOD mg/l	Nitrate mg/l	20C Viable Units/l
5/10/68	11.1	14.4	0.4	0.3240	-	-	-	-	
6	11.7	14.4	2.9	0.1850	-	-	-	-	
7	11.7	15.6	0.9	0.1510	-	-	-	-	
8	4.4	11.1	-		4.7×10^3	4.0×10^2	0.7	0.4	
9	12.2	16.7	-	0.0690	3.2×10^3	3.6×10^2	1.4	0.6	3.0×10^5
10	10.0	13.3	9.5	0.0730	4.7×10^4	6.0×10^3	1.4	0.6	
11	7.8	15.0	2.2	0.1740	2.0×10^4	3.8×10^3	1.6	0.5	2.4×10^6
12	10.0	16.7	5.5	0.0280	-	-	-	-	
13	9.4	13.3	2.8	0.1130	-	-	-	-	
14	10.6	13.3	11.2	0.0910	-	-	-	-	
15	6.7	11.1	11.3	0.3940	3.9×10^4	6.6×10^3	1.3	1.0	5.8×10^5
16	5.0	12.2	4.6	0.2780	1.7×10^4	1.4×10^3	0.8	1.0	4.0×10^5
17	9.4	12.2	7.5	0.2780	7.6×10^3	1.4×10^3	1.3	0.9	
18	6.1	12.2	-	0.2200	1.3×10^3	3.0×10^2	0.3	1.2	
19	3.9	12.2	-	0.1160	-	-	-	-	
20	8.3	15.6	11.9	0.1620	-	-	-	-	
21	11.7	16.1	-	0.2200	-	-	-	-	

Table ii. Data from the experimental pasture, Brickrow 22/10/68 - 1/3/69 (Fig. 15).

Date	Atmospheric Temp °C Min.	Max.	Rainfall mm	Flow l/s	*E. coli*/l	Enterococci/l	BOD mg/l	Nitrate mg/l	20C Viable Units/l
22/10/68	7.8	15.6	-	0.116	5.2×10^3	1.5×10^3	0.9	0.8	5.4×10^5
23	11.1	15.0	-	0.073	3.2×10^3	5.6×10^2	2.6	0.8	5.2×10^5
24	6.7	14.4	-	0.035	1.8×10^3	2.6×10^2	2.2	0.9	2.3×10^5
25	9.4	12.2	-	0.018	1.5×10^3	1.3×10^2	0.6	0.8	
26	10.0	11.1	0.1	0.006	6.6×10^2	7.0×10^1	0.9	0.8	
27	11.1	12.8	3.6	0.002	8.9×10^2	4.0×10^1	0.8	0.5	
28	12.2	-15.0	3.6	0.012	2.3×10^3	1.1×10^2	0.6	0.2	
29	12.2	-15.6	1.2	0.022	2.2×10^3	1.0×10^2	0.9	-	
30	10.0	12.2	6.9	0.023	8.5×10^2	1.6×10^2	-	-	
31	10.9	15.0	1.2	0.056	3.3×10^3	1.3×10^2	0.7	-	
1/11/68	2.2	12.8	25.2	0.347	1.4×10^5	1.4×10^4	-	-	
2	2.8	4.4	9.0	0.637	2.9×10^4	5.5×10^3	1.2	0.9	
3	-1.7	5.0	-	0.298	4.8×10^3	1.2×10^3	0.9	1.2	
4	-1.7	5.0	-	0.111	4.0×10^3	2.4×10^2	1.6	-	
5	-1.1	3.9	-	0.056	2.8×10^3	2.0×10^2	0.7	1.2	
6	2.2	7.8	-	0.030	1.8×10^3	2.1×10^2	0.6	-	
7	5.0	9.4	-	0.012	1.9×10^3	9.0×10^1	9.3	-	
8	1.1	7.8	-	0.003	9.4×10^2	5.0×10^1	-	-	
9	-2.8	5.6	-	0.002	-	4.0×10^1	-	-	
10	0.0	4.4	-	0.001	8.6×10^2	5.0×10^1	1.1	0.6	

Table ii (continued)

Date	Atmospheric Temp °C Min.	Max.	Rainfall mm	Flow l/s	E. coli/l	Enterococci/l	BOD mg/l	Nitrate mg/l	20C Viable Units/l
11/11/68	5.6	7.8	13.5	0.001	9.9×10^2	5.0×10^1	1.5	0.5	
12	5.6	7.8	0.6	0.002	2.5×10^2	3.0×10^1	2.1	0.6	
13	8.3	10.0	-	0.002	-	-	1.0	-	
14	5.6	7.2	-	0.001	6.2×10^2	2.0×10^1	-	-	
15	2.2	7.8	-	0.001	2.6×10^2	3.0×10^1	1.4	0.6	
16	0.0	7.2	-	0.001	1.5×10^2	3.0×10^1	2.3	0.4	
17	-1.7	3.3	-	0.0003	4.1×10^2	2.0×10^1	-	-	
18	-0.6	5.6	-	0.0002	5.0×10^2	2.0×10^1	-	-	
19	0.6	5.6	-	0.0002	-	-			
20	3.3	8.3	1.0	0.0002	-	-			
21	6.7	11.1	5.1	0.0002	1.2×10^2	5.0×10^1	1.2	0.1	
22	8.9	12.2	13.3	0.072	4.0×10^3	4.0×10^3	1.5	0.2	
23	6.7	11.1	12.8	0.197	9.5×10^3	3.5×10^3	1.6	0.4	
24	5.6	11.1	3.0	0.289	1.3×10^4	5.2×10^3	1.4	0.3	
25	5.0	11.1	8.6	0.394	-	-	0.0		
26	8.3	14.4	2.1	0.231	2.8×10^3	7.2×10^2	0.5	1.2	
27	6.7	9.4	-	0.094	2.0×10^2	1.9×10^2	5.6	1.0	
28	4.4	8.3	21.9	0.370	4.6×10^4	9.7×10^2	1.1	1.1	
29	5.6	9.4	-	0.417	6.6×10^3	3.0×10^3			
30	1.7	6.7	-	0.098	1.2×10^3	1.7×10^2			

Table ii (continued)

Date	Atmospheric Temp °C Min.	Max.	Rainfall mm	Flow l/s	*E. coli*/l	Enterococci/l	BOD mg/l	Nitrate mg/l	20C Viable Units/l
1/12/68	1.7	5.0	0.3	0.054	6.2×10^2	1.8×10^2			
2	-8.3	-6.1	-	0.023	8.5×10^2	1.0×10^2	1.0	-	
3	7.2	11.1	-	0.010	3.3×10^2	6.0×10^1	1.3	-	
4	7.2	10.0	3.8	0.004	6.4×10^2	1.0×10^2	1.2	-	
5	7.2	11.5	4.0	0.015	2.8×10^2	7.0×10^1	1.2	3.0	
6	6.1	9.4	2.5	0.035		-			
7	6.1	6.7	-	0.038		-			
8	1.1	5.6	-	0.034		-			
9	3.3	5.0	-	0.018	9.5×10^2	4.5×10^2			
10	0.0	3.9	-	0.008		-			
11	3.9	4.4	-	0.002	1.5×10^2	1.0×10^1	1.5	0.5	
12	0.0	5.6	-	0.001	1.4×10^2	1.0×10^1	4.2	0.5	
13	0.6	8.3							
14	3.3	5.6		0.079			1.0	2.5	
15	-3.3	2.2	-	0.004			2.9	2.5	
16	-3.3	4.4	0.3	0.035					

Table ii (continued)

Date	Atmospheric Temp °C Min.	Max.	Rainfall mm	Flow l/s	E. coli/l	Enterococci/l	BOD mg/l	Nitrate mg/l	20C Viable Units/l
12/ 1/69	0.6	3.9	-	0.043					
13	-0.6	6.1	9.4	0.208					
14	4.4	14.4	-	0.162	9.0×10^1	3.0×10^1			
15	2.2	7.2	3.2	0.101	1.8×10^2	3.0×10^1			
16	1.7	5.0	1.8	0.035	6.0×10^1	3.0×10^1			
17	1.7	6.7	1.1	0.016	6.0×10^1	1.0×10^1	2.4	1.1	
18	0.0	3.3	1.3	0.012	6.0×10^1	4.0×10^1	2.9	1.0	
19	0.0	5.6	1.8	0.0075	-	-	-	-	
20	1.4	6.7	0.6	0.0067	5.0×10^1	3.0×10^1	1.2	1.1	
21	3.6	10.0	14.8	0.347	1.8×10^3	1.6×10^3	0.7	1.1	
22	8.3	8.9	6.9	0.301	9.0×10^2	8.5×10^2			
23	3.6	4.4	-	0.278	6.2×10^2	2.5×10^2	3.1	1.2	
24	5.6	10.0	1.2	0.088	9.0×10^1	6.0×10^1	1.1	2.2	
25	5.0	10.6	3.9	0.080	8.0×10^1	8.0×10^1	2.6	2.3	
26	7.2	10.0	0.1	0.046	1.6×10^2	4.0×10^1	2.2	1.1	
27	6.8	9.4	1.0	0.027	6.0×10^1	1.0×10^1	1.0	1.1	
28	5.0	9.4	8.3	0.068	4.2×10^2	3.6×10^2	1.6	1.1	
29	6.1	7.2	0.1	0.053	8.0×10^1	7.0×10^1	0.9	1.0	
30	3.3	7.8	3.4	0.028	8.0×10^1	2.0×10^1	0.7	1.4	
31	3.3	7.8	8.1	0.064	6.0×10^1	6.0×10^1	0.7	1.2	

Table ii (continued)

Date	Atmospheric Temp °C Min.	Max.	Rainfall mm	Flow l/s	*E. coli*/l	Enterococci/l	BOD mg/l	Nitrate mg/l	20C Viable Units/l
1/ 2/69	1.7	5.6		0.099	-	2.0×10^1	1.0	1.0	
2			3.3	0.151					
3			-	0.139					
4			-	0.068					
5	4.4	6.1	0.2	0.045	5.6×10^3	1.5×10^3	0.6	1.1	
6	3.3	7.8	4.5	0.043	2.8×10^3	8.6×10^2			
7	1.7	4.4	1.4	0.083	1.4×10^3	3.6×10^2	1.2	1.2	
8	-5.6	0.6	-	0.059	4.2×10^2	9.0×10^1	1.2	1.1	
9			-	0.023					
10			-	0.0087	2.1×10^2	3.0×10^1			
11	0.6	7.2	12.9	0.030	1.2×10^3	1.4×10^3			
12	0.0	6.7	1.1	0.313	2.8×10^3	9.7×10^2			
13	-2.8	5.6	-	0.065	1.4×10^3	1.4×10^2	0.7	1.1	
14	-2.8	2.8	-	0.0083	4.2×10^2	5.0×10^1	0.6	1.1	
15	0.0	6.1	-	0.002	3.4×10^2	1.0×10^1	1.2	1.0	
16			-	0.002					
17	-8.9	1.1	-	0.002	1.8×10^2	2.0×10^1			
18	-3.3	3.3	-	0.002	2.0×10^1	1.0×10^1	1.1	1.0	
19	-1.7	2.8	-	0.002	-	-	0.7	1.0	
20			-	0.002	4.0×10^1	1.0×10^1	0.9	1.0	

Table ii (continued)

Date	Atmospheric Temp °C Min.	Max.	Rainfall mm	Flow l/s	E. coli/l	Enterococci/l	BOD mg/l	Nitrate mg/l	20C Viable Units/l
21/ 2/69			-	0.002			1.1	1.0	
22			-	0.002				-	
23			1.7	0.002				-	
24			-	0.002					
25	3.3	5.6	-	0.002	4.0×10^1	1.0×10^1	0.5		
26	2.2	5.6	0.6	0.002			4.4		
27	0.6	5.6	0.1	0.002	6.0×10^1	1.0×10^1	1.5		
28	0.6	5.0	-	0.002	-	1.0×10^1	0.4	0.9	
1/ 3/69				Nil					

Table iii. Data from the experimental pasture, Brickrow 2/11/69 - 26/3/70 (Fig. 16).

Date	Atmospheric Temp °C Min.	Max.	Rainfall mm	Flow l/s	*E. coli*/l	Enterococci/l	BOD mg/l	Nitrate mg/l
2/11/69	8.9	13.3	8.64	0.0001				
3	7.8	15.0	9.90	0.004				
4	7.2	8.9	4.82	0.007	7.2×10^3	2.9×10^3	7.3	2.8
5	1.7	5.2	9.90	0.036	1.9×10^4	7.8×10^3	1.3	2.6
6	0.6	8.3	4.06	0.060	2.8×10^3	4.8×10^2	-	-
7	4.4	10.0	10.51	0.110	-	-	-	-
8	3.3	8.9	14.00	0.079	6.2×10^3	4.4×10^3	9.1	3.0
9	3.3	7.2	10.00	0.218	1.8×10^4	9.8×10^3	9.2	2.6
10	1.7	5.6	8.90	0.021	1.8×10^4	8.0×10^3	0.8	1.3
11	1.7	7.2	TR	0.158	2.3×10^3	1.1×10^3	0.4	2.5
12	4.1	6.1	1.27	0.078	8.0×10^2	2.5×10^2	0.9	3.3
13	0.6	6.7	1.02	0.087	1.6×10^3	1.5×10^2	0.7	3.5
14	2.2	6.7	4.06	0.120	1.8×10^3	8.0×10^2	-	3.2
15	0.0	2.8	5.33	0.222	4.5×10^3	2.6×10^3	2.0	2.1
16	0.6	7.2	2.29	0.075	1.4×10^3	1.1×10^2	3.0	3.4
17	-1.7	2.2	-	0.043	5.6×10^2	9.0×10^1	2.6	2.8
18	-4.4	9.4	TR	0.033	4.4×10^2	9.0×10^1	8.5	2.8
19	-2.2	10.6	10.20	0.200	4.6×10^2	1.3×10^2	1.7	2.1
20	8.3	10.0	4.57	0.255	-	-	3.9	3.7

Table iii (continued)

Date	Atmospheric Temp °C Min.	Atmospheric Temp °C Max.	Rainfall mm	Flow l/s	E. coli/l	Enterococci/l	BOD mg/l	Nitrate mg/l
21/11/69	4.4	6.7	11.90	0.324	6.6×10^3	1.4×10^3	0.7	2.5
22	1.7	6.7	6.10	0.452	-	-	0.8	2.5
23	1.7	6.1	5.58	0.461	-	-	-	-
24	3.3	5.0	-	0.226	2.3×10^3	5.0×10^2	2.6	-
25	-3.9	3.9	-	0.128	1.4×10^3	1.2×10^2	2.2	2.6
26	-1.7	3.9	-	0.065	1.4×10^3	1.0×10^2	1.5	1.8
27	-4.4	7.2	2.54	0.036	1.1×10^3	1.5×10^2	-	-
28	3.3	7.2	18.60	0.481	5.5×10^4	1.9×10^4	5.0	3.4
29	-3.9	1.1	-	0.417	9.2×10^3	3.2×10^3	1.2	4.1
30	-4.4	2.8	0.51	0.167	-	-	-	-
1/12/69	-3.9	8.3	2.03	0.117			3.8	2.8
2	2.2	8.3	1.27	0.112	1.9×10^3	7.0×10^2	7.4	3.2
3	6.1	8.3	8.40	0.209	1.7×10^3	4.4×10^2	0.5	3.8
4	5.6	6.1	-	0.205	1.1×10^3	2.5×10^2	1.6	1.6
5	-1.4	1.7	0.25	0.123	3.2×10^2	1.3×10^2	1.7	1.8
6 *	-3.9	7.8	1.78	0.088	-	-		
7	-3.0	10.0	1.02	0.085	1.5×10^3	4.1×10^2	-	2.1
8	0.6	10.0	TR	0.067	6.2×10^2	1.8×10^2	2.3	1.9
9 *	2.8	10.0	TR	0.038	2.9×10^3	7.8×10^2	0.5	2.0
10	7.2	7.8	TR	0.035	3.6×10^2	1.8×10^2	3.3	3.0

Table iii (continued)

Date	Atmospheric Temp °C Min.	Max.	Rainfall mm	Flow l/s	E. coli/l	Enterococci/l	BOD mg/l	Nitrate mg/l
11/12/69	5.6	8.9	7.88	0.098	3.3×10^3	9.5×10^2	0.6	1.3
12	2.8	7.2	TR	0.138	1.8×10^3	3.1×10^2	3.2	1.1
13	-2.2	2.8	TR	0.088	6.0×10^1	9.0×10^1	3.5	0.7
14	-2.8	5.6	15.50	0.244	1.1×10^4	2.4×10^3	10.4	0.9
15	2.2	7.8	17.00	0.088	7.7×10^3	3.0×10^3	2.2	1.0
16	2.8	3.9	4.32	0.903	3.5×10^3	1.0×10^3	4.5	1.2
17	-1.7	8.9	0.51	0.408	6.0×10^2	2.0×10^2	1.6	1.1
18	0.6	1.7	TR	0.224	3.4×10^2	7.0×10^1	1.8	1.1
19	-1.1	1.7	0.51	0.141	1.6×10^2	3.0×10^1	7.2	1.6
20	-3.3	4.1	3.81	0.120	-	-	-	-
21	1.1	9.4	6.35	0.154	8.8×10^2	6.0×10^2	5.9	2.4
22	2.2	10.6	18.60	0.524	6.5×10^3	2.7×10^3	10.0	2.6
23	6.7	7.8	0.51	0.583	1.2×10^3	1.4×10^3	8.4	2.9
24	5.6	9.4	4.06	0.312	2.0×10^2	1.4×10^3	5.1	1.8
25	6.1	8.3	TR	0.266	-	-	-	-
26	0.0	5.0	TR	0.183	-	-	-	-
27	-2.8	3.3	-	0.125	-	-	-	-
28	-2.8	3.9	0.25	0.088	-	-	-	-
29	-2.8	4.4	TR	0.062	-	-	1.0	2.1
30	-1.7	4.4	TR	0.037	5.2×10^2	4.0×10^1	2.6	1.8
31	2.2	4.1	TR	0.029	1.4×10^2	2.0×10^1	2.1	1.8

Table iii (continued)

Date	Atmospheric Temp °C Min.	Max.	Rainfall mm	Flow l/s	E. coli/l	Enterococci/l	BOD mg/l	Nitrate mg/l
1/ 1/70	1.1	2.8	-	0.013	-	-		
2	-1.1	6.1	TR	0.013	-	-		
3	1.1	6.7	TR	0.012	-	-		
4	-1.1	3.3	TR	0.010	-	-		
5	-1.1	2.8	-	0.007	-	-		
6	-6.7	2.2	2.79	0.005	-	-		
7	-8.3	0.6	-	0.003	-	-		
8	-10.0	1.1	-	0.002	-	-		
9	-7.2	2.8	1.78	0.003	-	-		
10	-2.2	5.6	8.64	0.004	-	-		
11	2.2	7.2	0.25		-	-		
12	1.1	7.8	5.85		-	-		
13	1.1	6.7	2.03		1.1×10^3	2.3×10^2	2.4	1.8
14	2.2	7.8	TR	0.138	1.3×10^2	6.0×10^1	2.0	1.9
15	1.7	8.3	1.02	0.096	6.2×10^2	5.0×10^1	5.6	1.6
16	6.7	8.3	1.78	0.062	1.2×10^2	3.0×10^1	4.2	1.0
17	-0.6	3.9	TR	0.042	8.0×10^1	1.0×10^1	8.5	1.7
18	1.7	6.1	9.90	0.162	1.0×10^3	3.6×10^2	9.2	1.8
19	0.6	9.4	1.52	0.258	1.4×10^3	3.0×10^1	2.2	1.8
20	3.3	8.9	1.27	0.117	3.2×10^2	2.0×10^1	5.1	2.0

Table iii (continued)

Date	Atmospheric Temp °C Min.	Max.	Rainfall mm	Flow l/s	*E. coli*/l	Enterococci/l	BOD mg/l	Nitrate mg/l
21/ 1/70	5.0	10.0	1.27	0.103	3.0×10^1	1.0×10^1	5.2	1.8
22	6.7	10.0	2.03	0.088	7.0×10^1	2.0×10^1	7.6	1.8
23	5.0	8.3	TR	0.075	1.3×10^2	1.0×10^1	2.3	1.7
24	3.3	7.8	-	0.050	7.0×10^1	1.0×10^1	2.0	1.7
25	3.2	7.2	1.27	0.028	9.0×10^1	1.0×10^1	2.9	1.7
26	3.9	8.9	4.83	0.013	7.0×10^1	3.0×10^1	4.8	1.6
27 *	4.4	5.6	5.58	0.211	6.4×10^2	2.5×10^2	5.5	1.7
28	1.7	7.2	TR	0.163	3.8×10^2	2.9×10^2	5.6	1.8
29	0.6	6.1	-	0.108	1.4×10^2	6.0×10^1	3.4	1.7
30	1.7	4.4	0.25	0.077	2.0×10^1	2.0×10^1	2.0	1.7
31	2.2	4.4	TR	0.047	3.0×10^1	2.9×10^1	4.9	1.7
1/ 2/70	1.7	5.0	0.25	0.030	5.0×10^1	2.0×10^1	5.8	1.7
2	1.1	9.4	15.50		9.4×10^4	1.8×10^5	8.7	1.8
3	4.4	7.2	1.78		1.7×10^4	2.2×10^4	1.4	1.8
4	2.8	7.8	0.25		8.8×10^2	1.8×10^3	3.0	1.8
5	1.1	6.1	-		7.0×10^2	4.8×10^2	7.4	-
6	0.0	3.3	-		3.3×10^2	2.3×10^2	4.4	-
7	-5.6	4.4	6.35		4.2×10^2	3.1×10^2	5.1	1.7
8	-4.4	5.6	7.62		2.4×10^3	3.2×10^3	9.6	1.8
9	1.1	1.4	11.70		9.3×10^3	1.4×10^4	5.4	1.7
10	0.0	3.3	2.79		1.7×10^3	2.4×10^3	2.6	1.6

Table iii (continued)

Date	Atmospheric Temp °C Min.	Atmospheric Temp °C Max.	Rainfall mm	Flow l/s	E. coli/l	Enterococci/l	BOD mg/l	Nitrate mg/l
11/ 2/70	-3.9	3.3	-		2.5×10^2	2.5×10^2	4.2	1.7
12	-6.1	3.3	-		1.0×10^2	9.0×10^1	6.6	1.6
13	-5.0	2.2	-		8.0×10^1	7.0×10^1	3.0	1.6
14	-7.2	1.7	-		6.0×10^1	7.0×10^1	2.8	1.8
15	-6.1	2.8	-		4.0×10^1	6.0×10^1	3.3	1.7
16	-4.4	2.8	-		3.0×10^1	6.0×10^1	7.5	1.8
17	-3.3	2.2	0.15		-	5.0×10^1	6.9	1.6
18	-2.2	5.6	0.51		-	-	-	-
19	1.1	6.1	5.35		2.5×10^3	8.5×10^3	1.5	1.9
20	1.7	6.1	15.20		3.5×10^3	1.7×10^4	1.6	1.9
21	0.0	7.2	2.54		1.3×10^3	5.4×10^3	3.6	1.8
22	2.2	10.0	14.70		8.4×10^2	2.9×10^3	3.6	1.8
23	2.2	7.2	6.10		-	-	-	-
24	1.1	6.7	4.58		1.3×10^2	5.3×10^2	3.1	1.7
25	2.8	2.2	0.18		1.0×10^2	1.1×10^2	3.1	1.7
26	-2.2	6.1	TR		6.0×10^1	4.0×10^1	1.8	1.9
27					2.0×10^1	6.0×10^1	1.5	2.0
28					1.0×10^1	4.0×10^1	2.9	2.1

Table iii (continued)

Date		Atmospheric Temp °C Min.	Max.	Rainfall mm	Flow l/s	E. coli/l	Enterococci/l	BOD mg/l	Nitrate mg/l
1/ 3/70						3.0×10^1			
2		0.0	8.3	1.0		1.0×10^1	4.0×10^1	4.9	1.9
3		0.6	4.4	TR		3.0×10^1	3.0×10^1	3.3	2.0
4		-2.2	2.8	5.0		3.0×10^1	2.0×10^1	1.8	2.0
5	*	-2.2	3.3	TR		2.0×10^1	3.0×10^1	4.4	2.1
6		-5.0	4.4	-		2.1×10^3	2.8×10^3	1.1	2.0
7		-3.3	7.2	0.75		2.2×10^2	1.4×10^2	9.9	2.0
8		-3.3	7.2	TR		1.8×10^2	1.4×10^2	3.3	2.1
9		3.3	6.7	TR		1.0×10^2	4.0×10^1	2.8	2.3
10		-0.6	6.7	-		4.0×10^1	1.0×10^1	5.9	2.1
11		-0.6	6.1	5.0		6.0×10^1	2.0×10^1	0.9	2.0
12		1.1	5.6	1.25		1.0×10^2	3.0×10^1	0.9	2.0
13		0.6	5.6	TR		7.0×10^1	2.0×10^1	1.3	2.0
14		1.7	6.1	-		2.0×10^1	1.0×10^1	3.7	2.0
15		3.3	7.2	TR		2.0×10^1	1.0×10^1	4.3	2.1
16		1.1	9.4	TR		7.0×10^1	1.0×10^1	2.2	2.0
17		5.0	9.4	4.5		2.0×10^1	1.0×10^1	3.7	2.0
18		6.7	9.4	6.6		1.8×10^2	2.0×10^1	4.0	2.0
19		1.1	6.7	1.25		1.0×10^2	6.0×10^1	1.3	1.9
20		1.7	5.9	7.0		3.0×10^2	1.6×10^2	1.3	2.1

Table iii (continued)

Date	Atmospheric Temp °C Min.	Max.	Rainfall mm	Flow l/s	E. coli/l	Enterococci/l	BOD mg/l	Nitrate mg/l
21/ 3/70	4.1	9.1	0.5		5.0×10^1	2.0×10^1	7.7	2.0
22	0.6	4.4	1.25		1.0×10^1		3.8	2.0
23	2.8	8.3	TR				3.0	2.0
24	-0.6	8.3	-		4.0×10^1	2.0×10^1	1.4	2.1
25	-2.2	8.3	-		0	1.0×10^1	1.9	2.0
26	-1.7	8.3	1.25		2.0×10^1	0	1.8	2.0

*pig excrement applied to pasture.

Table iv. Data from experimental pasture, Brickrow, October 1970 (Fig. 17).

Date	Atmospheric Temp °C Min.	Max.	Rainfall mm	Flow l/s	*E. coli*/l	Enterococci/l
1/10/70	9.4	14.4	14.0	0.235	2.4×10^3	1.5×10^2
2/10/70	7.2	11.7	1.3	0.220	5.1×10^3	1.9×10^3
3/10/70	6.7	14.4	3.6	0.120	1.6×10^3	5.4×10^2
4/10/70	8.3	14.4	8.6	0.120	2.2×10^3	1.8×10^3
5/10/70	8.9	12.2	2.0	0.238	1.1×10^2	4.6×10^2
6/10/70	7.8	10.6	2.5	0.140	-	-
7/10/70	7.2	13.3	TR	0.085	8.2×10^2	2.6×10^2
8/10/70	7.8	11.7	-	0.036	8.2×10^2	1.1×10^2
9/10/70	4.4	12.8	0.1	0.017	3.8×10^2	6.0×10^1
10/10/70	8.9	14.4	-	0.012	4.0×10^2	5.8×10^1
11/10/70	6.7	15.6	3.6	0.012	3.2×10^2	5.1×10^2
12/10/70	9.4	16.7	TR	0.010	3.9×10^2	4.0×10^1
13/10/70	9.4	18.9	TR	0.012	3.8×10^2	3.0×10^1
14/10/70	8.9	18.3	-	0.006	3.0×10^2	4.0×10^1
15/10/70	9.4	13.9	-	0.005	3.9×10^2	2.0×10^1
16/10/70	7.2	14.4	-	0.003	2.4×10^2	4.0×10^1
17/10/70	8.9	12.8	TR	0.003	-	-
18/10/70	8.3	13.3	7.1	0.003	6.0×10^2	6.0×10^1
19/10/70	7.2	10.0	0.8	0.003	2.9×10^2	4.0×10^1
20/10/70	4.4	7.2	TR	0.003	4.1×10^2	2.0×10^1
21/10/70	1.1	8.9	-	0.003	2.4×10^2	1.2×10^1
22/10/70	5.0	11.1	-	0.003	1.3×10^2	5.0×10^1
23/10/70	7.8	11.7	6.9	0.003	2.8×10^2	1.1×10^2
24/10/70	8.9	12.2	15.0	0.120	2.0×10^3	3.5×10^3
25/10/70	7.8	10.0	2.5	0.265	4.4×10^3	-
26/10/70	6.1	8.9	1.3	0.110	5.5×10^2	4.1×10^2
27/10/70	3.3	11.7	4.1	0.148	2.0×10^3	2.1×10^2
28/10/70	1.1	5.0	6.6	0.190	3.2×10^3	1.0×10^3
29/10/70	2.8	3.3	11.7	0.330	9.4×10^3	5.0×10^3
30/10/70	9.4	13.9	4.6	0.775	5.0×10^3	4.2×10^3
31/10/70	8.3	15.6	27.9	0.850	9.8×10^4	9.4×10^3

Table v. Data from experimental pasture, Brickrow.

Experiment I. 55 m^3/ha pig excrement sprayed onto pasture (Fig. 18).

Date	Time h	Rainfall mm	Flow l/s	pH	E. coli colonies/l	Enterococci colonies/l
28/10/70	1400	0.1	0.125		1.0×10^3	2.3×10^2
	1600	0.4	0.125		6.6×10^5	3.2×10^4
	1700	TR	0.125		9.1×10^5	2.7×10^4
	1800	TR	0.125		6.2×10^5	1.2×10^4
	2000	2.8	0.130		2.0×10^5	1.1×10^4
	2200	0.9	0.142		2.7×10^5	1.0×10^4
	2400	1.4	0.142		2.1×10^5	6.9×10^3
29/10/70	0200	0.2	0.150		2.7×10^5	1.2×10^4
	0400	0.3	0.200		2.0×10^5	8.2×10^3
	0600	–	0.215		1.2×10^5	9.0×10^3
	0800	–	0.215		1.2×10^5	2.8×10^3
	1000	–	0.233		7.2×10^4	3.6×10^3
	1200	0.9	0.250		5.3×10^4	2.4×10^3
	1400	1.4	0.250		1.0×10^4	1.6×10^3
	1600	0.2	0.260		4.1×10^4	3.0×10^3
	1800	–	0.267		1.4×10^4	1.5×10^3
	2000	–	0.267		1.1×10^4	2.4×10^3
	2200	0.1	0.267		3.0×10^4	1.3×10^3
	2400	1.4	0.267		9.2×10^4	3.0×10^3
30/10/70	0200	2.3	0.267		1.7×10^4	1.7×10^3
	0400	0.2	0.300		2.3×10^4	4.3×10^3

Slurry application 1430–1730h on 28/10/70.

Table vi. Data from experimental pasture, Brickrow.

Experiment II. 55 m^3/ha pig excrement sprayed onto pasture (Fig. 19).

Date	Time h	Rainfall mm	Flow l/s	pH	E. coli colonies/l	Enterococci colonies/l
25/11/70	0010	-	0.45	6.7	8.4×10^2	1.5×10^2
	1145	-	0.42	6.7	1.6×10^3	4.0×10^2
	1215	-	0.40	6.7	6.0×10^3	2.8×10^3
	1245	-	0.40	6.7	4.5×10^4	2.9×10^3
	1315	-	0.38	6.7	5.0×10^4	3.2×10^3
	1345	-	0.38	6.7	4.1×10^4	2.9×10^3
	1415	1.3	0.37	6.7	3.4×10^4	2.4×10^3
	1445	1.1	0.36	6.9	3.0×10^4	1.5×10^3
	1515	0.9	0.34	6.9	3.1×10^4	1.4×10^3
	1545	-	0.34	6.9	2.0×10^4	1.9×10^3
	1615	-	0.34	6.9	4.2×10^4	1.1×10^3
	1645	-	0.33	6.7	6.5×10^4	1.7×10^3
	1715	-	0.33	6.7	4.8×10^4	6.2×10^3
	1815	-	0.33	6.7	6.1×10^4	7.2×10^3
	1900	-	0.33	6.7	5.3×10^4	8.2×10^3
	2000	-	0.33	6.7	5.2×10^4	1.6×10^3
	2200	-	0.33	6.7	3.8×10^4	1.8×10^3
	2400	-	0.33	6.7	3.5×10^4	1.8×10^3
26/11/70	0300	-	0.30	6.6	1.5×10^4	1.5×10^3
	0430	-	0.30	6.6	1.1×10^4	8.8×10^2
	0600	-	0.29	6.7	1.2×10^4	6.6×10^2
	0900	-	0.27	6.6	9.1×10^3	7.6×10^2
	1200	-	0.26	6.6	5.4×10^3	5.6×10^2
	1500	-	0.24	6.4	5.0×10^3	3.8×10^2
	1800	-	0.23	6.4	3.3×10^3	4.0×10^2
	2100	-	0.22	6.4	2.6×10^3	3.5×10^2
	2400	7.2	0.21	6.4	1.4×10^3	1.5×10^2

Slurry application 1015-1215h and 1315-1445h 25/11/70.

Table vii. Data from experimental pasture, Brickrow 17/11/71-24/3/72 (Fig. 20).

	Date	Flow l/s	Bacteria on m-PCB Viable Units/l	Soil Temp. 10cm °C	Soil Temp. 100 cm °C
1	17/11/71	0.0022	2.8×10^5	3.9	10.0
2		0.0022	7.0×10^5		
3	18/11/71	0.0780	6.4×10^7	3.3	10.0
4	19/11/71	0.0280	9.0×10^6	1.7	9.4
5		0.0740	4.3×10^7		
6	22/11/71	0.0960	4.6×10^7	-0.6	8.9
7		0.0740	4.3×10^7		
8	23/11/71	0.0600	3.0×10^7	8.9	10.0
9		0.0480	1.1×10^7		
10	24/11/71	0.0270	2.7×10^7	1.7	8.3
11		0.0220	6.5×10^6		
12	25/11/71	0.0190	1.1×10^7	6.1	8.3
13		0.0170	7.1×10^6		
14	26/11/71	0.0130	7.0×10^6	7.8	8.3
15	29/11/71	0.0220	6.5×10^6	2.2	8.3
16		0.0220	6.3×10^6		
17	30/11/71	0.0220	3.4×10^6	4.4	8.3
18		0.0220	4.7×10^6		
19	1/12/71	0.0170	4.2×10^6	4.1	7.2
20		0.0170	3.7×10^6		
21	2/12/71	0.0150	2.1×10^6	3.9	7.1
22		0.0130	1.4×10^6		
23	3/12/71	0.0095	2.1×10^6	1.5	7.0
24		0.0095	1.3×10^6		
25	6/12/71	0.0130	1.4×10^6	6.0	7.9
26		0.0110	1.5×10^6		
27	7/12/71	0.0070	1.3×10^6	4.2	8.0
28		0.0070	1.4×10^6		
29	8/12/71	0.0070	8.9×10^5	6.5	8.0
30		0.0070	9.4×10^5		

Table vii (continued)

	Date	Flow l/s	Bacteria on m-PCR Viable Units/l	Soil Temp. 10 cm °C	Soil Temp. 100 cm °C
31	9/12/71	0.0070	1.7×10^6	7.6	8.0
32		0.0065	1.4×10^6		
33	10/12/71	0.0065	1.6×10^6	7.5	8.0
34		0.0055	2.4×10^6		
35	13/12/71	0.0022	2.2×10^6	7.1	8.2
36		0.0022	2.2×10^6		
37	14/12/71	0.0045	2.6×10^6	7.2	8.2
38		0.0055	2.7×10^6		
39	15/12/71	0.0045	2.2×10^6	6.5	8.3
40		0.0045	2.2×10^6		
41	17/12/71	0.0036	3.8×10^6	8.4	8.3
42		0.0036	1.3×10^6		
43	20/12/71	0.0095	6.5×10^6	6.6	8.5
44	21/12/71	0.0340	2.0×10^7	8.5	8.4
45		0.0030	2.0×10^7		
46	22/12/71	0.0095	1.4×10^6	5.4	8.5
47		0.0065	2.8×10^6		
48	5/1/72	0.0044	2.7×10^6	2.7	7.2
49		0.0036	1.5×10^6		
50	6/1/72	0.0003	1.5×10^6	2.0	7.1
51		0.0003	1.6×10^6		
52	7/1/72	0.0003	2.2×10^6	1.0	7.1
53	10/1/72	0.0003	2.4×10^6	5.0	7.0
54		0.0003	1.0×10^6		
55	12/1/72	0.0600	2.3×10^7	1.2	6.8
56		0.0550	1.6×10^7		
57	14/1/72	0.0250	5.5×10^6	5.4	6.8
58	17/1/72	0.0065	1.7×10^6	4.2	6.8
59		0.0065	1.6×10^6		
60	18/1/72	0.0065	1.9×10^6	3.0	6.7
61		0.0065	1.9×10^6		

Table vii (continued)

	Date	Flow l/s	Bacteria on m-PCB Viable Units/l	Soil Temp. 10 cm °C	Soil Temp. 100 cm °C
62	19/1/72	0.2400	2.0×10^8	3.1	6.7
63		0.2200	1.3×10^8		
64	21/1/72	0.1100	1.4×10^8	1.4	6.7
65		0.0940	8.5×10^7		
66	21/1/72	0.0510	4.9×10^7	2.1	6.6
67	24/1/72	0.0890	1.0×10^8	4.0	6.6
68		0.0720	1.2×10^8		
69	25/1/72	0.0480	9.5×10^6	2.1	6.5
70		0.0440	9.5×10^6		
71	26/1/72	0.0440	2.3×10^7	4.9	6.5
72		0.0480	9.5×10^6		
73	27/1/72	0.0480	6.1×10^6	2.7	6.6
74		0.0440	4.7×10^6		
75	28/1/72	0.0250	1.6×10^7	3.1	6.4
76		0.0440	2.8×10^6		
77	31/1/72	0.0027	1.3×10^6	-*	6.3
78		0.0027	1.3×10^6		
79	2/2/72	0.0012	1.3×10^6	-*	5.7
80		0.0012	5.1×10^5		
81	7/2/72	0.0045	1.3×10^6	2.4	5.3
82		0.0045	1.9×10^6		
83	8/2/72	0.0028	1.6×10^6	3.2	5.3
84		0.0028	1.3×10^6		
85	9/2/72	0.0022	1.3×10^6	2.3	5.4
86		0.0022	1.3×10^6		
87	10/2/72	0.0022	9.8×10^5	2.7	5.4
88	11/2/72	0.0370	3.5×10^7	2.0	5.4
89		0.0420	4.9×10^7		
90	14/2/72	0.0440	3.3×10^7	2.4	5.4
91		0.0440	1.8×10^7		

* Thermometer broken.

Table vii (continued)

	Date	Flow l/s	Bacteria on m-PCB Viable Units/l	Soil Temp. 10 cm °C	Soil Temp. 100 cm °C
92	15/2/72	0.0310	2.3×10^6	2.4	5.4
93	17/2/72	0.0450	3.1×10^6	4.0	5.4
94		0.0440	5.4×10^6		
95	22/2/72	0.0053	8.8×10^4	2.6	5.3
96	24/2/72	0.0027	6.8×10^4	1.4	5.3
97		0.0024	7.1×10^5		
98	25/2/72	0.0024	1.6×10^6	2.8	5.3
99	28/2/72	0.0036	1.7×10^6	4.7	5.4
100		0.0036	1.6×10^6		
101	29/2/72	0.0036	2.7×10^6	3.4	5.5
102		0.0036	2.3×10^6		
103	1/3/72	0.0036	1.3×10^6	2.4	5.6
104		0.0036	1.1×10^6		
105	2/3/72	0.0036	1.1×10^6	1.5	5.6
106		0.0036	1.1×10^6		
107	3/3/72	0.0036	1.2×10^6	3.1	5.6
108		0.0036	8.6×10^5		
109	6/3/72	0.0036	1.1×10^6	2.6	5.6
110		0.0036	1.1×10^6		
111	7/3/72	0.0016	1.1×10^6	3.2	5.5
112		0.0016	1.0×10^6		
113	8/3/72	0.0016	9.3×10^5	3.5	5.5
114		0.0016	9.6×10^5		
115	9/3/72	0.0016	8.3×10^5	3.8	5.5
116		0.0016	5.0×10^5		
117	10/3/72	0.0012	2.7×10^6	2.0	5.6
118		0.0012	7.7×10^5		
119	13/3/72	0.0007	9.0×10^5	0.9	5.6
120		0.0007	9.6×10^5		

Table vii (continued)

	Date	Flow l/s	Bacteria on m-PCB Viable Units/l	Soil Temp. 10 cm °C	Soil Temp. 100 cm °C
121	14/3/72	0.0005	1.6×10^5	1.2	5.1
122		0.0005	1.5×10^5		
123	15/3/72	0.0003	3.8×10^5	4.1	5.5
124		0.0003	3.9×10^5		
125	16/3/72	0.0003	5.0×10^5	2.1	5.4
126		0.0003	5.3×10^5		
127	17/3/72	0.0007	1.4×10^6	6.2	5.4
128	20/3/72	0.0003	9.3×10^5	7.1	5.9
129		0.0002	8.3×10^5		
130	21/3/72	0.0002	8.0×10^5	3.9	6.1
131	22/3/72	0.0003	8.2×10^5	6.5	6.2
132	23/3/72	0.0003	9.2×10^5	7.3	6.3
133	24/3/72	0.0002	1.2×10^6	6.6	6.4
134		0.0003	1.0×10^6		

Table viii. Data from Culbae Area A (Figs. 21 and 22).

Date	Atmospheric Temp °C Min.	Max.	Rainfall mm	Drainage Rate l/s, ha	*E. coli*/l	Enterococci/l	Bacteria on m-PCB/l	BOD mg/l	SS mg/l
7/11/72	2.2	12.8	TR						
8	3.9	13.3	4.3						
9	3.3	12.2	18.9						
10	3.9	11.7	11.2						
11	5.0	13.3	12.4						
12	4.4	12.2	9.1						
13	-1.1	11.7	6.4	0.63	1.9×10^5	-	$>10^8$		
14	-2.2	11.1	5.8						
15	-2.2	10.6	1.6						
16	0.0	10.0	1.6	0.37	4.4×10^3	3.9×10^3	1.9×10^6	6.2	<5
17	-3.3	9.4	-						
18	-1.7	8.9	4.6						
19	0.6	9.4	29.8						
20	2.2	8.9	7.8					6.0	18
21	1.7	8.3	3.7						
22	1.1	8.9	0.8	0.63	6.8×10^4	3.3×10^4	5.5×10^8		
23	-0.6	7.8	TR						
24	-3.3	8.3	0.7						
25	-1.6	7.8	3.5						

Table viii (continued)

Date	Atmospheric Temp °C Min.	Max.	Rainfall mm	Drainage Rate l/s, ha	*E. coli*/l	Enterococci/l	Bacteria on m-PCB/l	BOD mg/l	SS mg/l
26/11/72	0.0	8.3	TR						
27	3.3	7.8	1.3	0.37	4.4×10^3	4.0×10^3	3.3×10^6		
28	2.2	7.8	2.8					8	<5
29	1.7	8.3	24.7						
30	3.9	8.9	7.3	0.58	1.6×10^4	2.0×10^4	2.9×10^7	4.4	14
1/12/72	4.4	9.4	3.4						
2	-1.1	7.8	TR						
3	2.8	7.8	7.3						
4	2.8	8.3	11.5						
5	2.2	7.2	3.5						
6	3.3	8.3	5.2						
7	0.0	7.2	-	0.50	-	-	1.5×10^7		
8	-3.3	7.2	TR						
9	0.6	7.8	4.3						
10	2.2	7.2	6.4						
11	2.8	7.2	13.3						
12	1.7	7.8	10.6	0.50	1.0×10^4	9.2×10^3	5.9×10^6		
13	0.6	7.8	3.7						

Table viii (continued)

Date	Atmospheric Temp °C Min.	Max.	Rainfall mm	Drainage Rate l/s, ha	E. coli/l	Enterococci/l	Bacteria on m-PCB/l	BOD mg/l	SS mg/l
18/12/72				0.35	1.6×10^3	1.5×10^3	4.1×10^6		
21				0.28	2.0×10^2	7.2×10^2	4.3×10^5		
23	2.8	7.8	2.0						
24	-3.9	6.7	1.6						
25	0.0	7.2	2.9						
26	1.1	7.2	8.2						
27	3.3	6.1	3.4	0.37	5.5×10^3	1.5×10^3	2.6×10^6		
28	3.9	6.7	17.4						
29	2.2	6.7	TR						
30	2.8	6.7	1.6						
31	3.3	7.2	7.0						
1/1/73	2.8	7.2	2.2						
2	3.3	7.8	1.3						
3	-2.2	8.3	1.2						
4	4.4	7.8	-						
5	3.3	8.3	-	0.33	5.8×10^2	-	3.9×10^6		
6	1.7	7.8	-						
7	-1.7	8.3	-						
8	2.2	7.2	-						
9	3.3	6.7	TR	0.24	3.3×10^2	-	2.3×10^6		

Table viii (continued)

Date	Atmospheric Temp °C Min.	Max.	Rainfall mm	Drainage Rate l/s, ha	E. coli/l	Enterococci/l	Bacteria on m-PCB/l	BOD mg/l	SS mg/l
10/1/73	3.9	6.1	0.2						
11	1.7	6.1	-	0.19	6.8×10^1	2.1×10^2	1.9×10^6		
12	2.8	5.6	-						
13	1.7	6.1	2.2						
14	-1.1	5.0	3.7						
15	2.8	5.6	1.6						
16	-0.6	6.1	TR	0.16	7.6×10^1	8.0×10^1	-		
17	-4.4	7.2	-						
18	-5.6	7.8	5.2						
19	1.7	4.4	19.6	0.27	2.0×10^2	2.8×10^2	1.9×10^6		
20	2.8	5.0	43.6						
21	-0.6	4.4	5.2						
22	-2.8	5.6	8.7						
23			1.5	(1.2)	1.7×10^3	5.2×10^2	6.8×10^7	1	11
24			1.4	(See Table ix, excrement applied to field)					
25	1.1	5.6	7.0	0.53	9.0×10^3	2.1×10^3	2.0×10^7	5	<5
26	2.8	5.9	2.2	0.90	5.9×10^4	3.8×10^4	1.5×10^8		

Table ix. Data from Culbae, Area B (Figs. 23 and 24).

Date	Drainage Rate l/s, ha	E. coli/l	Enterococci/l	Bacteria on m-PCB/l	BOD mg/l	SS mg/l
8/11/72	0.06	9.6×10^1	-	4.8×10^5		
13/11/72	0.63	8.2×10^4	-	4.4×10^7		
16/11/72	0.37	2.2×10^3	1.2×10^3	3.8×10^6	2	<5
20/11/72					8	19
22/11/72	0.63	1.0×10^5	5.6×10^5	4.1×10^7		
27/11/72	0.33	9.0×10^2	1.1×10^3	1.3×10^6	9	9
30/11/72	0.58	1.6×10^4	2.1×10^4	2.7×10^7		
7/12/72	0.50	7.5×10^3	1.1×10^4	2.6×10^7		
12/12/72	0.50	3.6×10^3	2.4×10^3	1.1×10^7		
18/12/72	0.35	8.0×10^2	8.0×10^2	6.1×10^6		
21/12/72	0.22	1.2×10^2	-	3.8×10^5		
27/12/72	0.28	1.6×10^2	4.2×10^2	4.5×10^6		
5/ 1/73	0.35	3.5×10^2	3.5×10^2	1.2×10^6		
11/ 1/73	0.19	2.6×10^3	-	-		

Table x. Data from Culbae, Area A, after 160m^3 pig excrement sprayed onto the Longcastle field (Figs. 25 and 26).

Date	Time h	Drainage Rate l/s, ha	E. coli/l	Enterococci/l	Bacteria on m-PCB/l	BOD mg/l	SS mg/l
23/ 1/73	1030	1.20	1.7×10^3	5.2×10^2	6.8×10^7	1	11
	1100	Spraying started					
	1120	1.20	2.9×10^3	2.2×10^4	1.3×10^8	13	13
	1145	1.20	5.4×10^6	1.5×10^6	2.3×10^9	360	117
	1345	1.20	2.8×10^6	2.5×10^5	9.6×10^8	46	36
	1415	1.20	8.0×10^5	5.4×10^5	6.1×10^8	39	35
	1500	1.20	2.0×10^6	7.0×10^5	9.9×10^8	114	69
	1600	1.20	7.6×10^6	-	3.5×10^9		
	1700	1.20	2.2×10^6	1.9×10^6	9.0×10^8		
	1700	Spraying stopped					
	1800	1.20	1.0×10^6	7.9×10^5	6.2×10^8		
	1910	1.18	5.4×10^5	2.1×10^5	3.6×10^8	15	28
	2005	1.18	3.5×10^5	1.7×10^5	4.6×10^8	22	28
	2100	1.15	4.3×10^5	1.9×10^5	1.5×10^8	20	27
	2200	1.15	2.6×10^5	9.6×10^4	2.1×10^8	9	22
	2300	1.14	2.8×10^5	1.2×10^5	5.1×10^8	16	23
	2400	1.13	1.6×10^5	4.6×10^4	4.2×10^8	13	18

Table x (continued)

Date	Time h	Drainage Rate l/s, ha	E. coli/l	Enterococci/l	Bacteria on m-PCB/l	BOD mg/l	SS mg/l
24/ 1/73	0440	1.05	1.1×10^5	5.0×10^4	2.0×10^8	4	13
	0830	0.90	6.5×10^4	2.8×10^4	6.9×10^7	6	12
	1200	0.80	4.6×10^4	2.0×10^4	4.9×10^7	8	23
	2100	0.69	3.5×10^4	1.1×10^4	1.1×10^7	5	<5
25/ 1/73	1400	0.53	9.0×10^3	2.1×10^3	2.0×10^7		
26/ 1/73	1130	0.90	5.9×10^4	3.8×10^4	1.5×10^8		

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