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ABSTRACT OF A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF APPLIED SCIENCE

Studies on faecal coliform bacteria in sediment of the Avon-Heathcote Estuary, Christchurch, New Zealand.

by

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The shallow bar built, well flushed Avon-Heathcote Estuary is a popular recreational area. Water quality within the Estuary is reduced by inputs of enteric organisms, which may include pathogens, originating from the Avon and Heathcote Rivers, oxidation pond effluent (Christchurch Treatment Works), wildlife, recreational water users and runoff from the surrounding catchment. The sediments of estuaries have been shown to be a reservoir of bacteria of sanitary significance and so a study was undertaken to determine the importance of sediment in the Estuary.

Counts of faecal coliforms in sediment deposited at two intertidal sites during one tidal cycle ranged from 30 to 556 g^{-1} (dry weight) and numbers appeared to be influenced by factors including turbulence and sediment type. Experiments undertaken to determine the role of adsorption in the removal of faecal coliforms from the water column suggested that much higher densities than would normally be expected would be needed in the water overlying one intertidal site to explain

the observed counts unless other mechanisms were involved in the removal of faecal coliforms from the water column.

Movement of faecal coliforms through sediment packed into columns was studied after an *in situ* experiment suggested that faecal coliforms deposited onto surface sediment could move to deeper sediment. Experiments showed that sediment profiles appeared to retain relatively high proportions of the faecal coliforms applied to them. Enumeration of faecal coliforms in sections taken from sediment profiles showed that numbers of faecal coliforms retained in the sediment declined approximately logarithmically with depth. When sediment profiles containing high numbers of faecal coliforms were flushed with water of low conductivity relatively few faecal coliforms were removed from the sediment. Enumeration of faecal coliforms in sections taken from sediment profiles suggested that faecal coliforms removed from the surface could be retained further down sediment profiles. A second *in situ* experiment suggested that bacteria could potentially travel far below the surface layers of sediment if they remained suspended in percolating estuarine water.

A combination of gentle physical disturbance and reduction in the salinity (conductivity) of estuarine sediment-water mixtures resulted in substantial release of sediment bound faecal coliforms.

Sediment was demonstrated to extend the survival of faecal coliforms when estuarine water and sediment were incubated together at 15°C in the dark. This appeared to be substantiated by faecal coliform counts in sediment at an intertidal site which were higher than those in the overlying water according to a previous bacteriological survey.

Counts also varied seasonally while higher numbers of faecal coliforms were found in the 0-10 mm depth than at the 10-40 mm depth of sample cores.

The findings of this study appear to substantiate the belief that estuarine sediments can be reservoirs of bacteria of sanitary significance, therefore, further research on bacteria in estuarine sediment is warranted, especially in light of the extended survival of faecal coliforms in sediment and their potential release as shown in this study.

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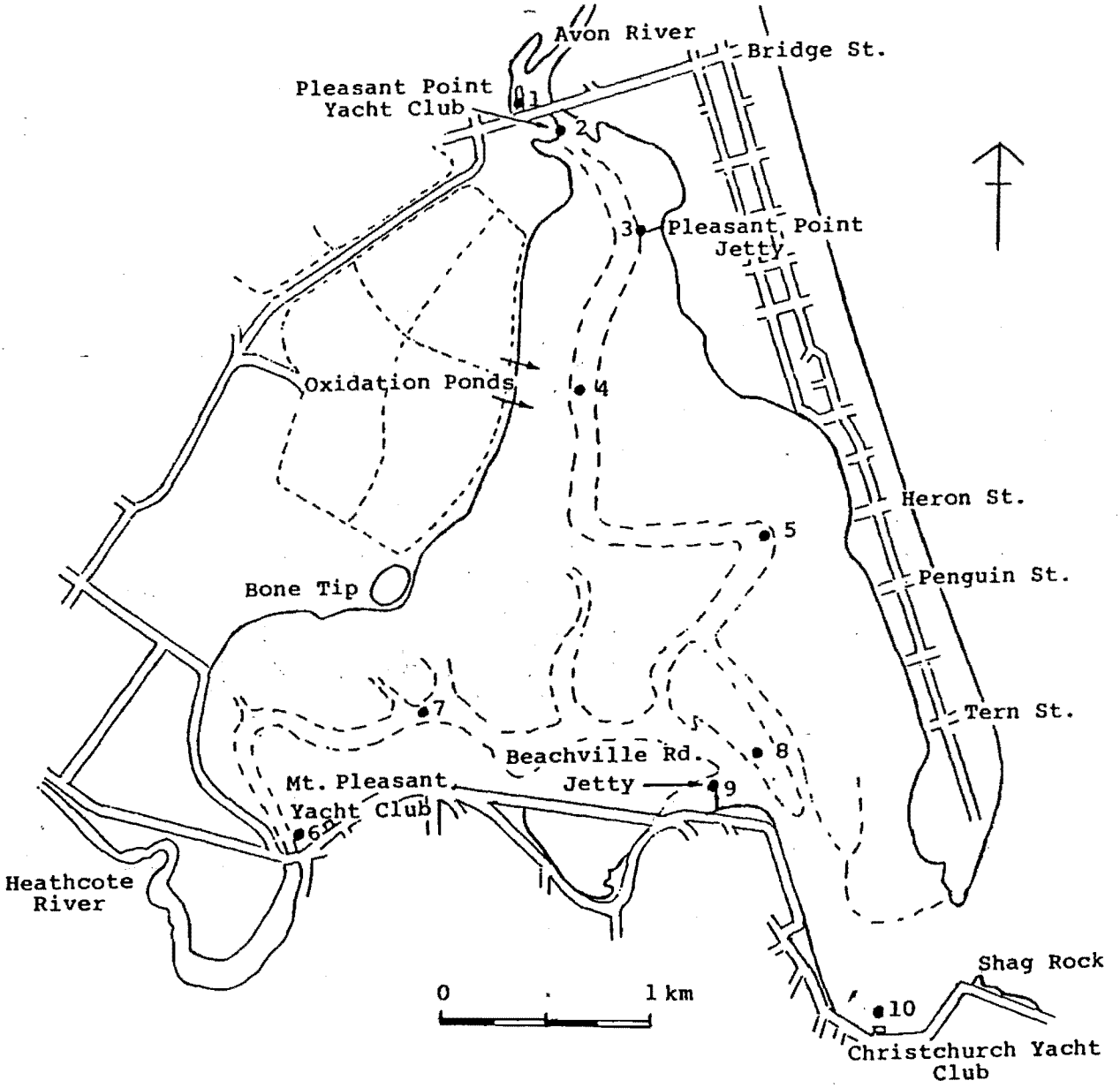
CHAPTER ONE

GENERAL INTRODUCTION

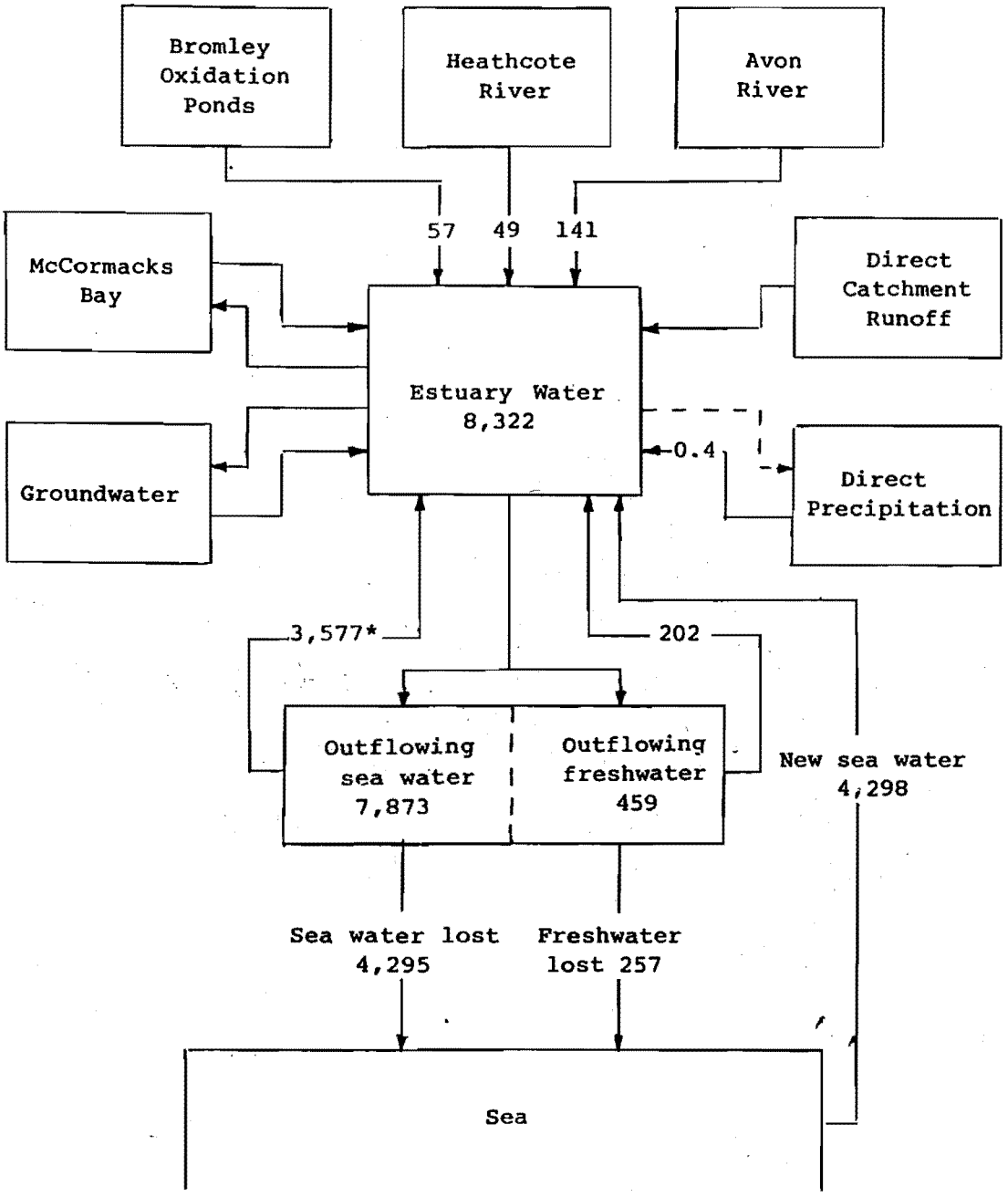
The Avon-Heathcote Estuary is situated approximately 6.5 km south-east of the centre of Christchurch City. It contains approximately 716 ha of tidal mudflats in the shape of an equilateral triangle with the Heathcote River entering at the western apex and the Avon River entering at the northern apex. The eastern margin is bounded by a narrow sandspit which separates the Estuary from the South Pacific Ocean (Christchurch Drainage Board, 1981) (Figure 1.1). The Estuary is a shallow bar built estuary with almost complete tidal exchange (Stephenson, 1980). The majority of the freshwater in the Estuary is contributed by the Avon and Heathcote Rivers (53 and 18%, respectively) while the Christchurch Drainage Board discharges most of the remainder from its treatment works at Bromley (Robb, 1974). The magnitudes of the freshwater and sea water loads are represented in Figure 1.2.

The sediment of the Estuary consists largely of mixtures of shell fragments (greater than 0.25 mm), fine sand (0.25 to 0.125 mm), very fine sand (0.125 to 0.0625 mm), silt (0.0625 to 0.0039 mm) and clay (0.0039 to 0.00006 mm) (Knox and Kilner, 1973). The distribution of these fractions is dependent on several factors including water velocity, the suspended particulate matter load in the water and water and wind action. This is reflected by the transition from sand at the mouth to essentially silt-clay at the head (Knox and Kilner, 1973) (Figure 1.3).



1. Bridge Street
2. Pleasant Point Yacht Club
3. Pleasant Point Jetty
4. Opposite outfalls from Christchurch Treatment Works
5. Opposite Heron Street
6. Mt. Pleasant Yacht Club
7. Opposite Bone Tip
8. Opposite Tern Street
9. Beachville Road Jetty
10. Christchurch Yacht Club

Figure 1.1 Sampling sites of the 1981 joint bacteriological survey of the Avon-Heathcote Estuary by the Christchurch Drainage Board and North Canterbury Catchment Board and the sampling sites used in this study.



Avon-Heathcote Estuary, Hydrology, Mean tide volumes (m^3 x 1000)

* based upon 44% return of freshwater

Figure 1.2 Hydrology of the Avon-Heathcote Estuary (Stephenson, 1980)

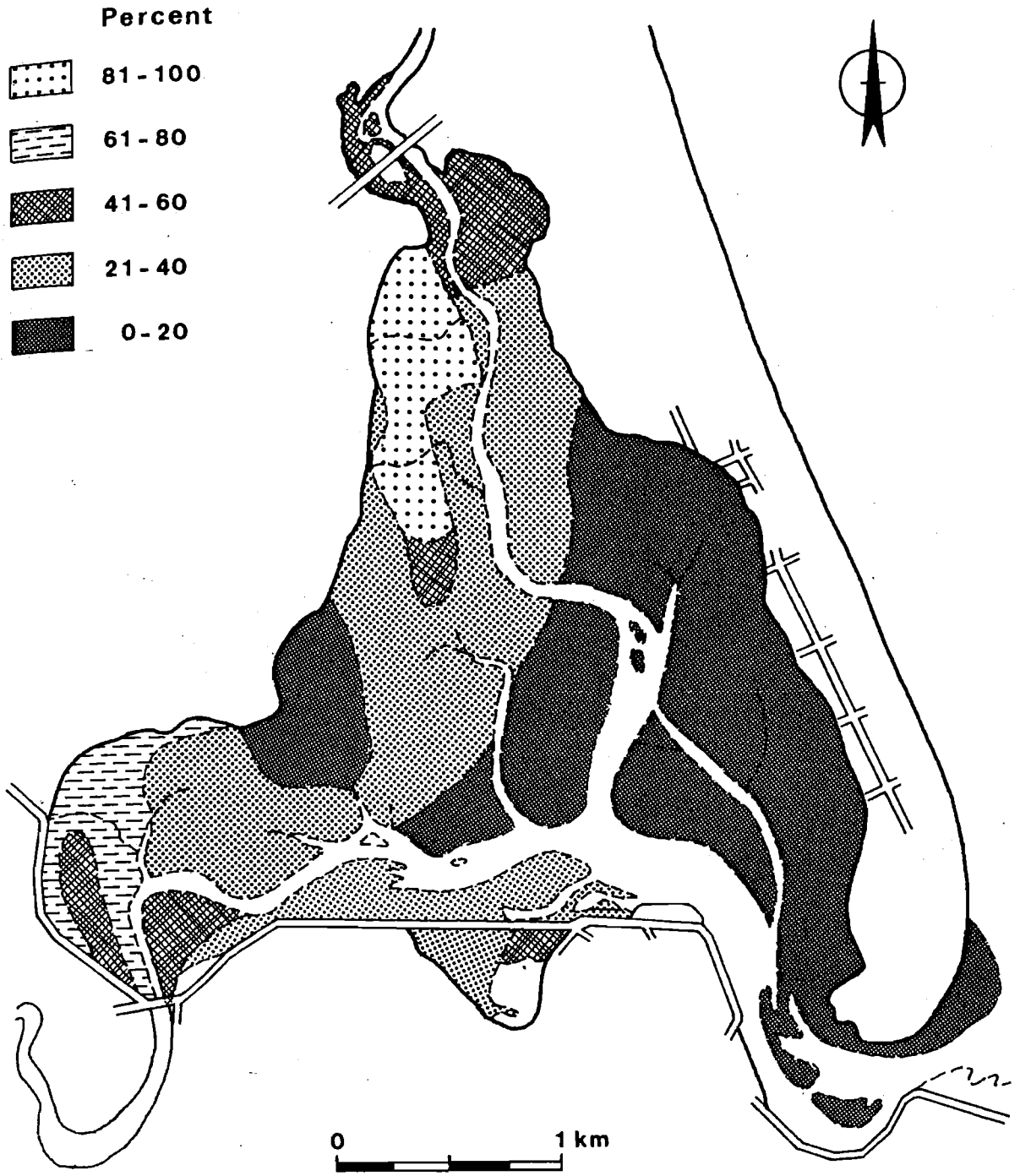


Figure 1.3 Sediment in the Estuary showing the percentage of the silt-clay fraction (Knox and Kilner, 1973)

The Estuary is a very important natural resource, serving as a convenient and popular recreational area for the people of Christchurch. It caters for a diversity of water sports including powerboating, water skiing, yachting, swimming, windsurfing and fishing. Continued use of the Estuary for recreation depends on maintenance of environmental quality as the two are closely linked (Knox and Kilner, 1973). Environmental quality of the Estuary is affected by its additional uses.

Both the Avon and Heathcote Rivers have in the past been used as convenient means of effluent disposal while treated sewage is released daily into the Estuary at high tide. Prior to 1970 no provision was made for the acceptance of industrial wastes from the heavily industrialised Woolston area into the Christchurch Drainage Board's sewerage system. In most instances wastes were discharged directly (frequently with little or no pre-treatment) into the lower reaches of the Heathcote River (Christchurch Drainage Board, 1981). A joint chemical and biological survey of the Avon and Heathcote Rivers by the North Canterbury Catchment Board and the Department of Industrial and Scientific Research (1975) illustrated the improvements of water quality in the two rivers, after comparsion of results with an earlier bacteriological survey by Hogan and Wilkinson (1959), since progressive incorporation of industrial discharges into the Christchurch sewerage system. A more recent bacteriological survey by the Christchurch Drainage and North Canterbury Catchment Boards in 1981 also concluded that the quality of water in the Estuary (particularly in the north-eastern sector) had improved significantly since 1973. This was attributed to to a corresponding improvement in the quality of effluent discharged from the Christchurch Treatment Works. The report also noted that within the Estuary-proper the highest bacterial concentrations

recorded were invariably from the two sites closest to the points of discharge of the two rivers (the Pleasant Point and Mount Pleasant Yacht Clubs) and that the SB standard was maintained over almost the entire Estuary throughout the sampling period. The results of this survey are summerized in Table 1.1.

Table 1.1 Summary of the joint bacteriological survey of the Avon-Heathcote Estuary by the Christchurch Drainage Board and the North Canterbury Catchment Board (1981). See Figure 1.1 for the location of sampling sites

	Faecal coliforms 100 ml^{-1} estuarine water			
	January 27 - March 3 1981		June 30 - August 12 1981	
Sampling Site	Range	Median	Range	Median
Pleasant Point Yacht Club	70-4145	590	45-3900	135
Pleasant Point Jetty	5-282	72	12-4040	162
Opposite Outfalls From Treatment Works	<5-405	72	Nil-1920	118
Opposite Heron Street	Nil-524	15	Nil-1010	36
Mt. Pleasant Yacht Club	<5-6300	85	5-1980	165
Opposite Bone Tip	Nil-35	4	Nil-1840	8
Beachville Road Jetty	<4-748	9.5	Nil-186	5
Christchurch Yacht Club	<4-508	8	Nil-196	8

environmental health implications of a catchment used for water supply contaminated by breeding gulls.

Enteric bacteria entering estuarine waters may remain in suspension or be deposited (sedimented) along with other particulate matter onto the underlying sediment as the salinity of the upper reaches of estuarine systems increase (Roper and Marshall, 1979). Investigators have attributed this phenomenon to processes such as adsorption of cells onto solid surfaces and flocculation. If faecal coliforms are deposited rapidly, then they may occur in relatively higher densities in the sediment compared to the overlying water. In this study, numbers of faecal coliforms were found in sediment deposited at two intertidal sites of the Estuary and the contribution of adsorption to the deposition of faecal coliforms was investigated. Densities of faecal coliforms enumerated in sediment samples were compared, on a volumetric basis, to the densities of faecal coliforms found in the water at similar locations in the Estuary by the 1981 Christchurch Drainage Board and North Canterbury Catchment Board bacteriological survey.

Once faecal coliforms and other enteric organisms are deposited onto sediment they may remain at the surface or be leached to deeper sediment depending on the "ability" of the sediment to retain them. If surface sediment does effectively retain most of the deposited enteric organisms then sediments may act as "sinks" or reservoirs of enteric organisms entering the estuarine environment. A number of investigators have found that enteric organisms do accumulate in sediments after deposition (Hendricks, 1971a; Van Donsel and Geldreich, 1971; De Flora *et al.*, 1975; Gerba and McLeod, 1976; Goyal *et al.*, 1977; Matson *et al.*, 1978; LaBelle *et al.*, 1980; Erkenbrecher, Jr., 1981; LaLiberte and Grimes, 1982;

Loutit and Lewis, 1985; Lewis *et al.*, 1986). These investigators have also found that survival of the enteric organisms is extended in the sediment. Experiments were conducted in this study to investigate the movement of faecal coliforms through sediment to determine if enteric organisms were likely to remain in surface sediment or be leached to deeper sediment. A survival experiment was also undertaken to compare the survival of faecal coliforms in estuarine water and sediment, incubated under controlled conditions of temperature and light, to determine if sediment extended the survival of faecal coliforms in the Estuary.

The extended survival of enteric organisms is of particular significance to public health because the release of sediment-bound enteric organisms to the water column has been reported after the physical disturbance of polluted sediment by dredging (Grimes 1975 and 1980). Wind-induced turbulence and motor boats, especially jet boats, are also capable of physically disturbing sediment in shallow waters and so may potentially create a temporary health hazard to water users in the vicinity of a polluted sediment. Release of *E. coli* from saline sediment upon reduction of the electrolyte concentration (conductivity) of the saline water overlying that sediment has also been demonstrsted by Roper and Marshall (1974). They believed desorption of saline sediment-bound enteric organisms could occur in estuarine systems and lagoons after dilution of the water during or after heavy rainfall or storms.

The health risk associated with recreational use of polluted waters stems from the fact that quantities of water are often unintentionally ingested by water users, particularly swimmers and water-skiers.

Quantities of polluted water may contain numbers of pathogenic agents sufficient to cause infection (infective doses). Diseases attributable to ingestion of polluted water range from typhoid to minor gastro-enteritis. Although severe infections appear to be rare, Shuval (1975) noted that there have been a number of studies that have implied that certain enteric disease outbreaks could be attributed to exposure to sewage contaminated sea water. The potential danger to water users from exposure to polluted water was emphasized in a programme that was initiated by the United States Environmental Protection Agency to re-examine the health effects, guidelines and standards for recreational waters in the United States. It was concluded from this programme that there was an increased risk of acute gastroenteritis associated with swimming in water polluted with wastewater (Cabelli *et al.*, 1983). It should also be mentioned that infections of a less serious nature are probably more frequent but tend not to get reported by the victim, consequently figures for such minor infections may underestimate the severity of the health risk associated with polluted recreational waters. The release of sediment-bound faecal coliforms was investigated in this study to determine if water users may be potentially exposed to water containing more pathogenic organisms during periods of high turbulence or large influxes of non-saline water.

CHAPTER TWO

DEPOSITION OF FAECAL COLIFORMS ONTO SEDIMENT

2.1 INTRODUCTION

The sediment of the Estuary originates from transport of particular matter by river flow, release of oxidation pond effluent from the Christchurch Treatment Works and marine currents. Although a variable proportion of the particulate matter is carried seaward each ebb tide, a proportion may also be deposited onto the sediment within the Estuary after flocculation of colloidal and near colloidal particles (Knox and Kilner, 1973). The sediment within the Estuary may also undergo relocation through wave action and wind-induced turbulence.

A number of investigators have suggested that the deposition or sedimentation of bacteria contributes to reduction of bacterial counts in natural waters (Rubentschik *et al.*, 1936; Weiss, 1951; Greenberg, 1956; Orlob, 1956; Rittenberg *et al.*, 1958; Carlucci and Pramer, 1959; Hendricks, 1971a; Jones, 1971; Matson *et al.*, 1978; Loutit and Lewis, 1985). Some of these investigators considered that adsorption of bacteria onto suspended particulate matter, enhanced the rate of deposition of bacteria and was an important process in their removal from the water column.

It was felt, that potentially high numbers of faecal coliforms entering the Estuary may be deposited onto the sediment and that adsorption was an important process in this phenomenon. In light of the

findings of Waksman and Vartiovaara (1938), Roper and Marshall (1974 and 1979) and Muller and Hickisch (1972), it was also considered that the salinity (conductivity) of the water and type of particulate matter suspended in the water, may influence the deposition of faecal coliforms onto the sediment.

In this chapter, an investigation is described which was undertaken to determine the numbers of faecal coliforms deposited onto two types of intertidal sediment in the Estuary, during one tidal cycle. Trays were put out at low tide and left over night at two sites. They were collected at the following low tide and their particulate matter and water contents analysed. Faecal coliforms were enumerated in samples of the sediment and water deposited in trays and the dry weight of sediment deposited was determined to estimate the number of faecal coliforms deposited onto an area of sediment. Two experiments were also conducted to determine the importance of adsorption in bacterial deposition. In the first experiment (I), faecal suspensions containing varying concentrations of faecal coliforms were added to 10 g sediment samples in flasks that were left over night on an orbital shaker. The next morning, the flasks were removed from the orbital shaker and left to stand for a period after which, the supernatant liquid of each flask was decanted and faecal coliforms were enumerated in the remaining sediment to determine the number of faecal coliforms adsorbed by the sediment. In an attempt to determine whether the mixing in experiment I was too violent to allow adsorption and whether the measurement of bacteria in sediments was an overestimate because of bacteria in interstitial water or an underestimate because of failure of the method of enumeration to remove adsorbed bacteria, a second experiment (II) was carried out. In this experiment, faecal coliforms were enumerated in samples of supernatant liquid from mixtures of faecal suspension and varying

concentrations of sediment, that had been gently rotated then left to stand for a period, to determine the removal of faecal coliforms and, therefore, the number of faecal coliforms adsorbed by the sediment. The concentrations of both faecal coliforms and sediment in mixtures were varied to determine if they influenced the degree of adsorption, suggested by the work of Hattori (1970) and Cooper (1977).

2.2 LITERATURE REVIEW

Over the years, the deposition of faecal coliforms and other enteric or indicator bacteria onto bottom sediment of natural waters has been investigated. From these investigations several processes have been suggested to contribute to their removal from the water column. Rubentschik *et al.* (1936) considered that adsorption onto sediment resuspended by tide and wave action was an important factor in the removal of *E.coli* from salt lake waters. Later, Weiss (1951) investigated the adsorption of *E. coli* on river and estuarine silts. In his experimental work, he discarded any silt fraction (usually sand grains) which settled immediately from suspension and fractionated some of the remaining silt into four particle sizes according to settling times (sedimentation). The silts and silt fractions were concentrated to a sufficiently high turbidity to allow preparation of any desired concentration by dilution. Volumes of silt suspensions were inoculated with aliquots of a stock suspension of *E. coli* and centrifuged after a suitable contact time. Aliquots of the supernatant liquid were removed and diluted for plating to determine percent adsorption. He concluded from his results that:

1. In the range of turbidities usually encountered in natural waters, *E. coli* were adsorbed to the particulate matter.

2. The degree of adsorption was characteristic of the origin and particle sizes of the silt.

3. Adsorption to silt particles increased the rate of sedimentation of bacterial cells.

4. Increases in salinity up to a limit of 5,000 g kg^{-1} increased the rate of flocculation of silts but reduced the adsorptive capacity of silts and, in some instances, caused desorption of *E. coli*.

Greenberg (1956), Orlob (1956) and Carlucci and Pramer (1959) also considered adsorption and sedimentation were important factors in the removal of enteric bacteria from the sea. Rittenberg *et al.* (1958), however, believed flocculation was more important than adsorption onto inorganic particles in promoting the sedimentation of coliforms in the areas around three marine sewage outfalls off California. Likewise, Faust *et al.* (1975) and Roper and Marshall (1979) also believed flocculation and sedimentation played an important role in the removal of bacteria from the water column. The later investigators found that coliform bacteria were deposited in bottom muds of the estuarine system they studied once the salinity exceeded a conductivity of 2.5 mS cm^{-1}. They considered sedimentation of suspended particles in estuarine systems was dependent on increasing salinity levels in a tidal zone and that as the salinity increased in the lower reaches of the estuary they studied, flocculation and sedimentation of both bacteria and particulates occurred.

Roper and Marshall (1974) demonstrated the sorption of *E. coli* (M 13), its specific bacteriophage and a portion of the native sediment

particles in suspension. Likewise, Cooper (1977) found that in mixed suspensions of clay (allophane) and *E. coli* the degree of removal of *E. coli* from suspension appeared to be dependent on the concentration of both components. Marshall *et al.* (1971) confirmed earlier work by ZoBell (1943) suggesting that sorption consisted of two phases, a reversible phase and a time-dependent irreversible phase. They defined reversible sorption as an essentially instantaneous attraction of bacteria to a surface. Such bacteria were said to be held weakly near the surface, still exhibiting Brownian motion and readily removed by the washing of the surface with 2.5% sodium chloride (NaCl). Irreversible sorption was defined as sorption involving the firm adhesion of bacteria to a surface; such bacteria did not exhibit Brownian motion and were not removed by washing of the surface with 2.5% NaCl. They also found that the electrolyte concentration at which all bacteria were repelled from a glass surface depended on the valency of the cation and interpreted the reversible phase in terms of the balance between the electrical double-layer replusion energies at different electrolyte concentrations and the van der Waals attractive energies. Daniels (1972 and 1980) extensively reviewed the literature on bacterial attachment in aquatic systems. This author considered the adsorption of micro-organisms onto surfaces could be viewed in three ways: (i) a number of microbial cells could become attached to a single larger surface, (ii) adsorbent particles and microbial cells of equal size could mutually interact, or (iii) several adsorbent particles of dimensions smaller than the microbial cells could adhere to a single cell. Daniels also divided up the mechanisms of microbial sorption into four, somewhat arbitrary, categories (Table 2.1).

Other authors have also extensively reviewed the literature

concerning bacterial attachment to surfaces in the aquatic environment (Corpe, 1970 and 1980; Marshall, 1976 and 1980; Fletcher, 1979).

Table 2.1 Mechanistic divisions of microbial sorption (Daniels, 1980)

Sorption division	Sorptive interaction	Sorption energy	Forces	Sorption sites
Chemical sorption	Specific; irreversible	High	Multiple; covalent bonding	Fixed
Ion exchange	Specific; reversible	Variable	Electrostatic	Fixed
Flocculation	Nonspecific; reversible + irreversible	Variable	Electrostatic; London-van der Waals	Variable
Physical sorption	Nonspecific; reversible	Low	London-van der Waals; interfacial tension	Variable

The most important factors involved in the sorption of bacteria onto particles appear to be the species of the bacteria and nature of the particle (adsorbent) (Daniels, 1972 and 1980). Weiss (1951) cited work by Dianowa and Woroschilowa (1925) who noted that different soil fractions adsorbed bacteria to different degrees, the finer fractions having a much greater adsorptive capacity than the coarser fractions. Waksman and Vartiovaara (1938) concluded that marine mud exerted an adsorptive effect upon bacteria in sea water but that sand had very little adsortive action upon bacteria in sea water. Muller and Hickisch (1972) tested 12 strains of bacteria and 9 adsorbents, including quartz, secondary clay minerals, artificial resins and loess subsoil. They

noted that the electrically neutral quartz with low cation exchange capacity (CEC) had the lowest adsorptive capacity (44%) of the adsorbents tested whereas the negativley charged secondary clay minerals with higher CEC had relatively high adsorptive capacities (75-92%). Bitton (1980) also noted that the assumption that the presence of clay minerals enhanced the adsorption of bacteria and viruses to soils was based on the high adsorptive capacity of clay minerals toward viruses which was attributed to the significant surface area and ion-exchange capacity of the minerals.

It appears from the literature that bacteria can be regarded as living colloids which benefit from attachment onto solid surfaces in the aquatic environment and that a number of mechanisms of attachment may exist.

2.3 ENUMERATION OF FAECAL COLIFORMS

The New Zealand Microbiological Society's Committee on Coliform Bacteria (1976) defined the faecal coliform group as all those organisms that are aerobic, facultatively anaerobic, Gram-negative, non-sporeforming, rod-shaped bacteria able to ferment lactose in 48 $\pm$ 2 h at 37 $\pm$ 0.5°C and in addition capable of fermenting lactose with the formation of gas in 24 $\pm$ 2 h at 44 $\pm$ 0.2°C. The bacteria that exhibit these characteristics belong mainly to the genera *Escherichia* and *Klebsiella*. The presence of faecal coliforms in the aquatic environment is generally considered indicative of faecal contamination by warm-blooded animals and, therefore, the potential presence of pathogens. Dufour (1977) noted, however, that situations have arisen where positive tests for faecal coliforms were observed in waters despite evidence suggesting no

faecal contamination had occurred. These aberrations were usually made on samples taken from waters receiving industrial effluents containing high concentrations of carbohydrate and were invariably due to *Klebsiella* species.

Enumeration of faecal coliforms usually involves the multiple tube or membrane filter techniques. Methods employing both of these techniques were used to determine the densites of faecal coliforms in samples of sediment, supernatant liquid and water analysed in this study.

The multiple tube technique for the enumeration of faecal coliforms gives a result expressed as the most probable number (MPN). The MPN method used to enumerate faecal coliforms in this study was based on the one recommended by the New Zealand Microbiological Society's Committee on Coliform Bacteria (1976) with one exception, an additional test was performed to exclude *Klebsiella* species which are indole negative (American Public Health Association, 1985), ie. the faecal coliforms enumerated were mainly *Escherichia* species (indole positive).

The membrane filtration method used was that given in "Standard Methods For the Examination of Water and Wastewater 16th Edition" (American Public Health Association, 1985). This method does not distinguish between faecal coliforms belonging to the genera *Escherichia* and *Klebsiella*.

Experiments in this study generally employed either the MPN or membrane filtration methods to enumerate faecal coliforms but sometimes both methods. It was considered that faecal coliform counts attained by both methods could be compared providing it was remembered that faecal

coliform counts attained by the membrane filtration method could include *Klebsiella* species and, therefore, be higher than faecal coliform counts attained by the MPN method, although there was unlikely to be high levels of carbohydrates in the water which would encourage the growth of *Klebsiella* species.

2.4 NUMBERS OF FAECAL COLIFORMS DEPOSITED ONTO SEDIMENT

2.4.1 Materials and Methods

2.4.1.1 Sites

The Tern Street site (refer to Figure 1.1) was located approximately 750 m due west of Tern Street (South Brighton Spit) and 1,200 m north-northwest of the mouth of the Estuary, on the edge of a large intertidal sand bar. This site was submerged for approximately 8 h each tidal cycle. The Bridge Street site (refer to Figure 1.1) was located approximately 30 m due north of the bridge across the Avon River, on intertidal mudflat which was submerged approximately 5 h each tidal cycle.

2.4.1.2 Sediment

According to Knox and Kilner's (1973) description of the sediment distribution within the Estuary, the sediment at the Bridge Street consisted predominantly of fine river borne particles, with a silt-clay fraction of 41-60%, while the sediment at the Tern Street site consisted predominantly of sand, containing less than 20% silt-clay fraction (refer to Figure 1.3) and between 1.0-1.75% organic matter (dry weight).

The organic matter content of the sediment at the Bridge Street site was not given in Knox and Kilner's description, but a similar sediment they described, 300 m southeast of the Bridge Street site, contained 3.0-7.0% organic matter (dry weight).

2.4.1.3 Sediment properties

Sediment was collected from the Tern Street site at low tide with a garden trowel and transferred into a plastic bucket. Approximately 2,000 ml of estuarine water was also collected and transported with the sediment back to the laboratory.

In order to determine some of the properties of the sediment from the Tern Street site, sediment was put into a plastic tray and allowed to dry for two days. A quantity of the sediment was removed from the tray and weighed on a Mettler PC 4400 balance before being added to a hollow glass column (22.5 mm diameter) with an angled outlet at one end, above which a fitted piece of filter paper (Whatman grade 17) and glass wool had been inserted. The bottom of the column and outlet were then bunged. Estuarine water was poured into the column while it was gently rotated washing the sediment down to the filter paper. The resulting sediment profile was stabbed with loop wire to remove any trapped air. The bottom of the column was un-bunged and left to drain. While the column was draining, sediment samples from the tray were transferred with a spatula to 10 drying tins, pre-weighed on a Mettler PC 4400 balance. The drying tins were re-weighed on the same balance then placed in a Qualtex oven set at 105°C and left to dry for approximately 24 h before they were re-weighed. After approximately 20 minutes the bottom of the column and outlet were re-bunged. Estuarine water was again added to the column but this time carefully so that the sediment

profile was not disturbed. The outlet was then un-bunged and the estuarine water allowed to drain until the top level of the water was just visible at the surface of the sediment. The top of the column was then bunged and the bottom unbunged allowing the trapped water beneath the filter paper and glass wool to escape. The depth of saturated sediment profile was measured and the glass wool and filter paper removed. The column was then weighed on the Mettler PC 4400 balance with and without the saturated sediment. From the measured parameters the water and particle components of the saturated sediment were determined and the bulk density (BD) calculated.

The same properties were determined for sediment at the Bridge Street site but because of its more structured nature a different procedure was used to determine the required parameters. When the tide no longer covered the sediment, five aluminiumn pipe segments were inserted into the sediment until they were level with the surface of the surrounding sediment. The segments were then carefully dug out with a garden trowel and any sediment stuck to the outside of the segments was removed. Each segment was put into a pre-weighed drying tin, taken back to the laboratory and weighed, put into a Qualtex oven set at 105°C for approximately 24 h and then reweighed. It was assumed that the sediment was saturated when removed.

2.4.1.4 Conductivities of overlying water

The conductivities of overlying waters were measured 3 h after high tide and at high tide at Tern and Bridge Street sites, respectively, with a portable HI 8333 conductivity meter (Hanna Instruments).

2.4.1.5 Sampling

Trays were left over night for one tidal cycle at the Tern Street site on April 20/21 and May 4/5 and at the Bridge Street site on May 4/5.

Holes were dug during low tide at the sites to fit plastic trays (374 mm long, 250 mm wide and 70 mm deep) so that the lip of each tray was 5 to 10 mm above the sediment surface. Each tray was then secured in position with wire tied around four metal rods which had been firmly inserted into the surrounding sediment to prevent any movement of the tray. Trays were collected the following low tide and a volume (approximately 200 ml) of the water left in each tray by the tide was collected in a sterile container while the trays were drained. The water samples were then placed in a plastic bucket (covered to prevent exposure to sunlight) and the trays put into plastic bags. Trays and water samples were transported back to the laboratory (40 minutes) where bacterial analysis of their contents was carried out within 4 h.

2.4.1.6 Bacterial analysis

Tray water samples and trays were immediately put into a cool room (7°C) on arrival to the laboratory and left there until required.

Tray water samples were analysed first. The method used to enumerate faecal coliforms was the most probable number (MPN) method described in section 2.3.

Minerals modified glutamate (MMG) (Oxoid) was used in a five-tube, mutliple-dilution multiple tube fermentation. Tubes were inoculated

with aliquots (drawn with sterile pipettes) of the appropriate dilution and placed in a Clayson air incubator set at 37°C for 48 ± 2 h. These were inspected for presumptive coliforms after approximately 24 and 48 h incubation. For each positive tube (yellow colour and gas production indicating lactose fermentation) a tube of BGBB (Oxoid) and a tube of peptone water (casein hydrolysate, Gibco) were inoculated with a loopful of culture from that positive tube and incubated in a water bath (Grant) set at 44.5 ± 0.2°C for approximately 24 h. Lactose fermentation (gas production) was deemed a positive reaction in BGBB tubes while indole production, tested for with Kovacs' Reagent (pink ring), was deemed a positive reaction in peptone water tubes. If both tubes gave a positive reaction then faecal coliforms, mainly of the genus *Escherichia*, were assumed to be present. The media used for this method were prepared according to the instructions given in Appendix II.

Dry weights of the sediment deposited in trays were determined by the method described in section 2.4.1.3.

To determine the density of faecal coliforms in sediment deposited in each tray, samples were removed with sterile spatulas and added to sterile 250 ml Kinex flasks, pre-weighed on a Mettler PC 4400 balance. The flasks were re-weighed on the same balance before 90 ml of 0.5% peptone water (bacteriological peptone, Oxoid) was added to each. They were then sealed with parafilm M (American Can Company) and placed on a Gallenkamp orbital shaker set at 180 rpm for 5 minutes. Faecal coliforms were also enumerated in aliquots of the supernatant liquid from each flask by the pre-described method.

2.4.1.7 Quantitative analysis

For each tray, the remaining sediment was emptied on to an Endecotts sieve (2.80 mm aperture) that had been placed on top of an open container which had been pre-weighed with its lid on a Mettler PC 4400 balance. The sediment was then searched for extraneous matter including shellfish, large shell fragments, seaweed and plant debri. This was removed with tweezers after any attached sediment had been carefully washed off. Containers were covered and left over night to allow suspended sediment to settle. Excess water was siphoned from each container and their lids inserted. The containers were then put into a Qualtex oven set at 160°C and removed for reweighing approximately 48 h later. The higher drying temperature and longer drying period were used to minimize the possible contribution of any extraneous organic matter that may have escaped sorting.

2.4.1.8 Statistical analysis

Statistical analysis was performed on data using Minitab on the Vax computer system at Lincoln College.

2.4.2 Results and Discussion

Table 2.2 shows the properties of sediment from the Tern and Bridge Street sites. The conductivities of the water at the Tern and Bridge Street sites were 9.0 and 40.7 mS cm^{-1}, respectively (equivalent to salinities of 5,296 and 27,719 g kg^{-1} or 15.1 and 79.2% sea water; calculated using the computer programme shown in Appendix III).

Table 2.2 Properties of 100 cm^3 of saturated sediment from the Tern and Bridge Street sites

Sediment property	Tern Street site	Bridge Street site
Moisture content (%)	35.57	57.64
Water volume (cm^3)	41.33	59.52
Dry weight volume (cm^3)	58.67	40.42
Dry weight (g)	116.17	103.64
Bulk density (g cm^{-3})	1.16	1.04

Table 2.3 Results of the bacterial analyses of water and sediment deposited in trays left at the Tern Street site on April 20/21 and May 4/5

		MPN estimates of faecal coliforms		
		Tray water	Tray sediment	
Tray	Date	100 ml^{-1}	100 ml^{-1}	g^{-1} dry wt.
1	April 20/21	7,000	31,914	275
2	April 20/21	3,300	11,598	100
3	April 20/21	7,900	28,450	245
4	April 20/21	4,900	22,786	196
5	May 4/5	490	3,485	30
6	May 4/5	1,700	8,016	69

Table 2.4 Results of the bacterial analyses of water and sediment deposited in trays left at the Bridge Street site on May 4/5

	MPN estimates of faecal coliforms		
	Tray water	Tray sediment	
Tray	100 ml^{-1}	100 ml^{-1}	g^{-1} dry wt.
7	950	57,624	556
8	790	32,750	316

Table 2.5 Quantitative analyses of sediment deposited in trays and the estimated numbers of faecal coliforms deposited per surface area of sediment (refer to Tables 2.3 and 2.4 for details of trays)

Tray	Dry weight of deposited sediment (g)	Number of faecal coliforms deposited x 10^5 m^{-2}
1	125.8	3.7
2	136.3	1.5
3	197.8	5.2
4	170.2	3.6
5	1,982.3	6.4
6	1,767.4	13.0
7	20.2	1.2
8	23.0	0.8

Tables 2.3 and 2.4 show the results of the bacterial analyses of tray contents from the Tern and Bridge Street sites, respectively. Table 2.5 shows the quantitative analyses of the sediment deposited at both sites and the estimated number of faecal coliforms deposited per surface area of sediment. Statistical analysis of data is shown in Appendix IV.

Densities of faecal coliforms in tray water samples of both sites are high compared with the densities of faecal coliforms found in water from nearby sites sampled in a bacteriological survey of the Estuary in 1981 (refer to Table 1.1). This may have been due to the resuspension of sediment containing high numbers of faecal coliforms when the water in each tray was drained into sterile containers (refer to section 2.4.1.5).

The amounts of sediment deposited in trays at the Tern Street site were significantly lower on April 20/21 than those on May 4/5 ($P = 0.04$) but were significantly higher than those at the Bridge Street site on both dates ($P = 0.0037$ and 0.037 for April 20/21 and May 4/5, respectively).

The significantly lower amount of sediment deposited at the Tern Street site on April 20/21 than on May 4/5 may be explained by the different water conditions experienced at the Estuary on the two dates. The weather during April 20/21 was cloudy (middle and high cloud) but calm. The maximum wind gust recorded was 29 knots but generally the breeze was around 6-15 knots, initially from the northeast then later from the northwest (Christchurch Meterological Service, 1986). This resulted in generally calm water conditions at the Estuary. On May 4 the weather was fine at first then cloudy. The wind changed to the

southwest at mid-day and then later a fairly strong Southerly breeze developed with a maximum recorded wind gust of 43 knots. On May 5 the weather was cloudy but calm and the wind had changed to a gentle Northerly (Christchurch Meterological Service, 1986). The weather on the night of May 4/5 resulted in turbulent water conditions and it appears that the greater turbulence experienced on May 4/5 compared with that on April 20/21 clearly resulted in the greater disturbance and relocation of sediment. The significantly lower amount of sediment deposited in trays at the Bridge Street site than at the Tern Street site on May 4/5 appears to be inconsistent with the suggestion that increased turbulence led to a greater disturbance and relocation of sediment but may be explained by the relative locations of the two sites and the nature of the sediments.

The Tern Street site was in open ground near the mouth of the Estuary and was exposed to weather from all directions whereas, the Bridge Street site was sheltered from southerly weather by a large man-made bank which underlies the road up to the bridge across the mouth of the Avon River. This appears to suggest that the Bridge Street site may have experienced less turbulence than the Tern Street site on the night of May 4/5. The significantly higher amount of sediment deposited at the Tern Street site on April 20/21 than at the Bridge Street on May 4/5 may also be explained if it is assumed that the turbulence at the Bridge Street site was less than at the Tern Street site on April 20/21. If this was not so, then the significant difference in the amount sediment deposited at the two sites on the two dates would appear to be caused by some other factor or factors influencing the deposition of suspended sediment. These may include the comparative periods the two sites were submerged, the resistance to physical disturbance of the sediment at the two sites and the salinity of the overlying water at the two sites.

The Bridge Street site, located at the northern head of the Estuary, was submerged for approximately 3 h less than the Tern Street site (refer to section 2.3.1.1). Therefore, the amount of sediment deposited at the Bridge Street site should have been less than that at the Tern Street site, assuming that turbulence and the rate of deposition of particulate matter were similar at the two sites on the different dates. The sediment at the Bridge Street site was more structured and more tightly packed than the loosely packed sediment at the Tern Street site and so may have been less susceptible to physical disturbance through turbulence. This also suggests that the sediment deposited at the Bridge Street site may have originated mainly from flocculation of particulate matter in the water overlying the site and not from the relocation of resuspended sediment. If, according to Roper and Marshall (1979), flocculation and deposition of suspended particular matter in estuarine systems is dependent on increasing salinity levels in the tidal zone, then the rate of deposition of particulate matter at the Tern Street site may have been higher than at the Bridge Street site because of the higher salinity of water overlying the Tern Street site.

Densities of faecal coliforms were significantly higher in deposited sediment than those in water from trays left at the Tern Street site on April 20/21 on a volumetric basis ($P = 0.029$) while densities of faecal coliforms were also higher in deposited sediment than those in water from trays left at the Bridge and Tern Street sites on May 4/5 on a volumetric basis but not significantly ($P = 0.17$ and 0.30, respectively).

Densities of faecal coliforms were significantly higher in water from trays left at the Tern Street site on April 20/21 than on May 4/5 ($P = 0.030$) and those in water from trays left at the Bridge Street site

on May 4/5 ($P = 0.018$). Densities of faecal coliforms were not significantly different in water from trays left at the Tern and Bridge Street sites on May 4/5 ($P = 0.78$).

Numbers of faecal coliforms g^{-1} of sediment (dry weight) deposited in trays left at the Tern Street site on April 20/21 and May 4/5 were not significantly different to those at the Bridge Street site on May 4/5 ($P = 0.32$ and 0.19, respectively).

The lack of significant difference observed between densities of faecal coliforms in deposited sediment and water from trays left at the Bridge and Tern Street sites on May 4/5 may be due to the small number of samples ($n = 2$) and the variability of samples used to compare the mean densities of faecal coliforms found in the deposited sediment and overlying water from trays (values for the coefficient of variation (CV) for the Bridge and Tern Street site water samples were 13.0 and 78.1%, respectively, and for the sediment samples they were 38.9 and 55.7%, respectively). Regardless of this, the results appear to suggest that faecal coliforms do not remain in suspension but are deposited along with particulate matter onto the sediment, however, the resuspension of sediment-bound indicator bacteria after physical disturbance of sediment has been reported (Grimes, 1975 and 1980). It would be expected, therefore, that tray water from the Tern Street site should contain higher densities of faecal coliforms on May 4/5 than on April 20/21, this was not observed. This discrepancy may, in part, be explained by the different amounts of solar radiation the trays received before collection on the two dates.

Trays were collected at approximately 9.00 am from the Tern and Bridge Street sites on April 21 and May 5 and between 9.30 and 10.00 am

from the Tern Street site on May 5. The amount of solar radiation the trays received before collection (Table 2.5; Christchurch Meterological Service, 1986) was approximately 0.5 MJ m^{-2} at the Tern Street site on April 21 compared with 0.6 MJ m^{-2} at the Bridge Street site and between 1.0 and 1.4 MJ m^{-2} at the Tern Street site on May 5. The greater solar radiation received at the Tern sites on May 5 than on April 21 may have caused greater die-off of faecal coliforms (Gameson and Saxon, 1967; Gameson and Gould, 1975; Bellair *et al.*, 1977; Chamberlin and Mitchell, 1978; Fujioka *et al.*, 1981; McCambridge and McMeekin, 1981). Bellair *et al.* (1977) derived an equation for the relationship between the time required for the density of faecal coliforms to decrease by 90% (T_{90}) in sea water and solar radiation. The equation

$$T_{90} = 3.4I^{-0.42} \qquad (2.1)$$

where T_{90} is in hours and I is the hourly solar radiation in MJ m^{-2}, can be used with the data in Table 2.6 to determine the die-off rate coefficient ($k = 1/T_{90}$) of faecal coliforms in the tray water. In turn, k can be substituted into Chick's Law (refer to equation 5.2) to estimate the initial densities of faecal coliforms present in the tray water at various times before collection (Table 2.7)

From Table 2.7 it would appear that the initial densities of faecal coliforms in tray water from the Tern Street site on May 4/5 are still lower than on April 20/21 even when the increased die-off of faecal coliforms in tray water from the Tern Street site on May 4/5 is taken into consideration. It should be noted, however, that this may not be the case if the die-off of faecal coliforms due to solar radiation is greater than that determined by Bellair *et al.*.

Table 2.6 Solar radiation (MJ m^{-2}) received by trays at the Tern and Bridge Street sites before collection

Time (am)	April 20/21	May 4/5
7.00 - 8.00	0.1	0.1
8.00 - 9.00	0.4	0.5
9.00 - 10.00	1.0	0.8

Table 2.7 The estimated mean densities of faecal coliforms 100 ml^{-1} tray water after the ebb tide

	Tern Street site		Bridge Street site
Time (am)	April 20/21	May 4/5	May 4/5
7.00	11,840	4,054 - 4,358	1,867
8.00	9,152	3,134 - 3,367	1,443
9.00	-	1,890 - 2,030	-

The suggestion that the greater turbulence of water overlying the Tern Street site led to greater resuspension of sediment-bound faecal coliforms appears to be substantiated by the numbers of faecal coliforms found in sediment (g^{-1} dry weight) deposited in trays left at the Tern Street site on both dates. It may also explain the lower numbers of faecal coliforms found in the sediment (g^{-1} dry weight) deposited at the Tern Street site on May 4/5, however, in another way. Deeper sediment containing fewer bacteria may have been resuspended by the turbulent

water and later deposited onto the trays diluting bacterial counts (Van Donsel and Geldreich, 1971; Babinchak *et al.*, 1977). The lack of significant differences observed between numbers of faecal coliforms found in sediment (g^{-1} dry weight) deposited in trays left at the Bridge Street site on May 4/5 and those at the Tern Street site on April 20/21 and May 4/5, however, would appear to be inconsistant with this suggestion. The lack of significant differences may again be the result of the small number and variability of samples (CV = 38.9% for the Bridge Street site (n = 2) and 37.5 and 55.7% for the Tern Street site on April 20/21 (n = 4) and May 4/5 (n = 2), respectively) used to compare the mean numbers of faecal coliforms in the sediment (g^{-1} dry weight) deposited in trays at both sites. This is suggested by the different mean numbers of faecal coliforms found in the sediment (g^{-1} dry weight) deposited in trays left at both sites (50 and 151 at the Tern Street site on May 4/5 and April 20/21, respectively, and 436 at the Bridge Street site on May 4/5). Alternatively, the lack of significant difference between densities of faecal coliforms found in sediment deposited in trays left at the Bridge Street site on May 4/5 and the Tern Street site on April 20/21 may also be explained if it is assumed that the turbulence at the sheltered Bridge Street site was similar to that at the Tern Street site and irrespective of the weather. If the deposition of bacteria is also dependent upon the salinity of water (Roper and Marshall, 1979) and submergence time, however, then it would be expected that fewer faecal coliforms would be deposited in trays left at the Bridge Street site than at the Tern Street because of the lower salinity of its overlying water and shorter submergence time. That this was not observed may be due to the influence the type of suspended matter has on the deposition of faecal coliforms, the densities of faecal coliforms in the water overlying the two sites or the comparative survival of faecal coliforms at the two sites.

The sediment at the Bridge Street site consisted of more clay and silt (refer to section 2.3.1.2) than the Tern Street site. If it is assumed that sorption of bacteria is enhanced by the presence of clay particles then suspended bacteria and clay particles would concentrate together, thereby, settling at a faster rate than discrete bacteria and particles of clay. From the 1981 bacteriological survey of the Estuary (refer to Table 1.1), the mean density of faecal coliforms in summer at a site nearby the Bridge Street site (the Pleasant Point Yacht Club) was 590 100 ml^{-1} of water while at a site nearby the Tern Street site (the Beachville Road Jetty) it was only 9.5 100 ml^{-1} of water. The likelihood that the overlying water at the Bridge Street site contained a greater concentration of clay particles and higher densities of faecal coliforms than the overlying water at the Tern Street site would appear to suggest that the influence of salinity on the deposition of faecal coliforms at the two sites was counteracted by these two factors. This would also explain the lower densities of faecal coliforms found in the tray water at the Bridge Street site on May 4/5. Alternatively, as a number of investigators have suggested, as bacterial survival decreases with increasing salinity (Carlucci and Pramer, 1960b; Hanes and Fragala, 1967; Faust *et al.*, 1975) any enhanced deposition at the Tern Street site may not have been found because of the higher mortality of faecal coliforms owing to the bactericidal effect of salinity.

The numbers of faecal coliforms in water overlying the two sites at any one time can also be estimated by using the densities of faecal coliforms found in water at nearby sites in the 1981 bacteriological survey and estimating the mean depth of the water overlying the two sites during the tidal cycle. From the Lyttelton tide tables (New Zealand Nautical Almanac (1986), 1985), the tide rose approximately 270 and 280 mm h^{-1} on April 20/21 and May 4/5, respectively. As the Bridge

Street site is submerged for approximately 5 h, it would be expected that the mean depth of the water overlying the site would be approximately 350 mm (depth at high tide would be approximately 700 mm). If the density of faecal coliforms in the water overlying the Bridge Street site was approximately 590 100 ml^{-1} (refer to Table 1.1), then the mean number of faecal coliforms in the water overlying 1 m^2 of Bridge Street site sediment would have been approximately 206,500. From Table 2.5, a mean of 100,000 faecal coliforms were deposited onto 1 m^2 of Bridge Street site sediment, therefore, it would appear that on average the water overlying the site held approximately twice the number faecal coliforms than the sediment deposited at the site.

If the density of faecal coliforms in the water overlying the Tern Street site on April 20/21 and May 4/5 was 9.5 100 ml^{-1} (refer to Table 1.1), then approximately 5,130 and 5,320 faecal coliforms would have been present in the water overlying 1 m^2 of Tern Street site sediment on April 20/21 and May 4/5, respectively (depth of water overlying the site at high tide would have been approximately 1,080 and 1,120 mm on April 20/21 and May 4/5, respectively). The high number of faecal coliforms estimated to be deposited onto the Tern Street site sediment (refer to Table 2.5) can not be attributed to deposition (removal) of the faecal coliforms estimated to be present in the water overlying the site alone. This appears to suggest that, either there were greater numbers of faecal coliforms present in the water overlying the site, or that most of the faecal coliforms estimated to be deposited were actually deposited previously but had been relocated along with resuspended sediment. This suggestion appears to be reasonable in light of the greater amounts of sediment found in trays at the Tern Street site than at the Bridge Street site. It would appear, therefore, that at the Bridge Street site where sediment and site characteristics were such

that little sediment was resuspended, the maximum number of faecal coliforms that may have been released into the water overlying sediment was only half the number held in the water but at the Tern Street site the numbers of faecal coliforms resuspended were many times the number in the overlying water. During resuspension, therefore, the net loss of faecal coliforms to the water from sediment is small unless die-off accounts for most of those released but since the trays were left out over night this should not be so.

The results also appear to be in general agreement to those of Erkenbrecher, Jr. (1981) who observed lower overall bacterial densities in coarse sediment of a site near the inlet of the estuary he studied, than at the headwater site characterised by decreased tidal action and lower salinity.

2.5 THE ROLE OF ADSORPTION IN THE REMOVAL OF FAECAL COLIFORMS FROM THE WATER COLUMN

2.5.1 Materials and Method

2.5.1.1 Faecal suspension

For experiment I, a mixture of estuarine water collected from a channel near the Tern Street site and bird faeces collected from the Estuary was shaken in a 1,000 ml screw cap laboratory bottle (Schott Duran) then left to settle for 5 minutes. The resulting suspension was decanted into a second 1,000 ml screw cap laboratory bottle and left to settle for another 5 minutes. The suspension was then filtered through an Endecotts sieve (250 µm aperture) into a third 1,000 ml screw cap laboratory bottle and diluted to 1,000 ml with estuarine water.

For experiment II, another faecal suspension was made up in the same way except the suspension was filtered into a 2,000 ml screw cap laboratory bottle and diluted to approximately 2,000 ml with estuarine water. Three 1 ml aliquots were drawn from the fecal suspension with a sterile pipette to determine the density of faecal coliforms present by the membrane filtration method described in section 2.3. The faecal suspensions were then stored in a cool room (7°C) until required.

2.5.1.2 Sediment

The sediment used in experiment I was collected from the Tern Street site then stored in a cool room (7°C) for 6 weeks. The sediment used in experiment II was also collected from the Tern street site, but was transferred to a plastic tray and air-dried for 7 days.

The moisture contents of both sediments were determined at the start of the adsorption experiments by the method described in section 2.4.1.3.

2.5.1.3 Experiment I

Ten g of sediment, weighed on a Mettler AC 100 balance, was added to each of 12 sterile 250 ml Kinex flasks. The flasks were divided up into six groups of two, each group receiving a different treatment (concentration of faecal suspension). Five groups of sediment received volumes of faecal suspension ranging from 10 to 10^{-3} ml that were diluted with autoclaved estuarine water (15 minutes at 121°C) to 100 ml. The remaining group received 100 ml of autoclaved estuarine water only. The flasks were then sealed with parafilm A (American Can Company) and left over night on a Gallenkamp orbital shaker set at 160

rpm. The next morning the flasks were removed from the orbital shaker and left to stand for 20 minutes. The supernatant liquid was decanted from each flask and faecal coliforms were enumerated in the remaining sediment within 4 h.

2.5.1.4 Experiment II

Eighteen sterile 250 ml polypropylene centrifuge bottles containing three replicates of six weights of air-dried sediment (6.25, 12.5, 25, 50, 100 and 200 g) from the Tern Street site were inoculated with 100 ml of faecal suspension. The centrifuge bottles were then tightly capped and placed upright in a plastic tray. A second tray was placed on top of the centrifuge bottles and fastened to the underlying tray. The "clamped" centrifuge bottles and remaining faecal suspension were then put into a cool room (7°C) where they were gently rotated (to simulate wave action) every 5 minutes for 1 h after which the top tray was removed. It was observed that the bottles containing the greater weights (100 and 200 g) of sediment had undergone poor mixing (the sediment had remained in a lump at the bottom of each centrifuge bottle) and so they were excluded from the experiment. The remaining centrifuge bottles were left to stand for 2 h. A 1 ml aliquot of the supernatant liquid was drawn from each centrifuge bottle with a sterile pipette and diluted to determine the density of faecal coliforms. Three 1 ml aliquots were also drawn from the remaining faecal suspension and diluted to determine they density of faecal coliforms added to the bottles.

2.5.1.5 Analysis of faecal coliforms

For experiment I, faecal coliforms were enumerated in the faecal suspension and sediment by the methods described in section 2.4.1.6.

For experiment II, the membrane filtration method described in section 2.3 was used to enumerate faecal coliforms in the supernatant liquid samples. Samples were diluted 10 and 100 fold based on the initial count made on the density of faecal coliforms in the faecal suspension (318,000 100 ml^{-1}) (refer to section 2.5.1.1). Faecal coliforms were also enumerated in samples of the sediment used in this experiment to determine initial numbers of faecal coliforms present by the membrane filtration method described in section 2.3.

2.5.1.6 Statistical analysis

Analysis of variance and regression analysis were performed on the data using Minitab.

2.5.2 Results and Discussion

The faecal suspension in experiment I contained 7 x 10^6 faecal coliforms 100 ml^{-1} of suspension. Table 2.7 shows the number of faecal coliforms added to the five groups of flasks in experiment I that received diluted volumes of faecal suspension and the mean number of faecal coliforms adsorbed g^{-1} (dry weight) of sediment. Only 2 faecal coliforms were enumerated in the 20 g of sediment that received autoclaved estuarine water only while no faecal coliforms were found in sediment that had been mixed with a suspension containing approximately 70 faecal coliforms. It was assumed that the numbers of faecal coliforms that may have been present in sediment used in this experiment were very low and could be neglected. The regression analysis (Appendix V) indicated that 79.7% of the variance in the number of faecal coliforms adsorbed g^{-1} of sediment (dry weight) was explained by the variation in the density of faecal coliforms in suspension. A

significantly higher number of faecal coliforms were adsorbed by the sediment as the density of faecal coliforms in suspension increased ($F = 31.45^{***}$). The proportion of faecal coliforms removed from suspension, however, was very small.

Table 2.8 The mean number of faecal coliforms adsorbed by sediment mixed with faecal suspensions of varying concentration

Concentration of faecal suspension faecal coliforms 100 ml^{-1}	Number of faecal coliforms adsorbed g^{-1}
70	0.0
700	0.3
7,000	4.4
70,000	157.3
700,000	1,784.3

The mean density of the faecal coliforms in the faecal suspension used in experiment II was 159,333 100 ml^{-1}. The moisture content of the sediment used was 0.83% (n=10) and no faecal coliforms were found in the samples of this sediment. Table 2.9 shows the mean densities of faecal coliforms removed from suspension for the different sediment-faecal suspension mixtures which were calculated by subtracting the mean densities of faecal coliforms found in the supernatant liquid samples from the mean density of faecal coliforms found in the faecal suspension. Analysis of variance (Appendix V) indicated that the densities of faecal coliforms in the supernatant liquid samples were not

significantly different to those the faecal suspension ($F = 1.79$, $P > 0.10$). The regression analysis (Appendix V) indicated that there was no relationship between the concentration of sediment in sediment-faecal suspension mixtures and the number of faecal coliforms adsorbed ($F = 3.22$, $P > 0.10$). It should be noted that the counts of faecal coliform colonies for all dilutions did not fall into the desired range of 20 to 60 faecal coliform colonies, ranging from 110 to 169, with values for CV ranging from 1.0 to 16.0%.

Table 2.9 Mean densities of faecal coliforms removed from suspension and the mean number of faecal coliforms adsorbed by sediment for varying sediment-faecal suspension mixtures

Sediment wet weight g	Mean density of faecal coliforms removed from suspension by sediment 100 ml^{-1}	Mean number adsorbed g^{-1} dry wt.
6.25	3,000	484
12.5	20,333	1,640
25	20,000	807
50	30,000	605

In section 2.3 it was suggested that sediment-bound faecal coliforms may be resuspended by physical disturbance of sediment and so the numbers of fecal coliforms in sediment used in these experiments were reduced with as little disturbance of the physical and chemical properties of the sediment as possible. For experiment I, the sediment was stored for 6 weeks before use. It was hoped that by this time all the faecal coliforms which may have been present in the sediment would

have died. From the results, however, it appears that even after 6 weeks cool storage of the sediment, some faecal coliforms survived. Although only small numbers of faecal coliforms did survive the storage, an alternative method of sediment sterilization was sought, therefore, the sediment used in experiment II was air-dried for 7 days. This was based on work by Beard (1940) who found that the survival time of *Salmonella typhosa* in sand was short during dry weather (between 4 and 7 d). As no faecal coliforms were isolated in samples of the sediment used in experiment II it was assumed that no faecal coliforms had survived the treatment and so air-drying appeared to be sufficient for this experiment. It should be mentioned, that the adsorptive capacity of the sediment may have been altered by the treatment process but that it was considered that sterilization of the sediment by autoclaving may have had a greater influence upon the adsorptive capacity of the sediment as heating of soil was reported to increase their adsorptive capacities (Daniels, 1972).

It was considered that any predators of faecal coliforms that may have been present in the sediment used in both experiments were present in very small numbers, if at all. Therefore, any potential removal of faecal coliforms from either sediment or supernatant liquid samples by predators introduced by the sediment could be neglected. The faecal suspensions, however, were made from bird faeces and water collected from the Estuary and so predators of faecal coliforms could be introduced into the sediment-faecal suspension mixtures by the faecal suspension and so the mean densities of faecal coliforms in the faecal suspensions were determined at the same time as the densities of faecal coliforms in the sediment and supernatant liquid samples. It was hoped that this would account for any potential removal of faecal coliforms owing to predation in the sediment-faecal suspension mixtures, assuming

that any potential predation of faecal coliforms in the faecal suspension occurred to the same extent in the sediment-faecal suspension mixtures. It was also hoped that determining the densities of faecal coliforms in the faecal suspensions at the same time as those in sediment and supernatant liquid samples would also account for any die-off of faecal coliforms that may have taken place during the experiments, assuming die-offs of faecal coliforms in the faecal suspensions were the same as those in the sediment-faecal suspension mixtures. In experiment II, if any differences were observed between the mean faecal coliform counts of supernatant liquid samples and the faecal suspension then it was assumed that the removal of faecal coliforms from suspension was due to adsorption between faecal coliforms and sediment particles.

The method used to mix sediment and faecal suspensions was changed in experiment II because it was felt that adsorption of faecal coliforms onto sediment in the experiment I may have been restricted by shear forces in the sediment-faecal suspension mixtures created by the orbital shaking of flasks. The method used to determine the number of faecal coliforms adsorbed by the sediment samples was also changed to see if both methods gave similar results. The supernatant liquid was analysed for faecal coliforms in experiment II in case the method used for elution of faecal coliforms from sediment in experiment I was ineffective.

The results are in general agreement with those of Waksman and Vartiovaara (1938) who found that sand exhibited little adsorptive capacity toward bacteria in sea water. The level of adsorption in experiment II was, therefore, too some extent, expected because the sediment used in this experiment consisted largely of sand with a silt-

clay fraction of less than 20% (refer to section 2.4.1.2). The particle size and surface area of these inorganic components are shown in Table 2.9. As the particle size and surface area of an adsorbent are important factors in adsorption, smaller particles and those with greater surface area are better adsorbents (Daniels, 1972), it would appear from this table that sand particles are much poorer adsorbents than clay particles. Therefore, as the bulk of the sediment particles in the sediment-faecal suspension mixtures of this experiment were sand grains, removal of faecal coliforms from suspension due to adsorption would have been low. Adsorption of bacteria onto particles also involves electrostatic attractions and so sand particles, which have little or no charge (electrically neutral), have low CEC which reflects their low adsorptive capacity. It would appear then, that the adsorption that may have taken place between faecal coliforms and sediment particles may have been due to the interaction of faecal coliform cells and clay particles.

Table 2.10 Characteristics of soil inorganic components (Burns, 1979)

Soil fraction	Diameter mm	Number of particles g^{-1} of particles	Surface area $m^2.g^{-1}$
Fine gravel	2.00-1.00	9.0×10^1	0.11
Coarse sand	1.00-0.50	7.2×10^2	0.23
Medium sand	0.50-0.25	5.8×10^3	0.45
Fine sand	0.25-0.10	4.6×10^4	0.91
Very fine sand	0.10-0.05	7.2×10^5	2.27
Silt	0.05-0.002	5.8×10^6	4.54
Clay	0.002	9.0×10^{10}	25-1000+

It should be noted that despite the observed insignificance of adsorption in experiment II, there did appear to be some relationship between the mean number of faecal coliforms removed from suspension and the concentration of sediment in sediment-faecal suspension mixtures. Although the regression analysis indicated that the relationship was not significant, the lack of significance may be due to the method used to determine the number of faecal coliforms adsorbed.

The significant relationship between the numbers of faecal coliforms adsorbed g^{-1} of sediment (dry weight) and the density of faecal coliforms in sediment-faecal suspension mixtures observed in experiment I was expected from the work of Hattori (1970) and Cooper (1977). This would also appear to suggest that the method used to determine the number of faecal coliforms adsorbed by sediment samples in experiment I was better that the one used in experiment II.

The numbers of faecal coliforms adsorbed g^{-1} of sediment (dry weight) in experiment II were variable, however, they were generally higher than those in experiment I taking into consideration the density of faecal coliforms in the faecal suspension used. It should also be remembered that the sediment and faecal suspensions were only mixed for 1 h in experiment II compared with over night (at least 14 h) in experiment I as mixing was by hand and not by machine. It would be expected, therefore, that more cell-sediment particle contacts (interactions) would have occurred in experiment I. It would appear then, that adsorption of faecal coliforms onto sediment particles may have been retarded too some extent in experiment I by the method used to mix sediment and faecal suspensions (orbital shaking). This may also explain why fewer faecal coliforms were deposited g^{-1} of sediment (dry weight) in trays left over night at the Tern street site on May 4/5 than

on April 20/21 in section 2.4 if the more turbulent water conditions had retarded adsorption of faecal coliforms onto sediment and "shaken-off" (released) loosely adsorbed faecal coliforms from sediment particles. It is also possible that the lower numbers of faecal coliforms found g^{-1} of sediment (dry weight) in experiment I could be explained if some of the faecal coliforms adsorbed by the sediment samples were not desorbed during the shaking of the sediment with peptone water.

Despite the apparent lack of adsorption, numbers of faecal coliforms removed g^{-1} of sediment (dry weight) in experiment II were generally higher than the numbers of faecal coliforms found g^{-1} of sediment (dry weight) deposited in trays left out over one tidal cycle at the Tern Street site in section 2.4. This suggests that the numbers of faecal coliforms deposited at the Tern street site could be attributed solely to adsorption, however, the density of faecal coliforms in the faecal suspension used in experiment II was much higher than would normally occur in the water of the Estuary. It would appear unlikely, therefore, that the number of faecal coliforms found g^{-1} of sediment (dry weight) deposited in trays could be explained solely by adsorption of faecal coliforms onto sediment particles.

If the presence of clay particles does increase adsorption of faecal coliforms then adsorption may play a significant role in the removal of faecal coliforms from the water overlying sediment of high clay content. Such sediment is mainly found in the vicinities of the Avon and Heathcote Rivers (refer to Figure 1.3).

If adsorption does not play a significant role in the removal of faecal coliforms from the water overlying sandy sediment of the Estuary then the numbers of faecal coliforms found in sediment deposited at the

Tern Street site (refer to section 2.4) may have been due to some other process. The sedimentation of free-living faecal coliforms appears to be unlikely because of their extremely slow settling times and because of the inhibiting effect of turbulence, however, if they are carried by currents (eddies) then they may be transported to the sediment surface and be filtered out from suspension. It may also be possible that faecal coliforms may enter the Estuary in aggregations (flocs) or in lumps of faecal material rather than as free-living cells and, therefore, settle more rapidly.

CHAPTER THREE

MOVEMENT OF FAECAL COLIFORMS THROUGH SEDIMENT

3.1 INTRODUCTION

Research on the movement of pollutant micro-organisms through natural soil systems has been mainly concerned with the possibility of groundwater contamination after wastewater application to various soils including sands. This is not normally a concern in estuarine systems, however, if pollutant micro-organisms move to deeper layers of sediment from the sediment surface they may represent less of a potential danger to water users. The horizontal movement or relocation of deposited faecal coliforms may result from wave action and wind-induced turbulence (refer to Chapter Two). It was felt, however, that faecal coliforms may also be transported down into deeper sediment layers via water infiltrating the sediment during the flood tide. Thus, the experiments described in this chapter were undertaken to study the vertical movement of faecal coliforms through sediment.

Initially, an experiment was undertaken to establish if there was evidence of *in situ* movement of faecal coliforms. Sediment was removed from two plots, autoclaved and then returned. Cockles (*Chione (Austrovenus) stutchburyi*) were inserted into the autoclaved sediment of one plot to determine if they influenced the leaching of faecal coliforms to deeper sediment through their activities. The cockle was chosen because it is the dominant species in the mudflat community of the Estuary (Knox and

Kilner, 1973), occurring in densities of greater than 2,000 m^{-2} (Stephenson, 1981). Faecal coliforms were enumerated in subsamples from 0-10 and 10-40 mm depths of sediment cores that were removed from the two plots to determine if their densities varied with depth and between plots. If faecal coliforms were found in samples from beneath the surface and numbers did not decline with depth, then it could be taken as evidence of replenishment or resupply from the surface. It should be noted, however, that if particulate matter was deposited at a fast rate and faecal coliforms survived for a long time in sediment, then the faecal coliforms found beneath the surface may have been buried rather than moved down with infiltrating water.

Later, experiments were conducted in the laboratory using columns packed with estuarine sediment consisting largely of sand. The removal of faecal coliforms from faecal suspensions by rapid infiltration (percolation) through sediment was studied by enumerating faecal coliforms in effluent samples. Faecal coliforms were also enumerated in samples of the sediment profile within columns after the percolation of the faecal suspensions to determine their distribution. Leaching of faecal coliforms from sediment to which faecal coliforms were applied was also studied by enumerating faecal coliforms from effluent samples after distilled water had percolated through the sediment. Faecal coliforms were again enumerated in samples of sediment profile after percolation to determine their distribution.

Finally, an attempt was made to determine the rate of infiltration of overlying water into estuarine sediment during one tidal cycle. Cylinders were inserted into the sediment and gradually filled with sea water (higher salinity than estuarine water), so that the water in the cylinders rose with incoming tide. After the tide had turned the

cylinders were allowed to drain, approximately maintaining the level of the outgoing tide. Sediment cores were taken from within and around the cylinders. Sediment from a particular depth of core was suspended in distilled water and the conductivity of the supernatant liquid measured. If the conductivity of the supernatant liquid of a suspension of sediment from within a cylinder was significantly different to that of surrounding sediment samples from a corresponding depth, it was assumed that sea water had infiltrated to that depth.

3.2 LITERATURE REVIEW

Land application has been used as a wastewater disposal method for many years (Schaub and Sorber, 1977). Thereby, studies on movement of micro-organisms through soil systems have been largely concerned with the potential contamination of groundwater by pathogenic micro-organisms. A number of these studies have attempted to evaluate the efficiency of sandy soil systems in the removal of micro-organisms from wastewater.

Butler *et al.* (1954) found that within the soil types they observed, bacteria removal in any given depth was appreciably less for soils of relatively large effective size than for finer soils. Dazzo *et al.* (1972) found that 90% removal of faecal coliforms from a percolating manure slurry occurred in the first 130 mm of fine sand that had been packed into a plexiglass column lysimeter. Bouwer *et al.* (1974) found that most of the faecal coliforms applied to rapid infiltration basins, consisting of sands and gravels, were removed in the top 600 mm of soil. Later, Gilbert *et al.* (1976) investigated the movement of bacteria in the same soil and found that 99.9% of faecal coliforms, faecal streptococci and

total bacteria were removed from wastewater filtered through 9 m of soil. Reneau, Jr. and Pettry (1975) studied the *in situ* movement of faecal and total coliforms in septic tank effluent through three Virginia Coastal Plain soils (two sandy loams and a loamy sand). They concluded that generally densities of both faecal and total coliforms decreased significantly with depth and horizontal distance from the source of the effluent. Schaub and Sorber (1977) found enteric indicator bacteria were readily concentrated on the soil surface by filtration of the soil surface mat formed by wastewater. The soil they studied was composed of silty sands and gravels of glacial origin underlaid by bedrock. Other investigators have also noted mat formation was significant in retaining bacteria (Krone *et al.*, 1958; Gerba and Bitton, 1984). It appears that the high solids content of certain percolates clog soil filtering sites, enhancing the filtration of bacteria.

Several investigators, however, suggest that soil systems may not be as efficient at removing micro-organisms from percolates as generally believed. Smith *et al.* (1985) noted that many studies of water movement and microbial and solute transport through soils were conducted on sieved, uniformly packed soil columns. They believed that when water flowed through macropores in soil it circumvented the soil matrix thus reducing the soils ability to remove dissolved or suspended matter carried in that water. In the soil they studied, they observed up to 96% of the bacteria applied to the surface of 280 mm deep intact columns leached out in the effluent. It should be noted that they used an antibiotic resistant strain of *E. coli* (K-12) and therefore any potential contamination of effluent samples by indigenous soil *E. coli* (introduced through faeces of warm-blooded animals) would have been avoided. Smith *et al.* also found that *E. coli* recovery in effluent increased as the rate of

water input increased. Likewise other investigators have also found infiltration rate influences the removal of bacteria in sand or sandy soils (Krone *et al.*, 1958; Gilbert *et al.*, 1976; Zohar *et al.*, 1971). In contrast to the findings of these investigators are those of Butler *et al.* (1954) who suggested there was no correlation between infiltration rate into various types of soil and the removal of bacteria during percolation of water through them.

Several investigators have noted the importance of the saturation status of soil in the movement of bacteria through soil. Schaub and Sorber (1977) cited work by McConkey and Krone (1967) who concluded that coliforms and other bacteria moved only a few feet in an unsaturated zone but several hundred feet in a saturated zone.

Investigators of bacterial movement in sand and other soils have consistently suggested the mechanisms of bacterial removal are straining or filtration, sedimentation, entrapment and adsorption. Krone *et al.* (1958) suggested that the accumulation of bacteria in the straining sites of soil enhanced the straining removal. Likewise, Gerba and Bitton (1984) believed that the straining or filtration of bacteria at the soil surface was a major limitation in their migration through soils. They also noted that adsorption was also a factor in the removal of bacteria by soil but that it played a more important role in the removal of micro-organisms in soils containing clays. Reneau, Jr. and Pettry (1975), in one of the sandy loams they studied, did not find any faecal coliforms beneath a pan, whereas faecal coliform densities above the pan were up to 2.4×10^6 100 ml^{-1} (water) or more.

It appears that a number of investigators who have studied bacterial movement in soil systems composed largely of sand, consider

that they are relatively efficient in removing bacteria from percolates and that distribution of bacteria in sandy soil systems rapidly declines beneath the surface.

The belief that sandy soils effectively remove bacteria from percolates is not surprizing as it has been recognized for many years that a sand filter will remove a proportion of the suspended solids in an effluent and that this, in turn, reduces the biochemical oxygen demand (BOD) and microbial population. Consequently, sand filters are often used in sewage treatment plants to purify or "polish" wastewaters and are capable of reducing total and faecal coliforms in wastewaters by 2 to 4 logs (United States Environmental Protection Agency, 1981).

According to Kreissl (1974) the major variables affecting performance of such filters can be grouped into three main categories: (i) influent solids characteristics (their physical and chemical nature as well as their concentration), (ii) liquid characteristics (filtration rate) and (iii) physical characteristics of the filter media (grain size and porosity). He considered that influent solids characteristics were the most important factors affecting filter performance as pretreatment of wastewater was the main determinant in filter performance (generally the concentration of suspended solids in a percolate determines the degree of filter surface clogging or surface mat formation and, therefore, the rate of infiltration of percolate).

In reality, the Estuary is like a slow or rapid sand filter with surface water movement that prevents surface clogging.

3.3 BUILD UP OF FAECAL COLIFORMS IN AUTOCLAVED SEDIMENT

3.3.1 Materials and Method

3.3.1.1 Sampling

The first plot was marked out at the Tern Street site at low tide on March 13. Sediment was removed from the plot to a depth of 50 mm with a garden trowel and searched for shellfish. Any shellfish found were counted and later discarded into a nearby channel. Shellfish-free sediment was transported back to Lincoln College in buckets and autoclaved for at least 2 h at 121 °C. The autoclaved sediment was returned to the plot on March 17 and compacted to the level of surrounding sediment after the site it was taken from was re-dug. Cockles were inserted (no deeper than 40 mm) into the autoclaved sediment in the density and approximate biomass they had been removed. A second plot was also marked out and sediment was removed and processed in the same way as in the first plot. The autoclaved sediment from the second plot was returned on March 19 and cockles were not replaced. Both plots were sampled on March 26 and April 14.

For sampling, the area within 100 mm of each plot edge was excluded. The remaining area was divided up into 64 squares of area 100 cm^2 using a string grid and numbered 1-64 from the top lefthand corner of each plot with respect to the tide. Random numbers from "Eaton's Statistical & Math Tables Fourth Edition" were divided up into two digit numbers excluding those less than 1 and greater than 64. Aluminium and PVC pipe segments of diameter 70 mm were pressed into the centres of grid squares (corresponding to the listed random numbers) to a depth of at least 50 mm. The pipe segments were then carefully removed with a

garden trowel ensuring that the sediment cores within remained intact. Four core samples were removed from each plot and transported back to the laboratory where bacterial analysis was carried out within 5 h.

3.3.1.2 Bacterial analysis

Core samples were stored in a cool room (7°C) until required. Sediment cores were paired and subsampled at 0-10 and 10-40 mm depths after removal from pipe segments. Two smaller cores were removed from the top centimetre of each core of a pair using segments of aluminium pipe that had been sharpened at one end. These were then transferred to a sterile 250 ml Kinex flask. The remaining sediment in the top 10 mm of each core was then sliced off with a sterile knife. One smaller core of depth 30 mm was removed from the remainder of each core and transferred to another sterile 250 ml Kinex flask. Faecal coliforms were enumerated in samples by the method described in section 2.4.1.6.

3.3.1.3 Statistical analysis

Statistical analysis was performed on data using Minitab.

3.3.2 Results and Discussion

Tables 3.1 and 3.2 show the mean estimates of faecal coliforms found in the 0-10 and 10-40 mm depths of plots 1 and 2 on both sampling dates. The wooden pegs used to mark the position of the plots were still visible, indicating that not more than 10 mm of sediment had been deposited onto the plots.

Table 3.1 Mean MPN estimates of densities of faecal coliforms 100 ml^{-1} of sediment samples collected from autoclaved sediment plots

Sediment Source	March 26		April 14	
	0-10 mm	10-40 mm	0-10 mm	10-40 mm
Plot 1 (autoclaved sediment and cockles)	10,215	5,000	42,560	2,838
Plot 2 (autoclaved sediment only)	18,571	4,381	109,226	4,941

Table 3.2 Numbers of faecal coliforms g^{-1} of sediment (dry weight) samples collected from autoclaved sediment plots

Sediment Source	March 26		April 14	
	0-10 mm	10-40 mm	0-10 mm	10-40 mm
Plot 1 (autoclaved sediment and cockles)	88	43	366	24
Plot 2 (autoclaved sediment only)	160	38	940	43

Analysis of data (Appendix VI) showed that densities of faecal coliforms in the 0-10 and 10-40 mm depths of plot 1 on March 26 were not significantly different to densities of faecal coliforms in those depths of plot 1 on April 14 ($P = 0.40$ and 0.51, respectively). Densities of faecal coliforms in the 0-10 and 10-40 mm depths of plot 2 on March 26 were also not significantly different to densities of faecal coliforms in those depths of plot 2 on April 14 ($P = 0.53$ and 0.84, respectively). Densities of faecal coliforms in the 0-10 mm depth tended to be higher

than those in the 10-40 mm depth of both plots but not significantly (P = 0.58 and 0.33 for plot 1 on March 26 and April 14, respectively, while P = 0.38 and 0.48 for plot 2 on March 26 and April 14, respectively). This may be the result of the small number (n=2) and varibility of samples used to compare mean counts of faecal coliforms for the two plots. For example, the densities of faecal coliforms in the 0-10 mm depth ranged from 3,762 to 208,333 100 ml^{-1} with values for CV ranging from 54.4 to 128.3%. The later count was much higher than the other counts of faecal coliforms for the 0-10 mm depth and markedly affected the statistical analysis of data. This higher count of faecal coliforms may have been due to enhanced survival or regrowth because of the increased availability of nutrients in autoclaved sediment (Gerba and McLeod, 1976). Marked numerical increases in *E. coli* have been observed in autoclaved sediment previously (LaLiberte and Grimes, 1982), while Hendrick (1971b) observed much higher respiration rates of *E. coli* in sediment eluted with phosphate buffer. He suggested that this was due to the increased availability of basal nutrients that were normally tightly adsorbed onto sediment particles and not readily available for metabolism by aquatic micro-organisms. Alternatively, it may have been the result of a bird dropping. When this count was omitted from the twosample *t*-test comparing the mean counts of faecal coliforms for the two depths, the probability of the means being different was almost significant at the 5% level (P = 0.065). The tendency for higher counts of faecal coliforms in the surface layers suggests that the upper layers of sediment may adsorb, readsorb and filter the majority of faecal coliforms deposited onto the sediment surface. The results also suggest that some faecal coliforms that are deposited onto the sediment surface do not remain there but infiltrate deeper into the sediment. The resupply of faecal coliforms to deeper sediment appears to maintain a reasonably uniform distribution of faecal coliforms found beneath the

surface. This was unaffected by the presence of cockles. It is possible, however, that the autoclaved sediment may not have been as tightly packed as surrounding sediment, being more porous and permeable to bacteria.

Densities of faecal coliforms in the 0-10 and 10-40 mm depths of plot 1 were not significantly different to densities of faecal coliforms in those depths of plot 2 on either March 26 ($P = 0.54$ and 0.82, respectively) or April 14 ($P = 0.63$ and 0.54, respectively). This was predictable from the characteristics of the cockle. According to Stephenson (1980 and 1981), cockles are relatively immobile, shallow burrowing (20 to 40 mm depending on age) filter-feeders. They may filter bacteria colonizing detritus and sediment particles but only assimilate a proportion of the filtered particulate matter, the rest being egested as pseudofaeces and faeces that settle onto the sediment. From these characteristics, cockles do not appear to physically disturb the sediment greatly which would provide access to deeper sediment. The majority of bacteria that are filtered from above the sediment surface would appear to be returned to the surface. The lack of significant differences between counts of faecal coliforms from plots 1 and 2 may also be explained by the small number ($n = 2$) and variability of samples used to compare mean counts of the two plots.

It should be noted, that although the level of the sediment surrounding the pegs used to mark out plots was similar throughout the experiment, suggesting that little or no new sediment had been deposited at the site, the possibility that all the autoclaved sediment was relocated by turbulent water and replaced with other sediment can not be ruled out. This would also account for the faecal coliforms observed beneath the surface and emphasizes the problems associated with *in situ* experiments.

3.4 MOVEMENT OF FAECAL COLIFORMS THROUGH SEDIMENT

3.4.1 Materials and Method

3.4.1.1 Sediment profiles

Sediment was collected from the Tern Street site, transported back to the laboratory and stored in a cool room (7°C) for approximately 5 weeks before it was used. On the day before it was required, the moisture content of the sediment was determined according to the method described in section 2.3.1.3 and was found to be 28.41%. From this value and parameters in Table 2.1, it was calculated that approximately 35 g of the sediment would produce a sediment profile of approximately 48 mm depth in the diameter of glass column used in this experiment (25 mm).

Fifteen glass columns were packed at one end with glass wool and sterilized in a Qualtex oven set at 160°C for 2 h. After the columns had cooled, 35 g of the sediment was added to each of them. Estuarine water, that had been autoclaved at 121°C for 15 minutes, was added to each column to suspend the sediment contained within. The sediment was allowed to settle as the autoclaved estuarine water drained and any air bubbles in the resulting profile were pierced with loop wire. The columns were then clamped into vertical positions onto a metal rod supported by two stands.

3.4.1.2 Treatments

A faecal suspension was made up according to the method described in section 2.4.1.1, except that autoclaved estuarine water was used.

Three volumes (400, 40 and 4 ml) of the faecal suspension were diluted with estuarine water to 4,000 ml, forming 10, 1 and 0.1% concentrations of faecal suspension.

Four groups of three sediment profiles received a total of 3,000 ml (approximately 420 pore volumes) of faecal suspension of different concentrations, intermittently, over a four day period. Twenty 50 ml volumes of a faecal suspension were applied to each sediment profile. The remaining group of three sediment profiles acted as a control receiving autoclaved estuarine water in the same volumes. Effluents of volumes 1, 5, 10, 15, and 20 were collected from each sediment profile in sterile 250 ml Kinex flasks and faecal coliforms were enumerated in dilutions or volumes of the effluent samples. Effluent samples were diluted according to the density of faecal coliforms in the faecal suspension applied.

The density of faecal coliforms in the 100% faecal suspension was determined the day before volumes of the faecal suspensions were applied. This was done so that the total number of faecal coliforms applied to each sediment could be calculated. The day after the last volumes of faecal suspension and autoclaved estuarine water were applied, the sediment profiles were sampled and faecal coliforms were enumerated to determine their distribution in the sediment profiles. Bacterial analysis of effluent samples was done within 2 h and sediment samples within 4 h.

3.4.1.3 Bacterial analysis

Densities of faecal coliforms in the 100% faecal suspension and effluent samples were determined according to the method described in section 2.4.1.4.

Total numbers of faecal coliforms were determined in sediment sections from three depths (0-5, 15-20, and 35-40 mm) of each group of sediment profiles according to the method described in section 2.4.1.4. Sediment profiles to which the 100% faecal suspension was applied were analysed first, all other sediment profiles were stored in a cool room (7°C) until required. The sediment profile in each column was pushed up with a section of 12 mm plastic tubing, inserted into the bottom of the column, until it was protruding approximately 5 mm above the lip of the column. The three protruding sediment sections were sliced off with a sterile knife into a sterile 250 ml Kinex flask. This procedure was continued and the 15-20 and 35-40 mm sediment sections from profiles were sliced off into a second and third sterile 250 ml Kinex flask. The dilution series of the multiple tube fermentation ranged from 10^{1} to 10^{-5} ml, depending on the concentration of faecal suspension applied to the sediment profiles.

3.4.2 Results and Discussion

Clogging became a problem in sediment profiles to which the 100 and 10% faecal suspensions were applied as the number of 50 ml volumes applied increased. Flow ceased from sediment profiles to which the 100% faecal suspension was applied after approximately 750 ml had been applied. The surface mats of the sediment were gently disturbed with a loop until flow returned. Infiltration rates of the 1 and 0.1% faecal suspension and autoclaved estuarine water were similar and relatively rapid, ranging from approximately 10 to 20 mm $min.^{-1}$. Table 3.3 shows the mean densities of faecal coliforms found in samples from the 100% faecal suspension before applications. These were used to estimate the total number of faecal coliforms applied to sediment profiles.

Table 3.3 Mean densities of faecal coliforms 100 ml^{-1} of the 100% faecal suspension over the period treatments were applied

Day 1	Day 2	Day 3	Day 4
350,000	143,000	89,000	46,000

Figures 3.1 to 3.4 show the mean proportion of faecal coliforms remaining in percolates of 100, 10, 1 and 0.1% faecal suspensions. No faecal coliforms were detected in effluent sampled from the sediment profiles to which autoclaved estuarine water was applied. Figure 3.5 shows the distribution of faecal coliforms in the sediment profiles to which faecal suspensions of different concentrations were applied. Only 2 faecal coliforms were found in the 9 sediment sections (22.1 cm^3) from the control sediment profiles. As no faecal coliforms were detected in effluent samples as well, it was assumed number of viable "indigenous" faecal coliforms in sediment profiles were negligible, thereby, all faecal coliforms enumerated in sediment samples from the other sediment profiles were considered to be introduced by the percolate.

The results showed similar trends to those of Krone *et al.* (1958). They applied diluted cultures of *E. coli*, isolated from sewage, to sieved sand (four different diameters, ranging from 0.063 to 1.1 mm) that had been poured into columns dry while the side of each column was tapped with a mallet. The porosites of the sand were 0.43 to 0.45. They found that *E. coli* numbers rose in column effluent abruptly until sufficient *E. coli* had accumulated in the filtering sites, enhancing removal by filtration. They noted a condition of mechanical instability of the accumulates in the presence of percolant soon followed resulting in a

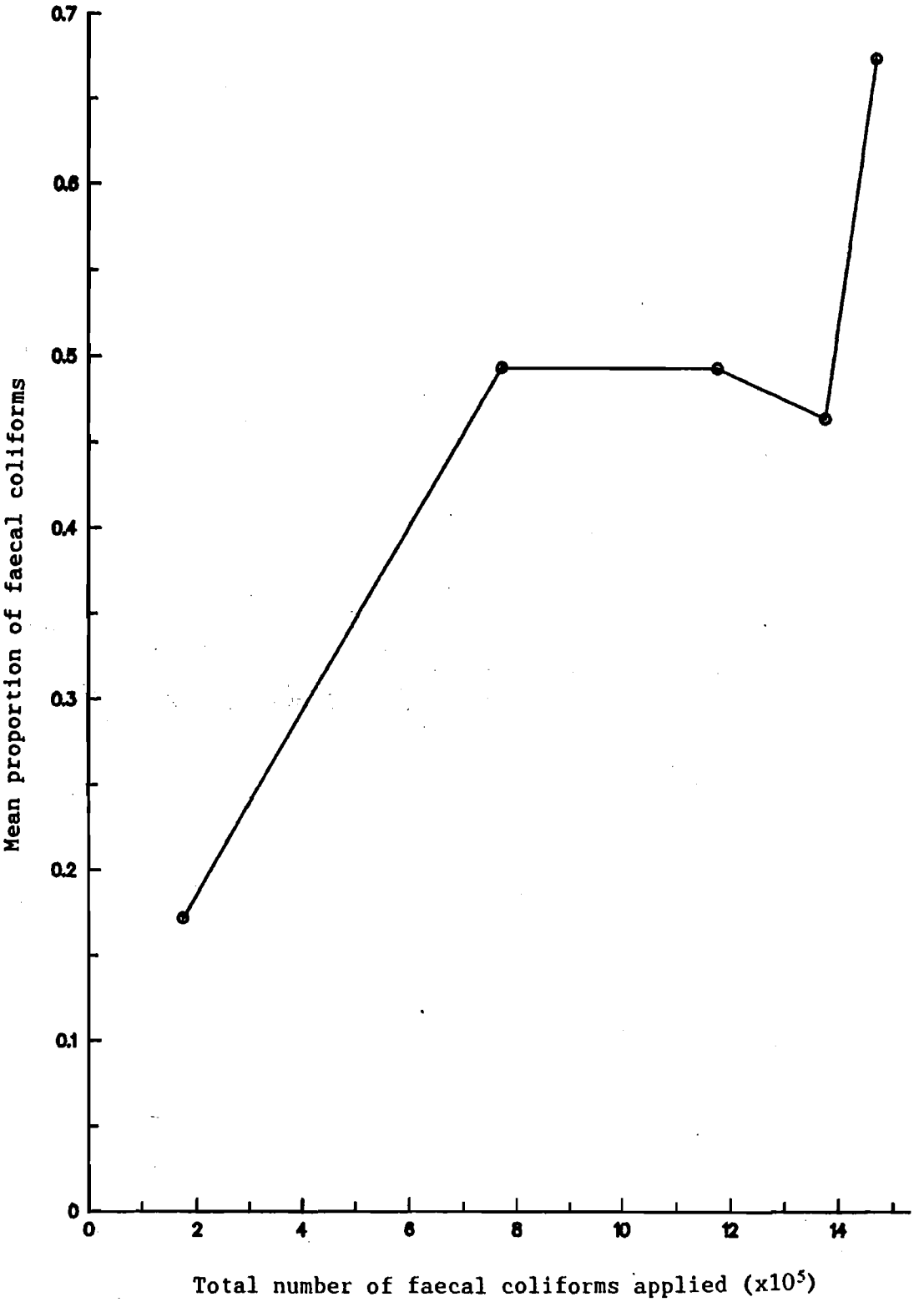


Figure 3.1 The mean proportion of faecal coliforms remaining in percolates of the 100% faecal suspension

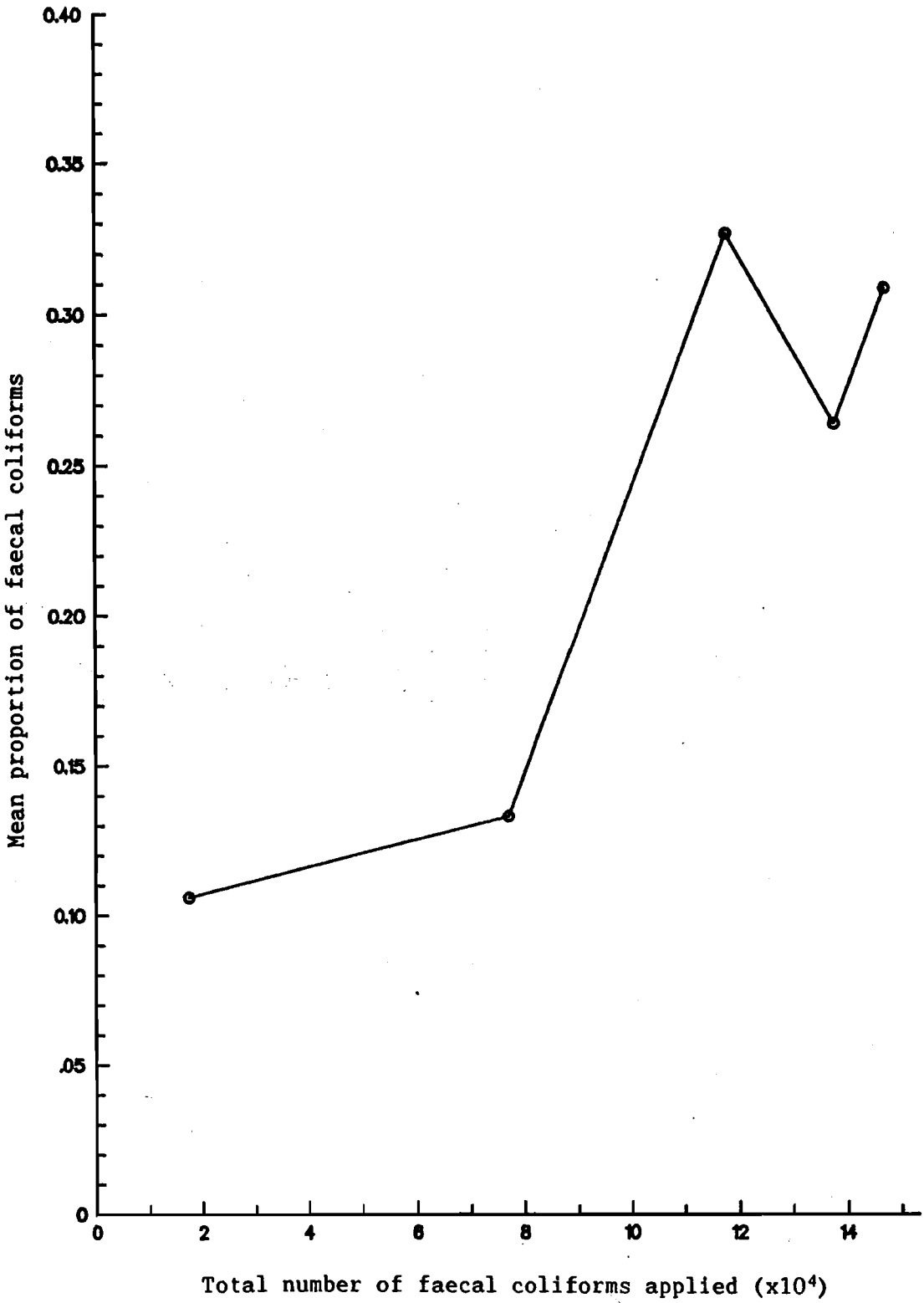


Figure 3.2 The mean proportion of faecal coliforms remaining in percolates of the 10% faecal suspension

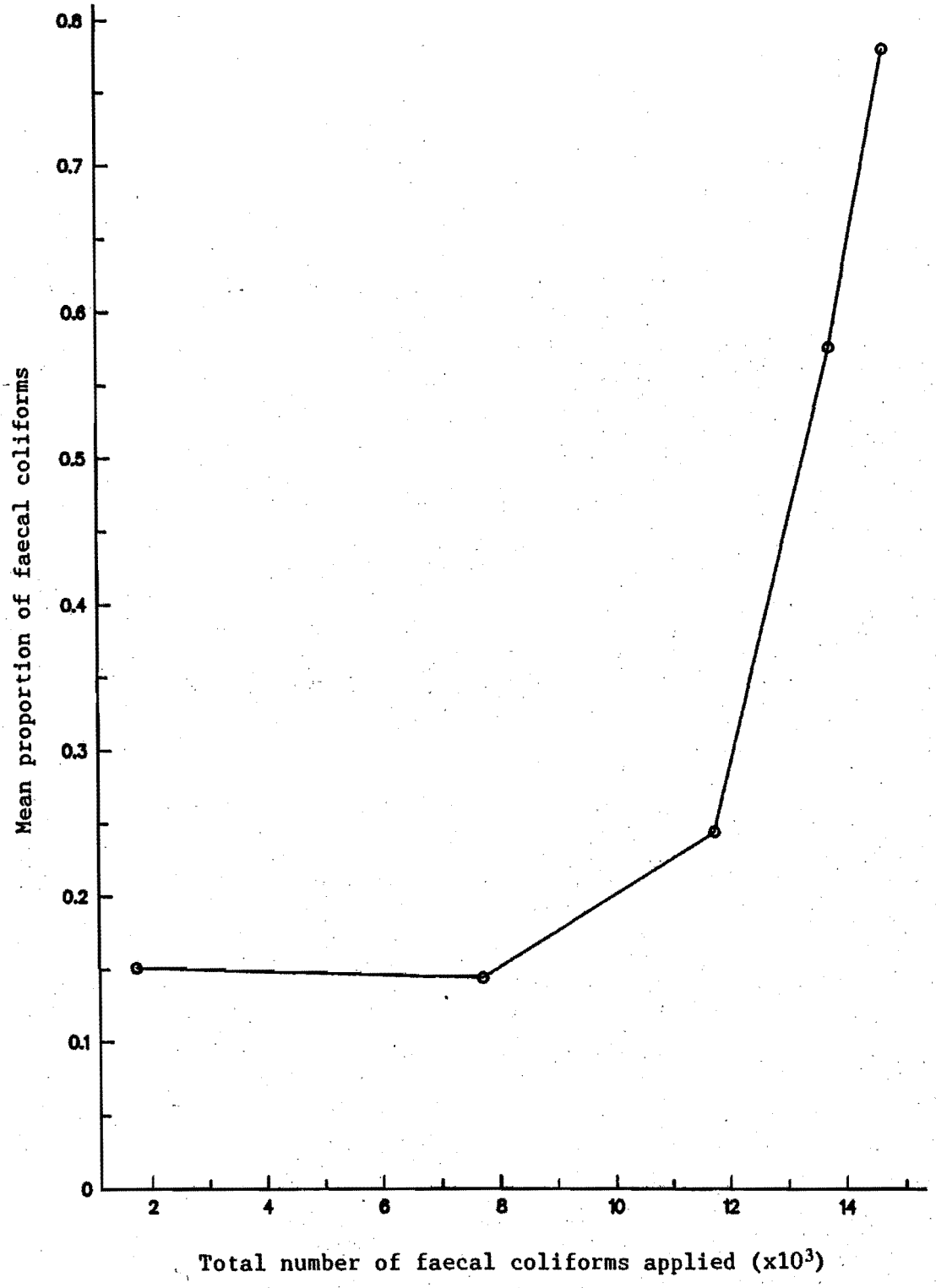


Figure 3.3 The mean proportion of faecal coliforms remaining in percolates of the 1% faecal suspension

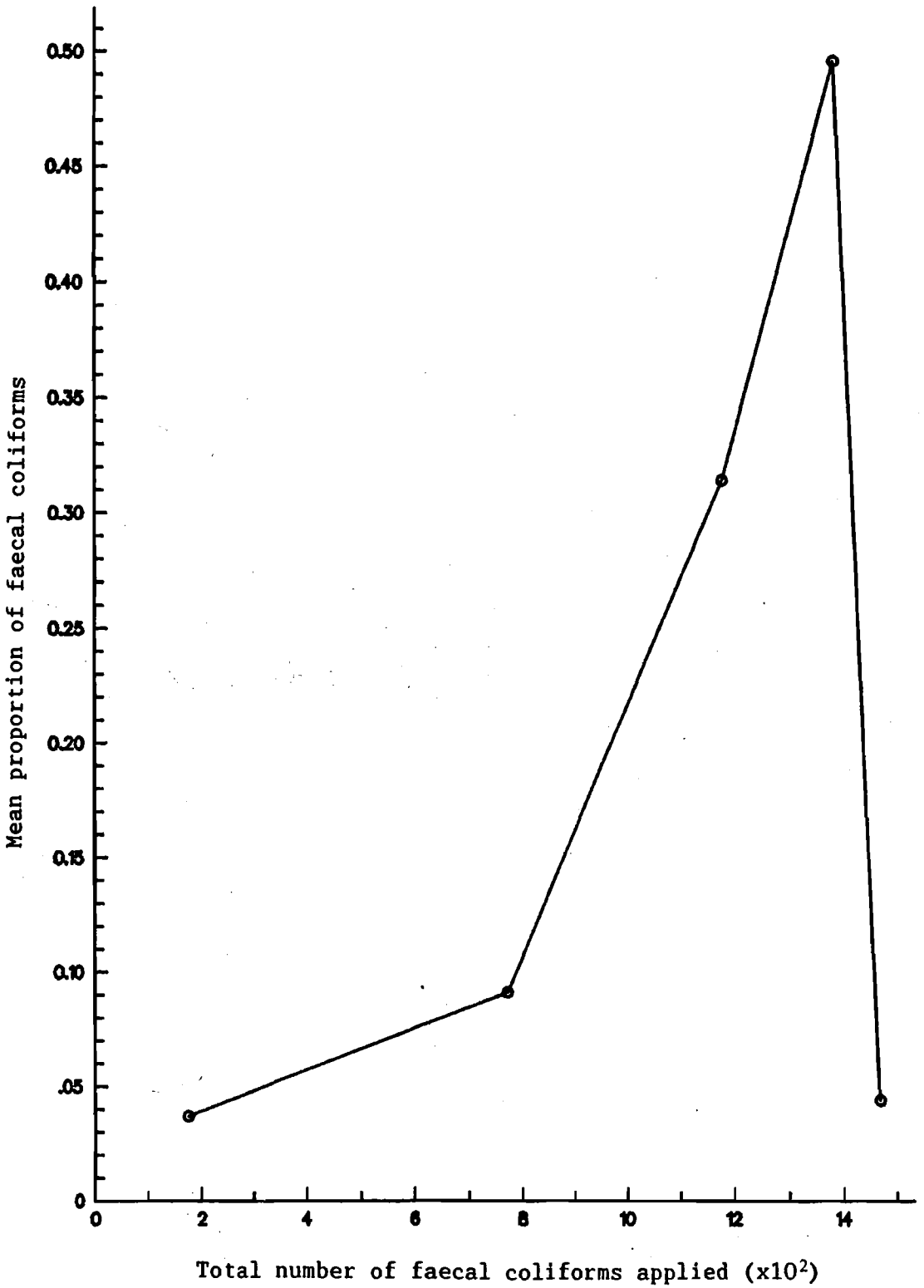


Figure 3.4 The mean proportion of faecal coliforms remaining in percolates of the 0.1% faecal suspension

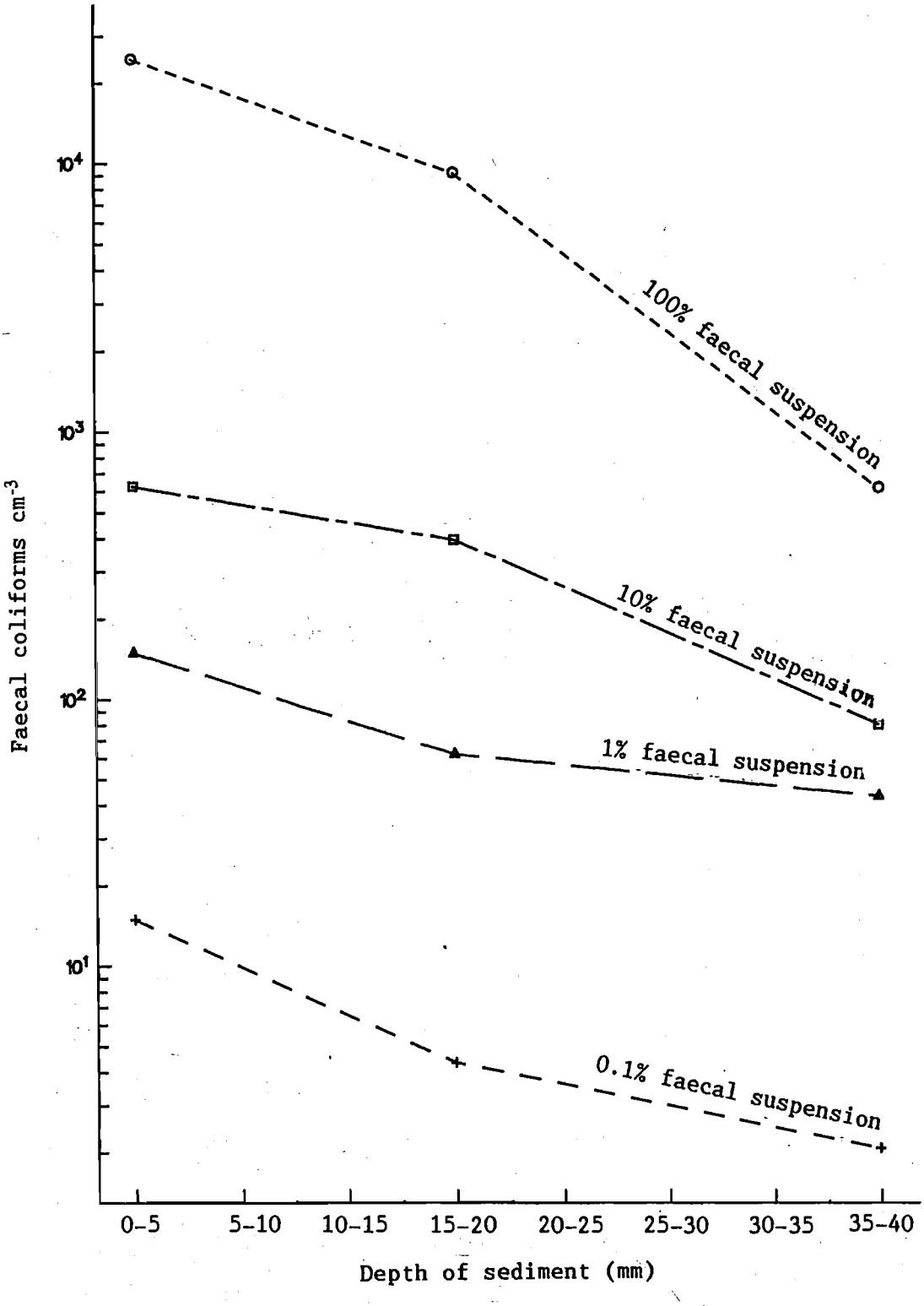


Figure 3.5 The distribution of faecal coliforms retained in sediment profiles to which faecal suspensions of varying concentration were applied

"cascade" of organisms and clusters of organisms and a rapid advancement of the "front of saturated straining (filtering) sites". Figures 3.1 and 3.2 appear to show this phenomenon, although the rise of faecal coliform numbers in the effluent is not as abrupt as the rise Krone *et al.* observed. This may have arisen from the nature of the sediment used in this experiment which was not sieved and so may have contained finer particles which would be more restrictive to bacterial movement, ie. the sediment may have been less permeable. Sediment profiles to which the 100% faecal suspension was applied (Figure 3.1) showed the "enhanced filtration" stage before sediment profiles to which the 10% faecal suspension was applied (Figure 3.2). This would appear predictable on the basis that the total number of faecal coliforms applied to the sediment profiles to which the 100% faecal suspension was applied at any one time was approximately one log higher than at the same time as sediment to which the 10% faecal suspension was applied. It is also likely that the 100% faecal suspension contained approximately a one log higher concentration of suspended solids (reflected by the relative degree of clogging between the profiles) that would also accumulate at filtering sites, thereby, enhancing filtration. This would also explain why no enhanced filtration stage was observed in sediment profiles to which the 1% faecal suspension was applied (Figure 3.3) suggesting that bacterial accumulation in pores had not reached a level that would restrict the passage of bacteria in the percolate. In contrast to this suggestion is the apparent enhanced filtration stage observed in sediment to which the 0.1% faecal suspension was applied. This occurred however, at a stage when only an average of 23 viable faecal coliforms were present in the 50 ml volumes of percolate applied. The low numbers of faecal coliforms, therefore, may have made enumeration inaccurate.

It should be noted that the increase, in the number of faecal coliforms in the last effluent sampled from sediment profiles to which the 100% faecal suspension was applied, was observed after the surface mats of the sediment were disturbed. The disruption of the surface mat would most certainly lead to the freer passage of bacteria carried in the percolate. This is probably similar to the disturbance of surface sediment layers which occurs when water currents are generated.

The distribution of faecal coliforms in sediment profiles to which different concentrations of faecal suspensions were applied was similar. Numbers were highest in the surface and declined with depth, approximately logarithmically, in agreement with the findings of Krone *et al.* (1958) and Zohar *et al.* (1971).

It was expected that numbers of faecal coliforms in sediment profiles to which the 100% faecal suspension was applied would be approximately one, two and three logs higher than those in sediment profiles to which the 10, 1 and 0.1% faecal suspensions were applied, respectively. This appeared to be generally true. The slight variations in the expected ratios may have been due to dilution errors and clumping of faecal coliforms. This does not explain, however, the higher than expected number of faecal coliforms observed in the 35-40 mm depth from sediment profiles to which the 1% faecal suspension was applied. The mean density of faecal coliforms found at that depth was relatively low (10 cm^{-3}) and, therefore, accurate bacterial enumeration would require precise technique. Sediment sections analysed were sliced off in approximately 5 mm depths and so it is possible that a slightly deeper section may have been sliced off, biasing the result. It may also be possible that a cluster of faecal coliforms may have avoided filtration until that depth. Although the number of faecal coliforms

found in the control sediment profiles was very small and considered negligible, the possible contamination of the count with indigenous faecal coliforms can not be ruled out.

The results also appear to suggest that the number of faecal coliforms retained by sediment profiles is relatively high compared with the total number of faecal coliforms applied. For example, the approximate number of faecal coliforms retained by each sediment profile to which the 100% faecal suspension was applied was 991,500 (calculated by subtracting the number of faecal coliforms enumerated in effluent samples from the total number of faecal coliforms applied) or 67.6% of the total number of faecal coliforms applied (1,466,500). In Figure 3.5, however, the number of faecal coliforms found in the first 40 mm of sediment profiles to which the 100% faecal suspension was applied was only 154,200 (area under the graph) or 15.6% of the estimated number retained. This discrepancy may be explained by three factors: (i) die-off, (ii) ineffective removal of faecal coliforms from sediment particles during enumeration and (iii) faecal coliforms retained in the sediment that was not analysed, ie. the 8 mm (2.8 cm^3) of sediment remaining in the columns. The later can be estimated if it is assumed that the distributions of faecal coliforms in the unanalysed sediment were the same as those found in the 20-40 mm depths of sediment profiles (ie. extrapolation). For example, the unanalysed sediment of sediment profiles to which the 100% faecal suspension was applied could have contained approximately 940 faecal coliforms or 0.1% of the estimated number retained by the profiles. Such a small proportion suggests that the numbers of faecal coliforms retained by unanalysed sediment in this experiment were insignificant in terms of the numbers of faecal coliforms estimated to be retained by sediment profiles to which the faecal suspensions were applied.

This experiment demonstrated that although faecal coliforms can pass through sediment packed in columns, a fairly high proportion are retained or die in the surface 40 mm (approximately) of sediment. If a similar pattern of faecal coliform removal takes place in estuarine sediment *in situ*, then faecal coliforms may be distributed mainly in surface sediment. It should be noted, however, that the sediment surface of the intertidal zone is regularly disturbed and, therefore, bacteria and suspended solids may not have time to accumulate at surface filtering sites and form a zone of saturated filtering sites where filtration is enhanced. Regardless of this, filtering sites deeper down in the sediment may gradually become blocked, restricting further movement of bacteria from above and, therefore, bacteria may still be distributed mainly in surface sediment.

3.5 LEACHING OF FAECAL COLIFORMS FROM SEDIMENT BY RAPID INFILTRATION OF DISTILLED WATER

3.5.1 Materials and Methods

Fifteen sediment profiles were constructed and four faecal suspensions were made up in the same way as in the previous experiment. The density of faecal coliforms in the 100% faecal suspension was determined as in section 3.4 and was found to be 3.5×10^7 100 ml^{-1}. Each concentration of faecal suspension was applied to three sediment profiles (replicates). Four 50 ml volumes (approximately 28 pore volumes) of the faecal suspension were applied to each sediment profile. The remaining three sediment profiles acted as a control, receiving four 50 ml volumes of autoclaved estuarine water. Sediment profiles were left overnight before application of distilled water.

Eighty 50 ml volumes of distilled water were applied to each sediment profile, intermittently, over a seven day period. Samples of effluents 1, 4, 7, 10, 20, 50 and 80 were collected on different days and faecal coliforms were enumerated as in section 2.4.1.4. The day after the last volumes of distilled water were applied to sediment profiles, the sediment profiles were sampled as in the previous experiment to determine the distribution of faecal coliforms in the sediment profiles.

3.5.2 Results and Discussion

Clogging was again a problem in sediment profiles to which the 100% faecal suspension was applied and so the surface mats of the sediment were gently disturbed at various intervals during the experiment when the flow of distilled water through sediment profiles was very slow. Figure 3.6 shows the mean densities of faecal coliforms in effluent samples after varying volumes of distilled water had percolated through sediment profiles to which different concentrations of faecal suspensions were applied. Faecal coliforms were enumerated in effluent samples from control sediment profiles only twice (effluents 1 and 7) and the total number enumerated in the 1,200 ml of effluent filtered was only 40. No faecal coliforms were enumerated in sediment samples from control sediment profiles. Therefore, it was assumed numbers of indigenous faecal coliforms initially present in sediment profiles was very low and were not considered to have contaminated counts significantly. Figure 3.7 shows the distribution of faecal coliforms in sediment after leaching.

Several trends were apparent in the results. The leaching of faecal coliforms appeared to decline approximately logarithimically in

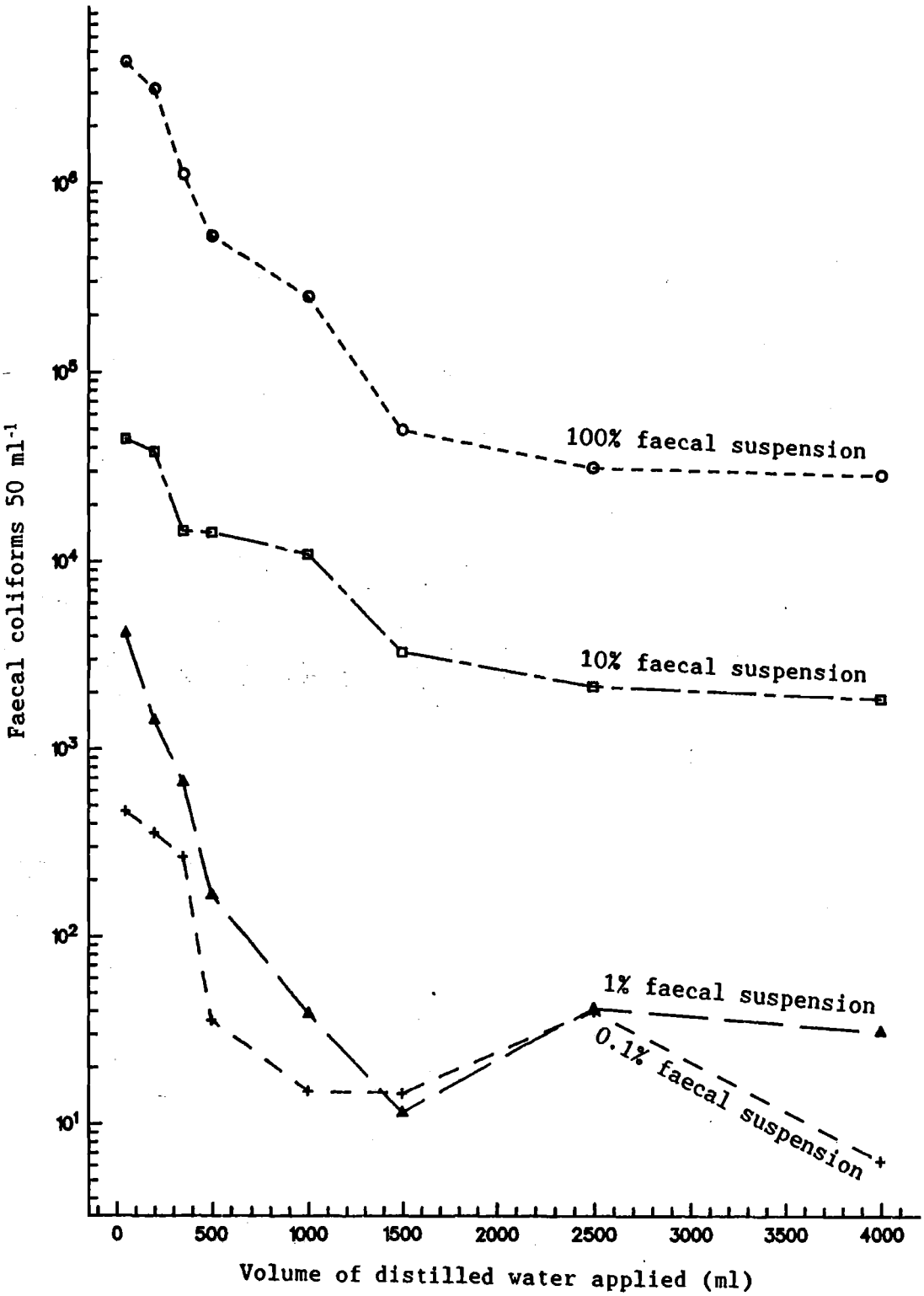


Figure 3.6 Mean densities of faecal coliforms in effluent samples of distilled water applied to sediment profiles to which faecal suspensions of varying concentrations had been applied earlier

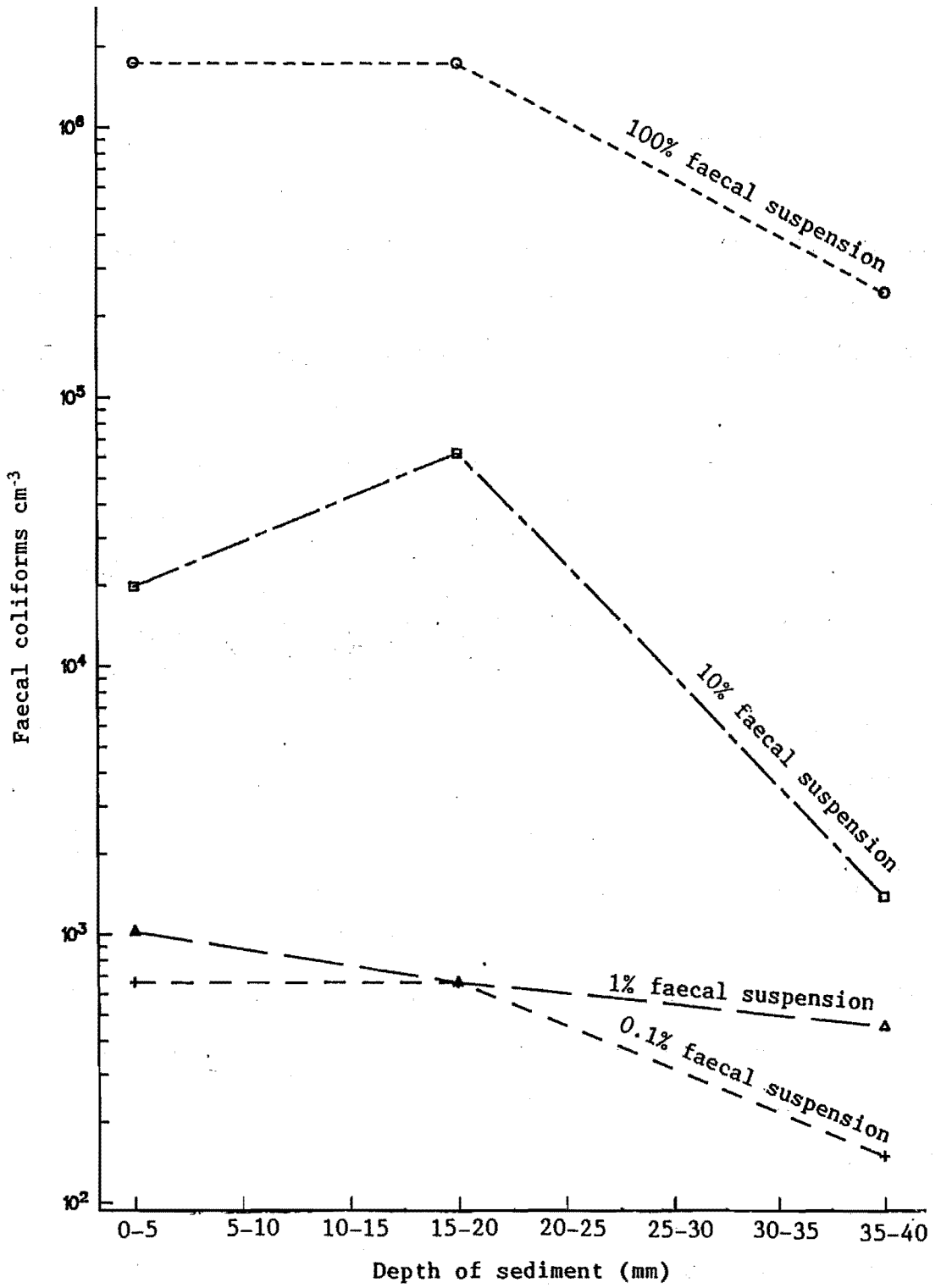


Figure 3.7 Distribution of faecal coliforms remaining in sediment profiles after 4,000 ml of distilled water had percolated through them.

sediment profiles to which different concentrations of faecal suspension were applied as the total volume of distilled water percolated through sediment profile increased until approximately 210 pore volumes of distilled water had percolated through sediment profiles. After this point, the faecal coliforms leached also appeared to decline slowly, again approximately logarithmically as the total volume of percolant increased in sediment profiles to which the 100 and 10% faecal suspensions were applied, but at a slower rate. Mean densities of faecal coliforms found in effluent samples from sediment profiles to which the 1 and 0.1% faecal suspensions were applied fluctuated after 210 pore volumes of distilled water had been applied.

The decrease in the densities of faecal coliforms in effluent samples was expected because fewer faecal coliforms are "available" to the leached after each "flushing", ie. most of the faecal coliforms are leached in the first few applications of distilled water. It should also be noted that the rate of decrease, before 210 pore volumes of distilled water had been applied to sediment profiles, appeared to be faster in sediment profiles to which the 1 and 0.1% faecal suspensions were applied than in the sediment profiles to which the 100 and 10% faecal suspensions were applied. This may be due to the higher suspended solids content of the 100 and 10% faecal suspensions. With a higher concentration of suspended solids (including bacteria) in the percolate a more rapid blocking of filtering sites further down the sediment profile would be expected. This would explain the distribution of faecal coliforms in sediment profiles and the change in rate of decrease of faecal coliforms enumerated in effluent samples. From the distribution of faecal coliforms in sediment profiles it would appear that faecal coliforms in the surface mat, physically disrupted by the percolate may only travel a small distance before they are filtered out

of suspension again. This was indicated by the apparent advancement of the zone of saturated filtering sites to at least the 20 mm depth of sediment profiles. Between the 20 and 40 mm depths of sediment profiles there was approximately a one-log reduction in numbers of faecal coliforms from the 0-20 mm depth to the 35-40 mm depth in sediment profiles to which the 100, 10 and 0.1% faecal suspensions were applied which suggests that the filtering sites were still unsaturated. The numbers of faecal coliforms remaining in the sediment profiles to which the 1 and 0.1% faecal suspensions were applied did not differ greatly. This may have arisen from the lack of a surface mat, ie. the absence of enhanced filtration.

In Figure 3.6, the mean number of faecal coliforms leached from sediment profiles to which 560 pore volumes (4,000 ml) of the 100% faecal suspension was applied was approximately 31,200,000 (area under the graph) or 44.5% of the total number initially applied. In Figure 3.7, however, the mean number of faecal coliforms found in the first 40 mm of sediment profiles to which the 100% faecal suspension was applied was only 19,300,000 (area under the graph), leaving approximately 19,500,000 faecal coliforms unaccounted for. As in section 3.4.2, the discrepancy may be explained by die-off, ineffective removal of faecal coliforms from sediment particles during enumeration and the numbers of faecal coliforms retained in the sediment that was not analysed. The number of faecal coliforms which may have been retained in unanalysed sediment of sediment profiles to which the 100% faecal suspension was applied, was estimated to be approximately 411,000 or 2.1% of the unaccounted faecal coliforms (calculated as in section 3.4.2). It is also possible that number of faecal coliforms unaccounted for may be an overestimate because although the initial number of faecal coliforms applied to the sediment is known, the number intially retained in the sediment is unknown.

Also, as in section 3.4.2, the sediment profile analysis suggests that even if a surface mat is not formed at the surface, the filtering sites deeper down eventually became saturated, retarding the further progress of bacteria from above. The zone of saturated filtering sites also appears to efficiently retain bacteria as in the sediment to which the 100% faecal suspension was initially applied, 19,300,000 faecal coliforms were still retained after application of 560 pore volumes of distilled water while the number of faecal coliforms found in the effluent at this stage was only 4,155 per pore volume (29,000 50 ml^{-1}).

3.6 THE RATE OF INFILTRATION OF ESTUARINE WATER THROUGH SEDIMENT *IN SITU*

3.6.1 Materials and Method

3.6.1.1 Experimental procedure

Three metal cylinders, approximately 290 mm in diameter and 330 mm in length, were inserted into the sediment near the Pleasant Point Jetty (Figure 1.1). This site was used because the jetty provided easy access to the cylinders and allowed easy pouring of sea water from containers into the beakers which were used to add sea water to cylinders. Earlier, a number of 8 mm holes had been drilled in a line up one side of each cylinder at 40 mm intervals. The holes were initially covered with adhesive tape so that water added to the cylinders when the tide was rising could not escape. Sea water that had been collected from the Pacific Ocean, was added to each of the cylinders as the level of the tide rose. Sea water was added until the water levels inside the cylinders were approximately the same as the level of the rising tide.

The conductivity of the sea water added to cylinders was measured with a portable HI 8333 conductivity meter (Hanna Instruments). The conductivities of the water within cylinders and the estuarine water surrounding cylinder were also measured at various times during the tidal cycle. After the tide had turned and the level of the water had started to recede, the drill holes of each cylinder were sequentially untaped, according to the level of the receding water.

3.6.1.2 Sampling

Three sediment cores were taken from the central area within each cylinder when the tide had receded below the level of the sediment surrounding the cylinders. The cores were extracted with PVC pipe segments by the method described in section 3.3.1.2 except that the PVC pipe segments were inserted to at least a depth of 70 mm. Six sediment cores of the surrounding sediment were also collected along a transect bisecting the three cylinders. Sediment cores were transported back to the laboratory for analysis.

3.6.1.3 Sediment core analysis

Sediment cores were pushed through the PVC tubes by inserting a plastic 250 ml centrifuge bottle beneath the cores. Sediment sections were sliced off at 10 mm intervals, with a knife into plastic beakers. The beakers were then filled with approximately 300 ml of distilled water and stirred vigorously for 10 s with a spoon. After the sediment had settled the conductivity of the supernatant liquid of each beaker was measured with the portable HI 8333 conductivity meter used to measure the conductivities of the cylinder and estuarine water. The temperature compensation knob of the conductivity meter was adjusted to

the temperature of the supernatant liquid while the probe was rinsed in distilled water (zeroed) before the supernatant of a beaker was measured.

3.6.1.4 Statistical analysis

Analysis of variance was performed on data and the mean conductivities of each depth sampled in different cylinders and surrounding sediment were compared in twosample *t*-tests using Minitab.

3.6.2 Results and Discussion

The conductivity of the sea water added to cylinders was 62.4 mS cm^{-1}. Sea water was initially added to cyinders between 10.35 and 10.40 am. The sediment surrounding and within each cylinder remained submerged for approximately 2.5 h. Cylinders were totally submerged for approximately 40 minutes. Table 3.4 shows the conductivities of water inside and surrounding the cylinders at various times during the tidal cycle. Conductivities of water inside and surrounding the cylinders measured after the tide had turned and the water had just receded below the level of the cylinders (12.15 pm), indicate that estuarine water had replaced sea water that had infiltrated through the sediment when the cylinders were totally submerged. Conductivities measured after the water level had receded a further 100 mm (12.30 pm) varied between cylinders while all those measured after the water level had receded 200 mm (12.45 pm) were similar to that of the sea water initially added. At 12.30 pm the conductivities of the water within cylinders 2 and 3 were approaching that of the sea water initially added while the conductivity of water within cylinder 1 was between that of sea water and estuarine water. These suggest that in the 70 minutes after the cylinders were

first submerged (11.35 am) the sea water may not have infiltrated more than 200 mm in cylinder 1 and 100 mm in cylinders 2 and 3, however, the later estimate may be inaccurate as the conductivities measured at 12.30 pm also also indicate that some mixing of estuarine and sea water occurred. The differences in conductivities of water measured in different cylinders but at approximately the same time may indicate different degrees of estuarine-sea water mixing or different rates of sea water infiltration through sediment within cylinders.

Table 3.4 Conductivities (mS cm^{-1}) of the water inside and surrounding the cylinders during submergence

Water	Time			
	11.35 am	12.15 pm	12.30 pm	12.45 pm
Surrounding	11.2	17.3	13.3	18.4
Cylinder 1	-	19.8	39.9	51.7
Cylinder 2	-	18.0	50.3	57.3
Cylinder 3	-	29.9	52.3	55.6

Table 3.5 shows the mean conductivities of supernatant liquid of mixtures of sediment samples from varying depths of cores and distilled water. Statistical analysis of data is shown in Appendix VII.

From the analyses of variance, supernatant liquid conductivities varied significantly with depth in surrounding sediment samples ($F = 5.21^{***}$). Supernatant liquid conductivities also varied significantly with depth of cylinder sediment samples ($F = 17.51^{***}$) and between

Table 3.5 Mean conductivities (mS cm^{-1}) of the supernatant liquid of mixtures of sediment samples from varying depths of cores, taken from outside and within cylinders, and distilled water

Depth (mm)	Outside	Cylinder 1	Cylinder 2	Cylinder 3
0–10	1.84	3.56	3.23	3.38
10–20	1.90	2.97	2.56	3.19
20–30	1.95	2.74	2.23	2.79
30–40	2.15	2.85	2.47	2.63
40–50	2.41	2.82	2.46	2.72
50–60	2.21	3.01	2.46	2.60
60–70	2.31	2.76	2.50	2.73

sediment samples from different cylinders ($F = 22.33^{***}$). Therefore, the supernatant liquid conductivities of mixtures of sediment samples and distilled water from different cylinders were compared separately with those of the surrounding sediment of corresponding depth. The twosample *t*-tests showed that 14 out of 21 mean supernatant liquid conductivities of sediment samples from the same depth were significantly different at the 5% level. Supernatant liquid conductivities of mixtures of sediment samples and distilled water for all three cylinders were significantly higher than those of the surrounding sediment in the top 30 mm of sediment cores. Supernatant liquid conductivities of mixtures of sediment samples from the 60–70 mm depth of sediment cores and distilled water for cylinders 1 and 3 were significantly higher than those from surrounding sediment ($P < 0.05$). Supernatant liquid conductivities of mixtures of sediment samples and distilled water for the cylinder 2 were not significantly different from

those of surrounding sediment beneath the 30 mm depth. These suggest that that the infiltration rates of sea water in all three cylinders were at least 12 mm h^{-1} (30 mm in 2.5 h) and in cylinders 1 and 3, may have been greater than 28 mm h^{-1}. The infiltration rate of sea water for cylinder 1 is less than that indicated by the conductivities of the water measured within this cylinder but may indicate that the infiltration rate of sea water through sediment is not constant. This appears reasonable as the hydrostatic pressure of water overlying inter tidal sediment will vary while sediment porosity may also vary with depth. The results also suggest, when differences in hydrostatic pressure are taken into account, that the infiltration rate of estuarine water through sediment may vary within short distances, presumably due to subtle differences in sediment structure and porosity, and that bacteria may potentially travel relatively long distances through estuarine sediment providing they are not adsorbed or filtered out from the infiltrating water. This would imply that estuarine water and, therefore, bacteria may penetrate deeper into sediment at the Tern Street site which is submerged for a longer period and at times by deeper water. Estuarine water may also penetrate deeper into sediment at the Tern Street site as the sediment at the Tern Street site may be more permeable than the sediment at the Pleasant Point Jetty site because it contains a lower silt-clay fraction (refer to Figure 1.3).

CHAPTER FOUR

RELEASE OF FAECAL COLIFORMS FROM SEDIMENT

4.1 INTRODUCTION

By definition an estuary is an area where sea water is measurably diluted by freshwater (Knox and Kilner, 1973). Therefore, a salinity gradient exists within an estuary which may change according to the proportions of sea water and freshwater entering the estuary. The proportion of sea water in an estuary depends largely on the level of the tide while the proportion of freshwater entering the estuary will vary according the flow of the freshwater inputs. River flow to an estuary will be regulated by the ways in which man uses that river, which may include water supply (irrigation, industrial and domestic), power generation and waste disposal, and by the weather. Generally, periods of dry weather result in low flow while periods of wet weather result in high flow. If the proportion of freshwater entering an estuary is higher than normal the range of salinities of the water within that estuary may be lower than normal.

Roper and Marshall (1974) suggested that a reduction in the salinity of water overlying a saline sediment may lead to the release (desorption) of bacteria adsorbed onto sediment particles. Desorption of bacteria could also result from the resuspension of phsically disturbed sediment particles which bacteria are adsorbed to, that are

transported (carried by currents) to areas within an estuary where the salinity of water is lower than that of the area the sediment particles originated from. If some of the bacteria in sediment are free-living (unattached), then the resuspension of sediment particles by physical disturbance may also result in the release of bacteria to the water column sediment.

It was considered that a potential risk to users of the Estuary may exist if sediment-bound faecal coliforms and the pathogens which may also be present, were released into the water column and remained in suspension upon reduction in the salinities of water within the Estuary or the resuspension of sediment by physical disturbance. If the bacteria remained attached to resuspended sediment particles then they may only remain in suspension for a short time. It was felt, however, that if they were desorbed from sediment particles or were free-living in sediment, then they may remain in suspension for some time before they were readsorbed, sedimented or filtered out by surface sediment.

In this Chapter, an experiment is described that was set up to determine the extent of release of faecal coliforms from sediment, slowly rotated in estuarine water of varying salinities (conductivities).

4.2 LITERATURE REVIEW

It appears that the bacteriological effects of physical disturbance of sediment have only recently been considered. Grimes (1975) was the first to report the release of indicator bacteria after physical

disturbance of sediment. He observed significantly higher densities of faecal coliforms downstream than upstream of a maintenance dredging operation in the Mississippi River nagivation channel. He attributed this to the disturbance of sediments by dredging and an accompanying release of sediment-bound faecal coliforms. He suggested that a large number of bacteria would be carried by currents and that a temporary health hazard could exist in recreational areas downstream of a dredging operation. Later, Grimes (1980) undertook a similar study and investigated the bacteriological effects of hydraulically dredged bottom sediment known to be heavily polluted with metropolitan sewage effluent. Results of this study substantiated his earlier work. He concluded that the turbulence created by the dredging process was sufficient to resuspend large numbers of sediment-bound bacteria, either as discrete bacteria or as bacteria still attached to suspended solids. He also suggested that it was probable that this turbulence was sufficient to desorb bacteria strongly attached to suspended solids.

In contrast to the findings of Grimes are those of Babinchak *et al.* (1977). They studied the effect of dredge spoil deposition on faecal coliform counts in sediments at a disposal site. Sediment containing a high density of faecal coliforms (14,000 100 ml^{-1}) was bucket dredged and then transported to a disposal site by bottom discharging barges. Faecal coliform counts in bottom water and sediment of the disposal site revealed that faecal coliforms counts were not increased by the deposition of dredge spoils. They attributed this to the dilution of surface sediment containing high densities of faecal coliforms by faecal coliform free subsurface sediment. It should be mentioned that Grimes (1980) noted that Babinchak *et al.* did not substantiate this claim as they only enumerated faecal coliforms in the top 10 mm of sediment cores and

did not state the depth of the deposition area. He argued that they did not adequately analyse the water overlying the disposal site as they only sampled at a 1 m depth above the sediment and, therefore, the question of whether sediment-bound bacteria were being released into the water column as the dredge spoil sedimented could not be fully addressed.

Sediment may also be physically resuspended by a number of other mechanisms. Matson *et al.* (1978) noted that resuspension of shallow water sediments could also be accomplished through increased river discharge, waves, wind-induced turbulence, motor boats, swimming, walking and the normal activities of aquatic macro-organisms. Sediment-bound bacteria, however, may also be resuspended by non-physical mechanisms.

Roper and Marshall (1974) investigated the effects of sorption phenomena on the interaction between *E. coli* (MC-6) and a bacteriophage in saline sediment. They found that both *E. coli* and the phage readily sorbed to saline sediments at high electrolyte concentrations (salinities) but were readily desorbed following dilution of the electrolyte below a critical concentration. This led them to suggest that when the electrolyte concentration was lowered beyond this critical concentration, the double-layer repulsion energies of bacteria and particles became more effective at a greater inter-particle distance, resulting in a repulsion of the bacteria from the particle surfaces. They also suggested that *E. coli* and other faecal bacteria accumulated in saline sediments may produce a potential health hazard in estuaries and lagoons if they were desorbed following dilution as a result of heavy rainfall. This phenomenon has also been observed in virus elution studies (Lance *et al.*, 1976 and Landry *et al.*, 1979).

From the available literature it is clear that indicator bacteria may be resuspended after deposition onto sediments, through physical and electro-chemical mechanisms. It is not clear, however, if the resuspension of indicator bacteria has corresponded with any outbreaks of disease among water users. It should be mentioned, however, that Grimes (1980) reported that he had obtained evidence that a private sand-and-gravel company was dredging for sand in the vicinity of Nine Mile Island, site of the 1974 shigellosis outbreak in Dubuque, Iowa. He noted that although the water in the Nine Mile Island area was sufficiently polluted to have been a possible source of *Shigella sonnei*, the dredging operation may have contributed to the waterborne shigellae in the area.

4.3 RESUSPENSION OF SEDIMENT-BOUND FAECAL COLIFORMS BY SLOW ROTATION OF SEDIMENT IN ESTUARINE WATER OF VARYING CONDUCTIVITIES

4.3.1 Materials and Method

4.3.1.1 Sediment

Sediment was collected in a plastic bucket from the Tern street site and transported back to the laboratory where it was transferred to a plastic tray and manually mixed with a spatula for several minutes.

4.3.1.2 Experimental procedure

Approximately 50 g of the mixed sediment, weighed on a Mettler AC 100 balance, was added to 18 sterile 250 ml polypropylene screw cap

centrifuge bottles. The centrifuge bottles were divided up into six groups of three replicates. Approximately 200 ml of autoclaved estuarine water (121°C for 15 minutes) was added to one group of centrifuge bottles while a second group received 200 ml of sterile distilled water. The remaining four groups received different volumes of autoclaved estuarine water diluted to 200 ml with sterile distilled water. The centrifuge bottles were then clamped between two plastic trays as described in section 2.5.1.3 and very slowly rotated, by hand, every 5 minutes for 1 h. After analysis of samples of the supernatant liquid for faecal coliforms by the membrane filtration method described in section 2.3, the conductivity of each supernatant liquid was measured with a portable HI 8333 conductivity meter (Hanna Instruments).

Three 10.0 g samples of the mixed sediment were added to three separate sterile 250 ml Kinex flasks. Approximately 90 ml of 0.5% peptone water (bacteriological peptone, Oxoid) was added to each flask before they were sealed with parafilm M (American Can Company) and placed on a Gallenkamp orbital shaker set at 180 rpm for 5 minutes. Faecal coliforms were also enumerated in three samples of the supernatant liquid by the membrane filtration method described section 2.3.

4.3.1.3 Statistical analysis

Data were subjected to regression analysis using Minitab.

4.3.2 Results and Discussion

The three membrane filter counts of faecal coliforms for the samples of supernantant liquid ranged from 49 to 170 100 ml^{-1} (mean = 96.3; CV = 67.1%) which suggested the sediment was not completely homogenous.

Membrane filter counts of faecal coliforms in the supernatant liquid samples ranged from 26 to 280 100 ml^{-1} while values for CV ranged from 15.3 to 46.8% between replicates. Conductivities of the supernatant liquids ranged from 3.48 to 55.44 mS cm^{-1} equivalent to salinities of 1.98 to 40.14 g kg^{-1}. The percentages of sediment-bound faecal coliforms released were calculated by dividing the faecal coliform counts of the supernatant liquids by the mean number of faecal coliforms in the sediment.

From the regression analysis (Appendix VIII), approximately 58.7% ($F = 22.73^{***}$) of the variance in sediment-bound faecal coliforms released was explained by the variation in the conductivity of the estuarine water. It should be noted that the regression analysis indicated that there was one observation with a large standard residual. When this value was omitted from a second regression, the variation in the conductivity of estuarine water explained 69.3% ($F = 33.76^{***}$) of the variance in sediment-bound faecal coliforms released. Figure 4.1 shows the percent release of faecal coliforms from sediment and the regression curve and equation from the second regression (refer to Appendix VIII).

As the sediment was not completely homogenous, it was not surprising that more of the variance in sediment-bound faecal coliforms

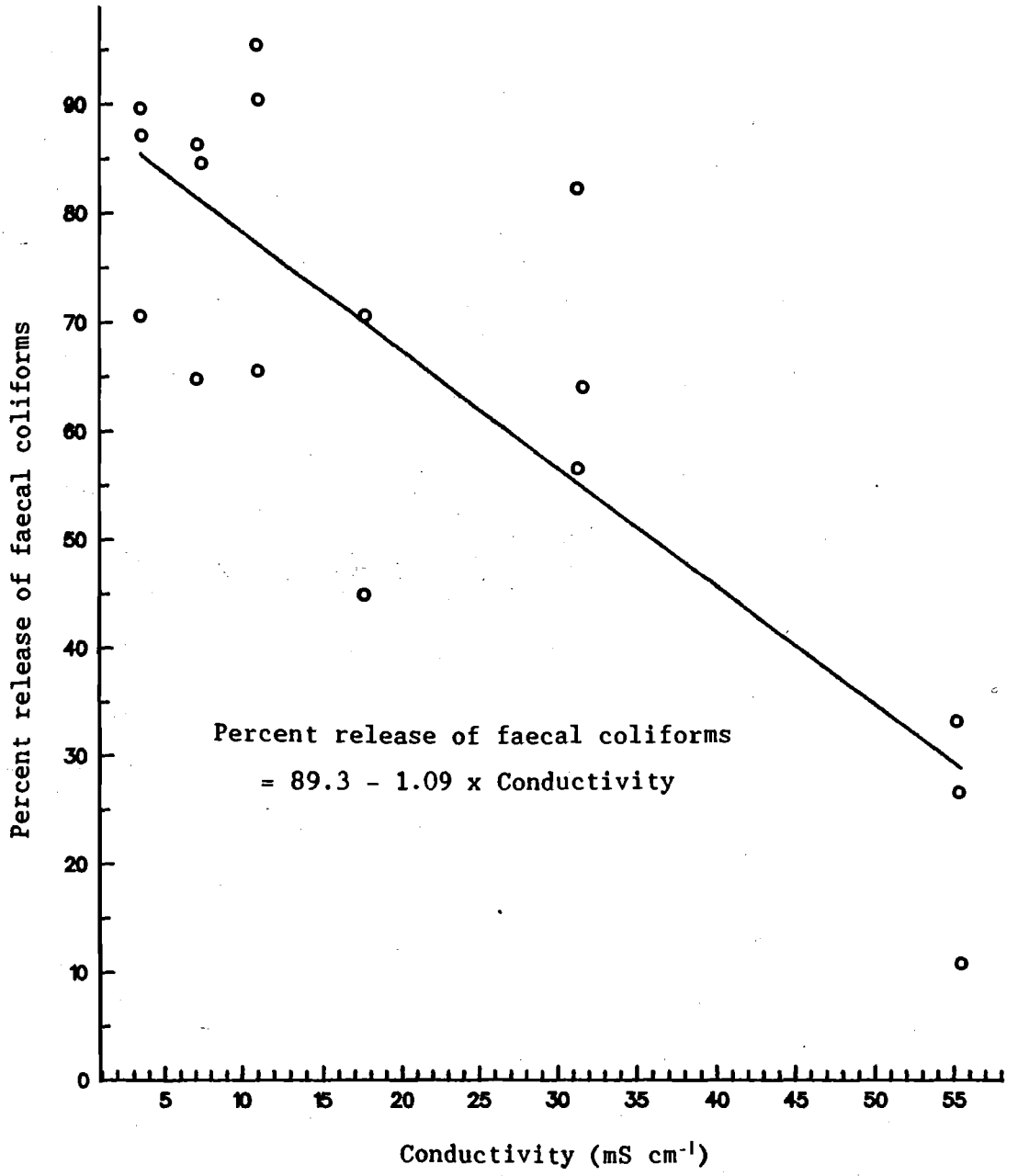


Figure 4.1 Percent removal of faecal coliforms from sediment gently rotated in estuarine water of varying conductivities

released was not explained by the variation in conductivities of estuarine water. This would explain counts that indicated greater than 100% (total) release of sediment-bound faecal coliforms, if it was assumed that the sediments had higher-than-average densities of faecal coliforms. Low counts could also be explained by lower-than-average densities of faecal coliforms in the sediment or if the peptone water did not release all the faecal coliforms from the sediment.

The conductivities of the supernatant liquid, from centrifuge bottles containing sediment and distilled water only, appeared to reflect the saline nature of the sediment at the Tern Street site. The elution of adsorbed ions raised the conductivity of the sterile distilled water from 0.00 mS cm^{-1} to between 3.48 and 3.54 mS cm^{-1}. It should be noted that conductivities were comparable to those of a mixture of sediment saturated with sea water and distilled water (refer to section 3.6). The high conductivities of the supernatant liquid from centrifuge bottles containing sediment and 100% estuarine water suggest that the autoclaving process increased the salinity of the estuarine water as the highest conductivity measured was 55.44, equivalent to a salinity of 40.14 g kg^{-1} or 114.7% sea water. Presumably this was due to the evaporation of water during autoclaving.

The results agree with those of Roper and Marshall (1974), although the method used to determine the release of sediment-bound bacteria differed. Roper and Marshall centrifuged saline sediment containing *E. coli* (adsorbed) that had been resuspended in distilled water and left to stand for 10 minutes. After the suspension had been centrifuged the supernatant liquid was decanted and numbers of *E. coli*, conductivity and absorbance (at 420 nm) were determined. The procedure was repeated

until after the dispersion of colloidal materials in the sediment. They observed that this corresponded to conductivities of 0.5 to 0.7 mS cm^{-1}. As in this experiment, they found few bacteria were released at high conductivities and that desorption of the bacteria coincided approximately with the dispersion of colloidal material in the sediment as the electrolyte concentration was lowered. The maximum number of cells were desorbed at a conductivity of approximately 2.22 mS cm^{-1} and the numbers of bacteria desorbed decreased rapidly in later sediment washings.

In this experiment, indigenous bacteria were used and, therefore, free-living faecal coliforms may have also been released because of the slow rotation (physical disturbance) of sediments. This could explain the counts observed at high conductivities if some of the faecal coliforms enumerated had not been released from sediment through desorption. It may also explain some of the variance in the number of sediment-bound faecal coliforms released, if numbers of free-living and adsorbed faecal coliforms differed between sediment samples added to the centrifuge bottles. The slow rotation was used to simulate gentle wave action so that the results could be related to the *in situ* situation.

If a similar pattern of release of sediment-bound bacteria occurs naturally in the Estuary then there are several public health implications. From this experiment and the work of Roper and Marshall (1974), bacteria adsorbed onto sediment may desorb upon reduction of the salinity of the overlying water. This would be expected to occur in tidal areas near the Avon and Heathcote Rivers during the ebb tide or when the discharge of the two rivers increased. Knox and Kilner (1973) compared the variation in salinites at the points where the tidal rivers

entered the Estuary proper (the bridges over the Avon and Heathcote Rivers) (refer to Figure 1.1). They noted that salinities recorded during a period of low flow (2.3 $m^3 s^{-1}$) for the Avon ranged from 0 to 26 g kg^{-1} at low and high water, respectively, while those of the Heathcote ranged from 0 to 30 g kg^{-1} at low and high water, respectively. During a period of high flow (11.3 $m^3 s^{-1}$), however, the maximum salinities recorded for the Avon and Heathcote were 5.5 and 2 g kg^{-1}, respectively, approximately 1/5 and 1/15 of the maximum salinities recorded during a period of low river flow. This would suggest that there may be a greater threat to water users during low tide or immediately after a storm or heavy rainfall. Alternatively, if sediment-bound bacteria are resuspended by physical disturbance then a potential health hazard may exist in certain areas of the Estuary after windy conditions or where there are motor boats. The hazard to water users arises because water users may unintentionally swallow mouthfalls of water than contain pathogenic micro-organisms. This may not only happen to swimmers but to water users such as windsurfers, water-skiers and yachtsmen who may also enter the water . Although the risk of infection from ingestion of saline water contaminated with pathogens is generally considered low (Moore, 1975), it should not be ignored.

CHAPTER FIVE

SURVIVAL OF FAECAL COLIFORMS IN ESTUARINE WATER AND SEDIMENT

5.1 INTRODUCTION

The potential for resuspension of sediment into the water column, thus exposing water users to sediment-bound indicators and pathogens, was discussed in the previous chapter. Matson *et al.* (1978) suggested that this was one of at least two factors determining the importance of aquatic sediments as reservoirs of health hazard indicators. The second factor they suggested was the possibility of extended survival and growth of indicators and possibly pathogens in sediments.

In this chapter, an experiment is described that was undertaken to determine if faecal coliforms survived longer in estuarine sediment than in estuarine water under controlled conditions of temperature and in the absence of light. It was felt, however, that although the survival of faecal coliforms may be extended in sediment, it would be of little significance unless densities of faecal coliforms in sediment were relatively high. Therefore, faecal coliforms were enumerated in sediment samples collected from the Tern Street site at various times of the year.

A seasonal variation in counts of faecal coliforms was observed in sediment taken from the Tern Street site sediment samples and this is discussed in terms of factors affecting faecal coliform survival and

numbers. From the results of this survey and the survival experiment, the numbers of faecal coliforms needed to be present in the water overlying the Tern Street site in order to maintain the numbers observed in the sediment at the site were estimated after variables influencing the resupply of bacteria to sediments had been taken into consideration. These numbers were compared with the numbers of faecal coliforms observed in the water of the Estuary in an extensive bacteriological survey carried out in 1981.

5.1 LITERATURE REVIEW

Nicati and Reitsch (1885) first recognized the beneficial effect of heat sterilization on the increased survival of non-marine bacteria in sea water (Carlucci and Pramer, 1960a) and hence discovered the biological bactericidal action of sea water on non-marine bacteria. Numerous investigators have since searched for agents or factors responsible or contributing to the bactericidal action of sea water. Not only have biological factors been implicated but chemical and physical factors have been suggested as well. Biological and non biological factors include pH of sea water (Carlucci and Pramer, 1960b), salinity (Carlucci and Pramer, 1960b; Hanes and Fragala, 1967; Faust *et al.*, 1975; Goyal *et al.*, 1977; Erkenbrecher, Jr., 1981), competition for nutrients with the indigenous microflora (Carlucci and Pramer, 1960b; Jannasch, 1968), nutrient deficiencies (Orlob, 1956; Carlucci and Pramer, 1960b; Savage and Hanes, 1971), heavy metals and other substances (Johannesson, 1957; Jones, 1964; Jones and Cobet, 1975), antibiosis (Rosenfeld and ZoBell, 1947; Vaccaro *et al.*, 1950; Aubert *et al.*, 1975), lysis (Mitchell *et al.*, 1967; Roper and Marshall, 1977) and

predation (Waksman and Carey, 1935; Ketchum *et al.*, 1952; Carlucci and Pramer, 1960d; Enzinger and Cooper, 1976; Roper and Marshall, 1978a; McCambridge and McMeekin, 1981). Other factors affecting the survival of non-marine bacteria in marine and estuarine environments, not specific to sea water, include water temperature (Nusbaum and Garver, 1955; Orlob, 1956; Carlucci and Pramer, 1960a; Hanes *et al.*, 1966; McFeters and Stuart, 1972; Gameson and Gould, 1975; Faust *et al.*, 1975; Babinchak *et al.*, 1977), solar radiation (Gameson and Saxon, 1967; Gameson and Gould, 1975; Bellair *et al.*, 1977; Chamberlin and Mitchell, 1978; Fujioka *et al.*, 1981; McCambridge and McMeekin, 1981) and dissolved oxygen (Faust *et al.*, 1975).

It is likely that a complex interaction of aforementioned factors is responsible for the often rapid die-off of non-marine bacteria, such as faecal coliforms, in marine and estuarine environments. Contribution to this interaction by specific components may vary, for example, atmospheric conditions, water quality, and geographical latitude are variables which may moderate the bactericidal effectiveness of sunlight in different areas of the world (Fujioka and Narikawa, 1982). The extensive literature on factors affecting the survival of faecal coliforms has been reviewed by several authors (Mitchell, 1968; Jones, 1971).

A number of investigators have observed an apparent inverse relationship between survival of *E. coli* and salt concentration (Hanes and Fragala, 1967; Faust *et al.*, 1975; Goyal *et al.*, 1977; Erkenbrecher, Jr., 1981). Prior to these observations, Carlucci and Pramer (1960b) found that the survival of *E. coli* was similar in natural sea water and in sodium chloride (NaCl) solutions of equal salinity. They concluded pH

and salinity of natural sea water did not favour survival of *E. coli* and significantly contributed to their rapid die-off; survival of *E. coli* decreased with increasing pH and salinity. Although they believed the effect of salinity was osmotic they did not discount specific ion toxicity. Other investigators, however, have suggested that salt does not exert a bactericidal action. For example, Vaccaro *et al.* (1950) believed since autoclaving destroyed a large part of the bactericidal activity of sea water, resulting in extented survival of coliforms in their experiment, salinity of sea water was not responsible for its bactericidal action. It would appear from much of the literature, that increased salinity may enhance die-off of faecal coliforms in saline waters, becoming more significant as other bactericidal agents act against and weaken them.

The oligotrophic nature of many aquatic environments appears to place stress upon faecal coliforms. Several investigators have suggested that *E. coli* is a poor competitor because of its relatively high growth parameters (Carlucci and Pramer, 1960b; Jannasch, 1968). These investigators noted that *E. coli* could compete or develop once the levels of the indigenous microflora were low or nutrients high. Earlier, Orlob (1956) had found that the maximum growth levels of coliform bacteria in sea water, supplemented with varying concentrations of lactose broth culture medium, were directly proportional to the concentration of nutrient added. Results of Savage and Hanes (1971) substantiated this claim.

Some investigators have studied the toxicity of heavy metals and other substances in natural sea water. Jones (1964) showed that growth of *E. coli* in natural and synthetic sea water was enhanced by the addition

of a number of chelating agents. The concentrations of chelating agents used to reverse the toxicity of filter sterilized sea water could be calculated on the basis of their stability constants. Jones and Cobet (1975) investigated the rapid die-off of non-marine bacteria in the Caribbean Ocean. They attributed the rapid die-off primarily to heavy metal ion toxicity and found addition of appropriate concentrations of chelating agents enhanced the survival of *E. coli* (ATCC 11229). Johannesson (1957) proposed that the toxicity of sea water for *E. coli* was caused by iodate (IO_3^-), however research by Jones (1964) did not support this claim.

Conflicting views on the importance of antibiosis in natural sea water exists. Carlucci and Pramer (1960c) dispelled the suggestion that antibiotics produced by marine micro-organisms were resposible, in part, for the bactericidal action of sea water (Rosenfeld and ZoBell, 1947; Vaccaro *et al.*, 1950) on failure to detect the production of antibiotics active against *E. coli* or *Bacillus subtilis* in tests involving 200 marine bacteria and on the premise that although Rosenfeld and ZoBell had found 9 out of 58 species of micro-organisms tested for antibiotic production to be antagonistic to non-marine forms when cultivated on agar media, little or no antibiotic was produced by these same organisms when grown in nutrient broth prepared with sea water. According to Aubert *et al.* (1975) studies in this field seem to establish beyond doubt the existence *in situ* of biochemical interactions between marine micro-organisms and terrestrial bacteria. Their own work revealed that the main bacterial groups responsible for antibiotic production in the Mediterranean Sea belonged to several genera, in particular *Pseudomonas, Achromobacter, Flavobacterium, Chromobacterium* and *Vibrio.* In contrast, Mitchell and Chamberlin (1975), on the basis of available studies, believed there

appeared to be no evidence that antibiotics were produced under natural conditions by marine bacteria, or that they contributed to the death of enteric bacteria in sea water. They also believed the significance of algal toxins in influencing the survival of enteric bacteria in sea water was unclear, excluding both algal and bacterial toxins (antibiotics) from their model of enteric bacterial die-off.

There also appears to be conflicting views on the importance of lysis and parasitism on the survival of *E. coli* in saline waters. Roper and Marshall (1977) found that *E. coli* numbers declined after 24 h incubation after addition of a myxobacter (*Polyangium* MR45) to autoclaved natural sea water. The decline of *E. coli* corresponded to an increase in the numbers of myxobacters. This myxobacter could also lyse a variety of other sewage micro-organisms. Lytic bacteria were also responsible for decline of *E. coli* in sea water according to Mitchell *et al.* (1967) who found that the decrease in numbers of *E. coli* was strongly affected by the size of the indigenous microbial population. Enzinger and Cooper (1976), however, found survival of *E. coli* was dependent on the presence of protozoan predators and not the presence of lytic bacteria in their studies involving estuarine waters. They believed bacterial competition, antagonism (antibiosis) and even bacterial predation were relatively unimportant in removing coliforms from estuarine water. Bacteriophages have also been isolated from natural sea water but were presumed by several investigators (Carlucci and Pramer, 1960d; Jones, 1971) to have little effect on bacterial survival because of the low levels of dissolved organic matter in sea water and, therefore, the absence of extensive host bacteria multiplication.

Ketchum *et al.* (1952) considered predation to be an important process contributing to the decline of coliforms in the tidal estuary they studied. Carlucci *et al.*(1960) attributed the beneficial effect of heat and filter sterilization on bacterial survival, in part, to the removal of predacious organisms. Roper and Marshall (1978a) suggested that predation and parasitism were significant in the destruction of faecal bacteria in sea water and that there was a range of organisms adapted to this role. They studied both filtered and unfiltered sea water, noting that microscopic observations of unfiltered sea water containing *E. coli* showed a sequence of micro-organisms which were apparently responsible for the decline in *E. coli* numbers. Initially smaller predators and parasites were responsible for destruction of *E. coli* in sea water. Large protozoa developed later in the succession, also helping in the restoration of the initial natural microbial balance by reducing numbers of the smaller predators and parasites. McCambridge and McMeekin (1981) also believed microbial predators contributed significantly to the decline of sewage bacteria in estuarine waters.

A number of investigators have considered temperature to be a very important factor in the survival of faecal coliforms in water (Nasbaum and Garver, 1955; Carlucci and Pramer, 1960a; Hanes *et al.*, 1966; Jones, 1971; McFeters and Stuart, 1972; Faust *et al.*, 1975; Gameson and Gould, 1975; Babinchak *et al.*, 1977). All these investigators believed that enteric bacterial survival is prolonged at low temperatures. Jones (1971) attributed this to the slow metabolic rate of bacteria in low temperature environments. Nusbaum and Garver (1955) found that coliform counts of sea water samples incubated at 5°C showed insignificant changes for periods up to 9 days whereas samples incubated at 18 and 30°C showed initial lag phases of 1-3 days followed by a rapid decline

in the numbers of coliforms. Results of Hanes *et al.* (1965) also showed that extended lag phases of coliform die-off curves corresponded to lower temperatures. Faust *et al.* (1975) found survival of *E. coli* MC-6 was closely and negatively correlated (linearly) with increasing water temperature ($r = -0.717$) but did not observe a lag phase. They also found survival of cells was directly proportional to dissolved oxygen concentration of the water. Gameson and Gould (1975) derived a regression equation describing the effect of temperature on coliform die-off in the dark from a series 188 experiments in sea water at Bridport (Dorset, England). Their regression equation was

$$\log T_D = 2.292 - 0.0295\Theta \qquad (5.1)$$

where T_D (T_{90}) was in hours and Θ was the temperature in °C. They also found from results of more than 400 experiments on the mortality (die-off) of coliform bacteria in the dark, that the die-off curves for coliform bacteria in natural sea water were approximately log-linear, following first order kinetics (Chick's Law) so that the number of organisms, N, remaining after time t was given by

$$N = N_0 e^{-kt} \qquad (5.2)$$

where N_0 was the initial number present and k is the die-off rate coefficient. Crane and Moore (1986) in their review on bacterial die-off noted that this law accurately described bacterial die-off.

Light has also been considered very important in determining the survival of faecal coliforms in water. Gameson and Saxon (1967) found reduction in coliforms was proportional to the intensity of short-wave

radiation received over a given period of time. Gameson and Gould (1975) found the rate of die-off of coliform bacteria in samples of sea water exposed to daylight was very much greater than in the dark. The rate of die-off was approximately proportional to the total radiation intensity. From a series of experiments they attempted to determine the wavelengths of radiation responsible for the lethal effect on coliform bacteria. On the basis of their results they attributed about half the lethal effect of solar radiation to wavelengths below 370 nm, a quarter to the near-visible ultraviolet (370-400 nm) and a quarter to the blue-green region of the visible spectrum (400-500 nm). Bellair *et al.* (1977) and McCambridge and McMeekin (1981) also found the die-off of coliform bacteria was proportional to the total radiation they received over a given period of time. Chamberlin and Mitchell (1978) suggested coliform decay was primarily the result of light-induced cell damage, noting that possibly other factors other than light accentuated or in fact were essential for the effect of light, citing predation as one such factor. They found daytime *in situ* die-off rates of coliforms in sea water were of the order of 1.0 h^{-1} and were in sharp contrast to those in freshwater (0.015-0.020 h^{-1}). From a comparison between die-off rates of coliforms and *E. coli* they concluded that there were no substantial differences between the sensitivities of coliforms and *E. coli* to light. Fujioka *et al.* (1981) also believed both visible and ultraviolet light were responsible for the bactericidal effect of sunlight. The effect was a major one and could be demonstrated at a depth of 3.3 m in clear sea water.

The role of deposition or sedimentation in the removal of faecal coliforms from the water column has been reviewed in some detail in Chapter Two. Although the factors influencing the survival of faecal

coliforms in water will also influence their survival in sediment, there is some evidence that sediments act to prolong the survival of faecal coliforms. Hendricks (1971a) observed higher *Salmonella* recovery from stream bottom sediments than from overlying waters. He suggested that it was entirely possible that sedimentation and adsorption of organisms to sands and clays could concentrate bacteria on the stream bottoms, increasing the recovery of bacteria studied, speculating that if the bacteria could find suitable nutrients present, growth might occur to further increase recovery. Results of Van Donsel and Geldreich (1971) and LaLiberte and Grimes (1982) substantiated this view. Van Donsel and Geldreich also found that survival of salmonellae in mud closely paralleled that of faecal coliforms. This suggestion of prolonged bacterial survival in bottom sediments after sedimentation and adsorption was reasoned by Roper and Marshall (1974 and 1978b) to be the result of protection of bacteria by particulate material from attack by parasites and possibly predators. Faust *et al.* (1975) found that clay (montmorillonite) addition to Rhode River water extended survival time of *E. coli* MC-6. Gerba and McLeod (1976) attributed the longer survival of *E. coli* in sediment to the greater organic matter content of sediment compared with that of sea water. Their results revealed that *E. coli* was capable of using nutrients adsorbed to estuarine sediments from areas where sewage effluents were discharged as well as from areas free of such pollution. Hendricks (1971b) had earlier shown through his respiration experiments that selected strains of *Enterobacteriaceae* (including *E. coli*) had the ability to metabolize substrates eluted with phosphate buffer. Gerba and Schailberger (1973) considered that the once sedimentation occurred, the fate of bacteria was governed by their ability to metabolize benthic nutrients, withstand predatory pressure and metabolically compete with other microbes.

Seasonal variations in the die-off of *E. coli* have been observed by a number of investigators in both sea water (Vaccaro *et al.*, 1950; Carlucci *et al.*, 1960; Gameson and Saxon, 1967; Faust *et al.*, 1975) and sediment (Sayler *et al.*, 1975; Goyal *et al.*, 1977; Erkenbrecher, Jr., 1981). Generally, *E. coli* die-off rate is greatest in summer months and lowest during winter months. Faust *et al.* (1975) found that the physical parameters of water (salinity, temperature and dissolved oxygen content) changed seasonally and most likely reflected the seasonal variation observed in *E. coli* die-off. This seasonal variation observed by several investigators seems understandable as water temperature is lowest, dissolved oxygen content is highest and sunlight hours and intensity are less during winter.

Survival of faecal coliforms seems likely to be a variable property on the basis that factors contributing to their die-off are likely to vary with time and geographical location, as well as with weather conditions.

5.3 COMPARATIVE SURVIVAL OF FAECAL COLIFORMS IN ESTUARINE WATER AND SEDIMENT

5.3.1 Materials and Methods

5.3.1.1 Experimental procedure

Surface sediment (0-20 mm) was removed from the Tern Street site at low tide with a garden trowel, transferred to a bucket and shaded from the sunlight. Water was collected from a channel (very close to where

the sediment had been removed) in a 1000 ml plastic beaker then poured into a large opaque plastic container. After arrival at the laboratory (40 minutes), water was poured from the container into a sterile 2000 ml screw cap laboratory bottle (Schott Duran) and its conductivity measured. Sediment was spread out in a sterile plastic tray and manually mixed with a spatula for several minutes. After mixing, the tray was covered with a plastic bag and placed, along with the laboratory bottle, into a Clayson air incubator set at 15°C.

Faecal coliforms were enumerated in the water samples from the laboratory bottle after 2, 26, 50, 74 and 96 h incubation by the membrane filtration method described in section 2.5.1.5. Faecal coliforms were enumerated in three 10 g sediment samples from the plastic tray at various times, over a four week period, by the method described in section 2.4.1.6. The moisture content of the sediment was determined before and after incubation by the method described in section 2.4.1.3.

5.3.1.2 Data transformation

The data were transformed using

$$Y = \text{LOGTEN}\ (X + 1) \qquad (5.3)$$

where Y is the logarithmic density of cells and X is the density of cells 100 ml^{-1}. This allowed the logarithmic transformation of zero counts.

5.3.1.3 Statistical analysis

Data were subjected to regression analysis using Minitab.

5.3.2 Results and Discussion

Faecal coliforms could not be isolated from the three water samples analysed after 96 h incubation while faecal coliforms could not be detected in any sediment samples after 431.5 h incubation. The conductivity of the estuarine water was 42.0 mS cm^{-1}, equivalent to 82.2% sea water.

The moisture content of the sediment before and after incubation was 28.94 (n = 10) and 28.47% (n = 10), respectively, indicating that little moisture was lost throughout the experiment. The highest density of faecal coliforms found in sediment samples was 11,373 ml^{-1} while the highest density of faecal coliforms found in water samples was 720 100 ml^{-1}. Values for CV of replicates ranged from 0 to 126.0% for water samples and from 0 to 115.9% for sediment samples. From the regression analysis (Appendix IX), 96.7 and 76.6% of the variance in logarithmic die-off of faecal coliforms in water and sediment, respectively, was explained by the variation in time. It should be noted that both regressions indicated there was one observation with a large standard residual. When these two observations were omitted from second regressions, 99.1 and 87.2% of the variance in logarithmic die-off of faecal coliforms in water and sediment, respectively, was explained by the variation in time. It was considered that the second regressions provided a better estimate of faecal coliform die-off in estuarine water and sediment, therefore, the die-off rate coefficients (k) of faecal

coliforms in estuarine water and sediment were derived from the second regressions (k = 0.719 and 0.122 d^{-1}, respectively). These give values for T_{90} of faecal coliforms in estuarine water and sediment of 1.39 and 8.20 d, respectively. Figures 5.1 and 5.2 show the die-off of faecal coliforms in estuarine water and sediment, respectively, the regression curves and equations from the second regressions are also shown. No lag-phase was observed in faecal coliform die-off in either estuarine water or sediment. Other investigators have also observed no lag-phase in faecal coliform die-off (Hanes and Fragala, 1967; Faust *et al.*, 1975), however, a number of investigators have observed a lag-phase before the die-off of faecal coliforms in saline water (Vaccaro *et al.* 1950; Orlob 1956; Calucci and Pramer 1960a) and sediment (Gerba and McLeod, 1976; LaLiberte and Grimes, 1982). These investigators have studied the survival of faecal coliforms by inoculating saline water or sediment with faecal coliforms grown in the laboratory while this experiment studied the survival of *in situ* faecal coliforms in estuarine water and sediment. Alternatively, the conflicting literature concerning the possible lag-phase before faecal coliform die-off in saline water and sediment, may indicate the inconsistent and variable response of coliform bacteria to different sources of sea water under varying conditions (Faust *et al.*, 1975).

The results appear to agree with the suggestion that Chick's Law accurately describes bacterial die-off (Crane and Moore, 1986). The values for k and T_{90} of faecal coliforms in estuarine water (82.2% sea water) incubated in the dark compare favourably with a number of such values given in the literature.

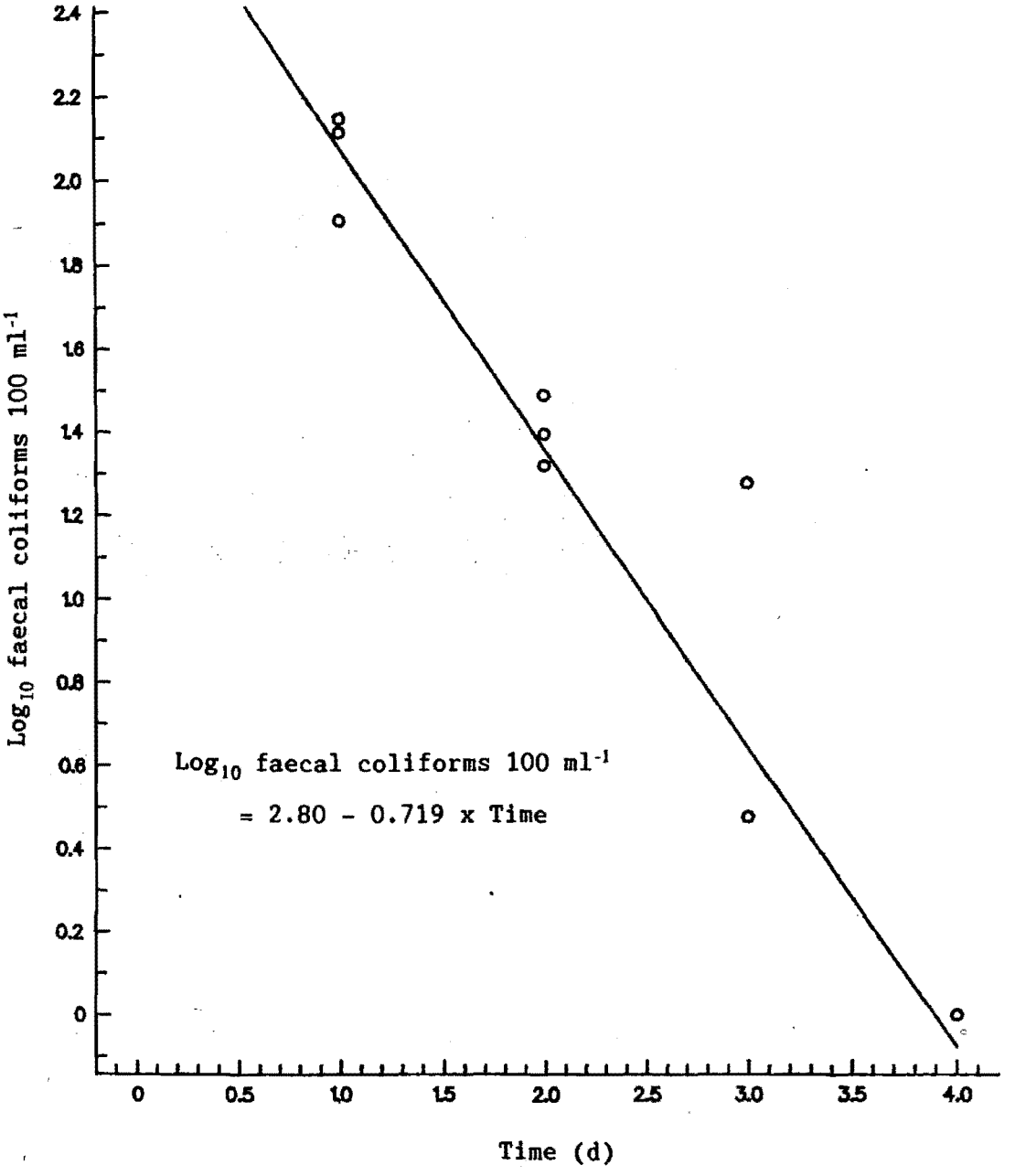


Figure 5.1 Faecal coliform die-off in estuarine water incubated at 15°C in the dark

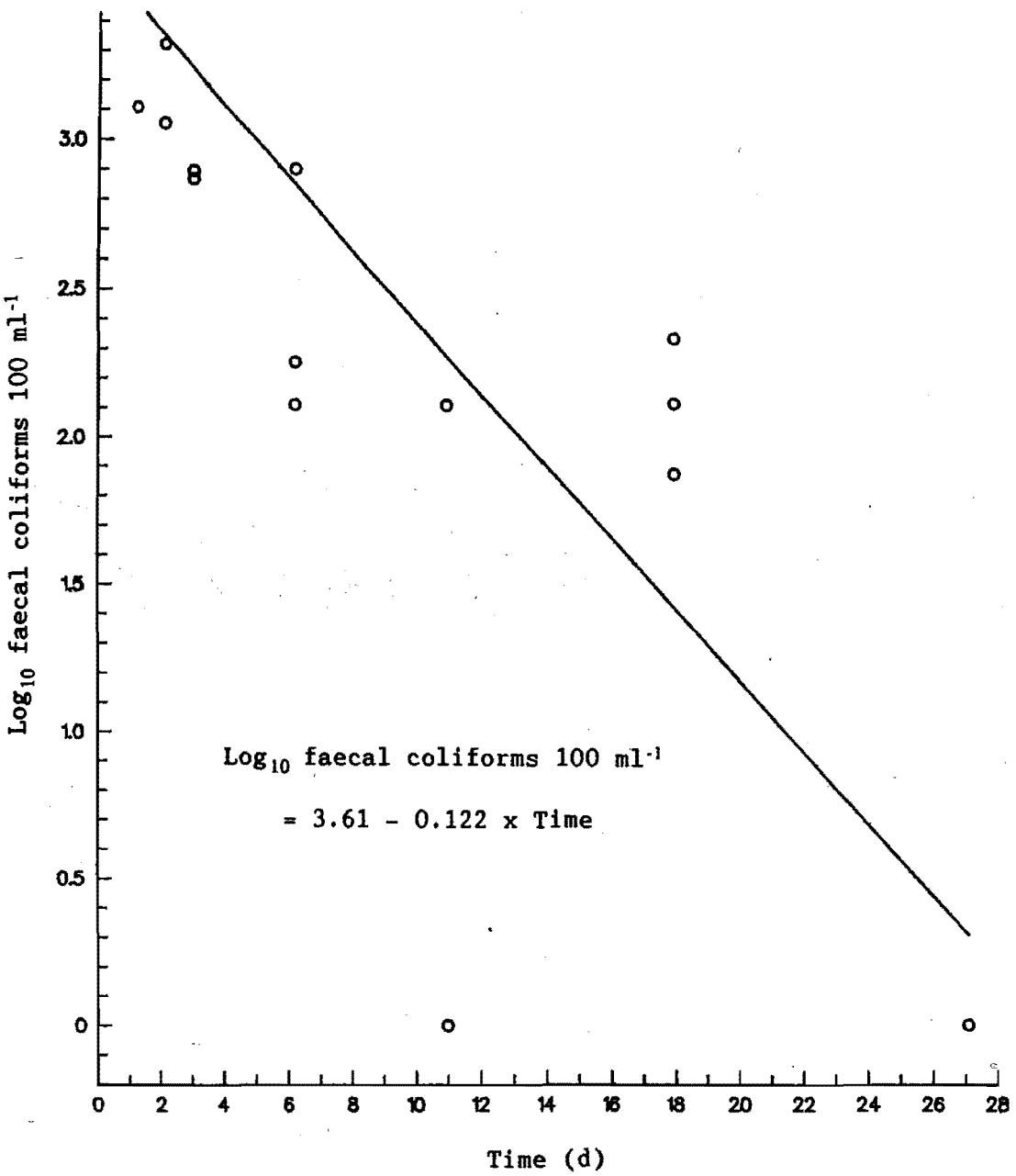


Figure 5.2 Faecal coliform die-off in estuarine sediment incubated at 15°C in the dark

Vaccaro *et al.* (1950) found the T_{90} and k of *E. coli* in untreated (raw) Massachusetts sea water, incubated in the dark at room temperature in spring, were 1.5 d (lag-phase of 0.3d) and 0.85 d^{-1}. If spring temperatures in Massachusetts are approximately 15°C, then these values are very similar, particularly when the lag-phase and differences in salinity are considered.

Hanes and Fragala (1967) found the values for k of *E. coli* (Type I) in 33, 67 and 100% sea water, inoculated with 1% sewage and incubated at 20°C, were 0.2741, 0.7735 and 1.3319 d^{-1}, respectively. These results indicate that the value for k of faecal coliforms in the estuarine water used in this experiment could be expected to lie between 0.7735 and 1.3319 d^{-1}. Although the observed value for k was only 0.719 d^{-1}, this may be the result of the enhanced survival of faecal coliforms at the lower incubation temperature used in this experiment. It may also be caused by the *E. coli* used by Hanes and Fragala as they used *E. coli* isolated from a nutrient rich environment (sewage) that may have undergone exaggerated die-off after the initial shock upon introduction to sea water. The faecal coliforms enumerated in this experiment were already present (*in situ*) and so may have survived the initial shock and acclimatized to the environmental stresses exerted by saline water.

It would appear from the comparison that values for T_{90} and k of faecal coliforms in saline water determined in the laboratory may vary, but that the variation may be explained in terms of the different conditions under which they were determined.

A comparison of the die-off of faecal coliforms in estuarine sediments is more difficult as estuarine sediments may vary in

composition within a small area (refer to Figure 1.3) while the studies dealing with indicator bacteria in the estuarine environment have been limited, of those only a few have been published on research conducted in estuarine sediments (Erkenbrecher, Jr., 1981). The values for T_{90} and k of faecal coliforms found in this experiment are consistent with the suggestion that sediments may prolong the survival of faecal coliforms in the aquatic environment (Hendricks, 1971a; Roper and Marshall, 1974 and 1978b; Faust *et al.*, 1975; Gerba and McLeod, 1976; Goyal *et al.*, 1977; LaLiberte and Grimes, 1982; Hood and Ness, 1982; Loutit and Lewis, 1985).

Although this experiment did not reveal why the survival of faecal coliforms is prolonged in sediment, it demonstrated the extended survival of faecal coliforms in sediment. The die-off of faecal coliforms was approximately six times faster in estuarine water than in estuarine sediment incubated at 15°C in the dark. It should be noted that the difference between die-off rates of faecal coliforms in estuarine water and sediment may be even greater *in situ*. In the presence of sunlight, the die-off of indicator bacteria such as *E. coli* and faecal coliforms in sea water is much faster (Gameson and Saxon, 1967; Gameson and Gould, 1975; Bellair *et al.*, 1977; Chamberlin and Mitchell, 1978; Fujioka *et al.*, 1981; McCambridge and McMeekin, 1981; Fujioka and Narikawa, 1982), however, in sediment the sunlight may only have a lethal effect on organisms near the surface, but not on those below (Van Donsel *et al.*, 1967). Sediment when submerged may also be protected from some of the lethal effect of sunlight by the overlying water.

The extended survival of faecal coliforms in sediment may, too some extent, explain the observations of the numerous investigators who have

found either high numbers of indicator bacteria in sediment (Savage, 1905; Nusbaum and Garver, 1955; Rittenberg *et al.*, 1958; Babinchak *et al.*, 1977; Grimes, 1980) or higher numbers of indicator bacteria in sediment than in overlying water (Hendricks, 1971a; Van Donsel and Geldreich, 1971; Gerba and McLeod, 1976; Goyal *et al.*, 1977; Matson *et al.*, 1978; Erkenbrecher, Jr., 1981; Loutit and Lewis, 1985; Lewis *et al.*, 1986). It should be noted, however, that the observation of large numbers of enteric bacteria in sediment does not, by itself, indicate extended survival or growth in that habitat. Mechanisms for concentrating enteric bacteria in sediment may simply be proceeding faster than factors causing die-off (Matson *et al.*, 1978). It is of interest also, that some investigators have found lower counts of indicator bacteria in sediments than in water (De Flora *et al.*, 1975; Sayler *et al.*, 1975).

If extended survival of faecal coliforms occurs *in situ* and large numbers accumulate, then physical disturbance of the sediment, whether naturally or by man, may lead to high numbers of faecal coliforms and, therefore, pathogens in suspension, thus creating a potential health hazard to water users. The extended survival of faecal coliforms in sediment appears to suggest that even when water quality indicator densities are low in the overlying water, a reservoir of indicator bacteria and pathogenic organisms may exist in the underlying sediment.

5.4 SURVEY OF THE DENSITIES OF FAECAL COLIFORMS IN THE SEDIMENT AT THE TERN STREET SITE

5.4.1 Materials and Method

5.4.1.1 Experimental procedure

Sediment cores were collected from the Tern Street site at low tide in the months of April, September and November 1986. Pipe segments (PVC and aluminium) were inserted into the sediment to a depth of at least 50 mm, dug out with a garden trowel and put into plastic bags. Core samples were transported to the laboratory where bacterial analysis was carried out within 5 h by the method described in section 2.4.1.6. Moisture contents of the sediment samples were determined as in section 2.3.1.3.

5.4.1.2 Statistical analysis

Data were subjected to analysis of variance and twosample *t*-tests using Minitab.

5.4.2 Results and Discussion

The moisture contents of the sediment cores collected from the Tern street site on the four dates (Table 5.1) were very similar, ranging from 31.07 to 31.90%. From the mean MPN counts of faecal coliforms of the four dates shown in Table 5.2, two trends were apparent: (i) the densities of faecal coliforms were higher in the 0-10 than the 10-40 mm depth and (ii) counts of faecal coliforms for both depths of sediment

Table 5.1 Moisture contents (%) of sediment samples at time of analysis

April 4	April 15	September 1	November 5
31.90	31.51	31.07	31.75

Table 5.2 Mean MPN counts of faecal coliforms in sediment samples

	April 4	April 15	September 1	November 5
0-10 mm	4,893	13,694	237	151
10-40 mm	3,778	1,310	49	84

samples collected in April were much higher than those in September and November. The later trend appeared to indicate that there was a temporal or seasonal variation in counts. When data were statistically analysed as "autumn counts of faecal coliforms" (April 4 and 15) and "spring counts of faecal coliforms" (September 1 and November 5) (Appendix X), spring counts of faecal coliforms for the 0-10 mm depth were significantly lower than autumn counts of faecal coliforms ($P = 0.023$) while for the 10-40 mm depth, spring counts of faecal coliforms were almost significantly lower than autumn counts of faecal coliforms ($P = 0.067$). Analysis of variance of spring counts of faecal coliforms showed that densities of faecal coliforms were significantly higher in the 0-10 than in the 10-40 mm depth ($F = 12.23^{***}$). In contrast, autumn counts of faecal coliforms were not significantly different between depths ($F = 2.53$, $P>0.10$). It should be noted, however, that autumn

counts of faecal coliforms were generally higher for the 0-10 than for the 10-40 mm depth but were more variable than spring counts of faecal coliforms for both depths (vaules for CV = 83.6 and 103.3% for the 0-10 and 10-40 mm depths, respectively, of autumn sediment samples while values for CV = 56.0 and 63.0% for the 0-10 and 10-40 mm depths, respectively, of spring sediment samples).

Seasonal variation in densities of indicator bacteria including faecal coliforms have been observed in sediment by other investigators (Sayler *et al.*, 1975; Babinchak *et al.*, 1977; Goyal *et al.*, 1977; Erkenbrecher, Jr. 1982). These investigators observed higher densities of indicator bacteria in sediment in winter than in summer months, suggesting survival was enhanced at lower temperatures. Sayler *et al.* and Erkenbrecher, Jr., however, observed a number of seasonal occurrences of indicator bacteria that did not support this suggestion. Sayler *et al.* observed a two log increase in faecal coliforms at one of their sediment sampling sites in summer while Erkenbrecher, Jr., observed peak densities of faecal coliforms in estuarine water in spring and later summer. Although neither sediment nor water temperatures were measured during the survey, meteorological data obtained from the Christchurch Drainage Board (Robb, 1986) and the Christchurch Meteorological Service (1987) (Tables 5.3 and 5.4, respectively) suggest that the temperature of the sediment at the Tern Street site may have been similar when sampled during the two seasons. Mean water temperatures for the Estuary were similar for April and November while solar radiation, air temperature and sediment moisture content (Table 5.1) appear to suggest that the sediment at the Tern Street site, when exposed, may have received a similar degree of heating before all sampling dates. Survival of faecal coliforms is likely to have been similar prior to

sampling as a consequence of the similarity of the meteorological data. If this was the case, then it would be expected that counts of faecal coliforms should be similar unless the numbers of faecal coliforms deposited onto sediment at the Tern Street site differ seasonally. This has been found in a previous bacteriological survey of the Estuary (Christchurch Drainage Board, 1981). In that survey, the mean daily faecal coliform input to the Estuary was calculated to be 2.20 and 1.45 x 10^{12} faecal coliforms in the summer and winter periods, respectively. If there is a greater input of faecal coliforms in autumn than in spring, then this may explain, too some extent, the higher counts of faecal coliforms observed in autumn. Alternatively, some other variable affecting the survival of faecal coliforms, such as predation or sediment nutrient status, may have differed during the survey period but this can not be tested. Despite the observed seasonal variation, densities of faecal coliforms even at their lowest, were high enough in the top 10 mm of sediment to warrant concern.

Table 5.3 Mean water and air temperatures (°C) for the Avon-Heathcote Estuary before and during sampling

Month	Water temperature	Air temperature
March	17.0	15.9
April	14.9	16.3
August	7.7	7.5
September	9.9	10.6
October	12.6	13.2
November	15.3	15.1

Table 5.4 Mean daily solar radiation (MJ m^{-2}) for the Avon-Heathcote Estuary before and during sampling

Month	Daily solar radiation
March	14.2
April	11.6
August	7.2
September	12.0
October	15.8
November	20.7

The legislation (Appendix I) defines the minimum bacterial standard for primary contact recreational use in saline water (SB) as "the median value of the faecal coliform bacteria content of the waters must not exceed 200 per 100 millilitres, based on not fewer than 5 samples taken over not more than 30-day period". The range of counts of faecal coliforms in the top 10 mm of sediment at the Tern Street site over the survey period was 116 to 18,179 100 ml^{-1}. These were generally higher than the densities of faecal coliforms found in the water of the Estuary by a previous bacteriological survey (refer to Table 1.1) and in some instances, at least one log higher. This is in accordance with the findings of Van Donsel and Geldreich (1971) and Lewis *et al.* (1986).

If sufficient sediment is resuspended and sediment-bound indicator bacteria are released to the water column (refer to Chapters Two and Four), then higher densities of indicator bacteria and, therefore, pathogens may occur in the water. This would appear to suggest that

water quality standards should take into account indicator bacteria densities in aquatic sediments.

The results also show a considerable variation in total counts of faecal coliforms among individual samples collected from the Tern Street site on April 4 and 15 (values for CV = 92.2 and 63.0%, respectively). This is also in agreement with the findings of Van Donsel and Geldreich (1971). The observation that counts of faecal coliforms were generally higher in the surface than subsurface sediment, appears to substantiate the suggestion that bacteria are largely removed from percolating water in the surface layers of sediments (refer to Chapter Three).

5.5 THE RESUPPLY OF FAECAL COLIFORMS NEEDED TO MAINTAIN THE NUMBERS OBSERVED IN THE SEDIMENT AT THE TERN STREET SITE

5.5.1 Die-off of Faecal Coliforms in the Sediment at the Tern Street Site

In section 5.3, it was found that Chick's law (equation 5.2) accurately described the die-off of faecal coliforms in sediment from the Tern Street site, incubated in the dark at 15°C If it is assumed that solar radiation does not significantly affect the die-off of faecal coliforms in sediment then the *in situ* die-off of faecal coliforms in sediment at the Tern Street site could also be determined using Chick's Law providing the values for N_o and k are known.

The maximum depth at which faecal coliforms occurred in the sediment of the Estuary was not found in this study and, therefore, the

values for N_0 of faecal coliforms in the sediment at the Tern Street site can only be estimated. Table 5.5 shows a range of such values for faecal coliforms in a area (1 m^2) of sediment at the Tern Street site during autumn and spring. These were calculated using the mean densities of faecal coliforms found in the 0-10 and 10-40 mm depths of sediment cores, removed from the Tern Street site during the survey described in Section 5.4, and under the assumptions that faecal coliforms are evenly distributed below the surface 10 mm of sediment and do not occur deeper than 100 mm below the surface.

Table 5.5 Estimated values for N_0 of faecal coliforms in 1 m^2 of sediment at the Tern Street site during autumn and spring. See text for details of calculation

	Accumulative numbers of faecal coliforms (x 10^4)	
Sediment depth (mm)	Autumn	Spring
40	166.2	3.9
50	196.9	4.5
60	227.7	5.1
70	258.4	5.7
80	289.1	6.4
90	319.8	7.0
100	350.6	7.6

As the mean water and air temperatures for the Estuary before survey sampling dates, except for September 1, were approximately 15°C (refer to Table 5.3) the temperature of the sediment at the Tern Street

site during autumn and spring may also have been approximately 15°C. If this was the case, the value for k of faecal coliforms in sediment from the Tern Street site, incubated in the dark at 15°C, 0.122 d^{-1} (refer to section 5.3.2), could be substituted along with individual values for N_o of faecal coliforms in sediment from Table 5.5 into Chick's Law to estimate the *in situ* daily die-off of faecal coliforms in the sediment at the Tern Street site during autumn and spring (Table 5.6). In order to maintain the numbers of faecal coliforms estimated to be in the sediment at the Tern Street site during autumn and spring, the daily resupply of faecal coliforms from water overlying the sediment when it is submerged must equal the daily die-off of faecal coliforms in the sediment.

Table 5.6 Estimated daily die-off of faecal coliforms in 1 m^2 of sediment at the Tern Street site during autumn and spring. See text for details of calculation

	Accumulative numbers of faecal coliforms (x 10^4)	
Sediment depth (mm)	Autumn	Spring
40	40.7	1.0
50	48.2	1.1
60	55.8	1.3
70	63.3	1.4
80	70.8	1.6
90	78.3	1.7
100	85.9	1.9

5.5.2 Minimum Initial Numbers of Faecal Coliforms in the Water Overlying the Sediment at the Tern Street Site Needed to Resupply the Numbers of Faecal Coliforms Which Die in the Sediment

Values for N_0 of faecal coliforms in the water overlying the sediment at the Tern Street site needed to maintain the numbers of faecal coliforms estimated to be present in that sediment would have to be somewhat greater than the numbers of faecal coliforms which die in the sediment. This is because faecal coliforms in water die-off more rapidly than those in sediment, especially during day because of the lethal effect of solar radiation (refer to sections 5.2 and 5.3). Although the values for k of faecal coliforms in estuarine water exposed to autumn and spring sunlight were not determined in this study they can be estimated using equation 2.1 (remembering the saline nature of the estuarine water overlying the Tern Street site) and the mean daily solar radiation data in Table 5.4. Using the mean daily solar radiation values for the months of March and April to represent autumn and those for the months of August, September, Octocber and November to represent spring, the values for mean hourly solar radiation in autumn and spring, assuming 12 h of daylight, are 1.1 and 1.2 MJ m^{-2}, respectively. These give mean values for T_{90} of faecal coliforms in water in autumn and spring during daylight of 3.27 and 3.15 h respectively, or mean values for k of faecal coliforms of 0.31 and 0.32 h^{-1}, respectively. If the mean temperature of the water overlying the Tern Street site does not change markedly during the night in autumn and spring, the value for k of faecal coliforms in water from the Tern Street site, incubated in the dark at 15°C, 0.719 d^{-1} or 0.030 h^{-1} (refer to section 3.5.2), could be used to determine the die-off of faecal coliforms in the water overlying

the Tern Street site at night. If the Tern Street site is submerged for approximately 8 h each tidal cycle (refer to section 2.4.2.1) and on average one tidal cycle spans the day and the other the night, then the minimum values for N_o of faecal coliforms in the water overlying the Tern Street site, needed to resupply the number of faecal coliforms which die in the sediment (N_m), can be calculated by rearranging Chick's Law. On an hourly basis, N_m would be derived as follows

$$N_m = \frac{N_d}{\sum_{1}^{n=8} 10^{-nk_d} + \sum_{1}^{n=8} 10^{-nk_n}} \qquad (5.4)$$

where N_d is the number of faecal coliforms that die in a day, n is the number of hours the faecal coliforms are in the water and k_d and k_n are the mean die-off rate coefficients of faecal coliforms in water during the day and night, respectively. Table 5.7 shows the values for N_m derived using equation 5.4 and values for N_d given in Table 5.6.

5.5.3 Minimum Densities of Faecal Coliforms in the Water Overlying the Sediment at the Tern Street Site Needed to Resupply the Numbers of Faecal Coliforms Which Die in the Sediment

The minimum initial densities of faecal coliforms in the water overlying the sediment at the Tern Street site needed to resupply the numbers of faecal coliforms which die in the sediment (D_m) could be calculated if the mean volume of water overlying the sediment during a tidal cycle is known. From section 2.4.2, the mean depth of water overlying the Tern Street site at any period during a tidal cycle in

Table 5.7 Minimum intial numbers of faecal coliforms in the water overlying the Tern Street Site needed to resupply the numbers of faecal coliforms which die in the sediment, N_m. See text for details of calculation

	N_m ($\times 10^3$)	
Sediment depth (mm)	Autumn	Spring
40	59.1	1.4
50	70.0	1.6
60	80.9	1.8
70	92.0	2.1
80	102.7	2.3
90	113.6	2.5
100	124.5	2.7

autumn and spring (if tides were similar) would be 0.55 m, ie. 0.55 m^3 of water would overlay 1 m^2 of sediment at the Tern Street site.

From Figure 1.2, however, at mean tide approximately 55% of the water in the Estuary is lost to the Pacific Ocean and replaced with new sea water. If the new sea water contained few or no faecal coliforms then the effective volume of water which would contain the faecal coliforms needed to resupply the faecal coliforms which die in the underlying sediment would be 0.25 m^3. Table 5.8 shows values for D_m based on this volume and the values for N_m in Table 5.7.

Table 5.8 The minimum initial densities of faecal coliforms in the water overlying the sediment at the Tern Street site needed to resupply the numbers of faecal coliforms which die in the sediment, D_m. See text for details of calculation

	D_m (100 ml^{-1})	
Sediment depth (mm)	Autumn	Spring
40	23.6	0.6
50	28.0	0.6
60	32.4	0.7
70	36.8	0.8
80	41.1	0.9
90	45.5	1.0
100	49.8	1.1

The values for D_m shown in Table 5.8 are similar in magnitude to the median densities of faecal coliforms found in the water at sites in the vicinity of the Tern Street site in a previous bacteriological survey (refer to Table 1.1). It would appear, therefore, from the crude calculations carried out in this section, that the numbers of faecal coliforms observed in the sediment at the Tern Street site during the survey could be accounted for by the removal of faecal coliforms from the water overlying the site.

Values for D_m could also be used to calculate the minimum mean hourly and daily inputs of faecal coliforms into the Estuary needed to resupply the faecal coliforms which die in the sediment as the mean

tidal volume of water in the Estuary is given in Figure 1.2 (8.332×10^6 m^3) and if it is assumed that faecal coliforms are evenly distributed in the water of the Estuary (Table 5.9).

Table 5.9 The minimum mean hourly and daily inputs of faecal coliforms into the Estuary needed to resupply the faecal coliforms which die in the sediment at the Estuary. See text for details of calculation

	Numbers of faecal coliforms			
	h^{-1} ($x10^{10}$)		d^{-1} ($x\ 10^{12}$)	
Sediment depth (mm)	Autumn	Spring	Autumn	Spring
40	196.9	5.0	47.2	1.1
50	233.2	5.0	60.0	1.1
60	270.0	5.8	64.8	1.4
70	306.6	6.7	73.5	1.6
80	344.9	7.5	82.8	1.8
90	379.1	8.3	91.0	2.0
100	414.9	9.2	99.6	2.1

It should be noted that mean daily inputs of faecal coliforms into the Estuary from the Avon and Heathcote Rivers and the Christchurch Treatment Works have been estimated to be approximately 2.20 and 1.45×10^{12} in summer and winter, respectively (Christchurch Drainage Board, 1981), which are similar to the estimated minimum numbers of faecal coliforms needed to enter the Estuary in order to resupply those which die in the sediment of the Estuary in spring shown in Table 5.9.

Although such estimates for autumn were calculated to be between 10 and 100 fold higher, the discrepancy could be account for by additional sources of faecal coliforms to the Estuary which may include animals inhabiting or visiting the Estuary and run-off from the surrounding catchment.

CHAPTER SIX

GENERAL DISCUSSION AND CONCLUSIONS

The Avon-Heathcote Estuary is an important recreational resource for the people of Christchurch. Despite a major improvement in the water quality of the Estuary since 1973, mainly because of an improvement in the quality of effluent discharged from the oxidation ponds of the Christchurch Treatment Works (Christchurch Drainage Board, 1981), the water within the Estuary still only meets the minimum standard recommended for primary recreation (SB). Occasionally densities of faecal coliforms in the water exceed the 200 100 m^{-1} median specified by the SB standard (Appendix I).

The Estuary is contaminated with faecal coliforms which originate from various sources (Figure 6.1). Faecal coliforms which enter the Estuary may remain in the water or be deposited onto sediment. The aim of this study was to determine what might happen to those faecal coliforms which were deposited onto the sediment. Faecal coliforms were studied specifically because they are generally indicative of faecal contamination from warm-blooded animals (and therefore, pathogens) and are easily isolated and enumerated.

An investigation is described that was undertaken to determine how many faecal coliforms were deposited onto the sediment at two intertidal sites within the Estuary during one tidal cycle (refer to Chapter Two). Faecal coliforms were found in densities ranging from 3,485 to 57,624

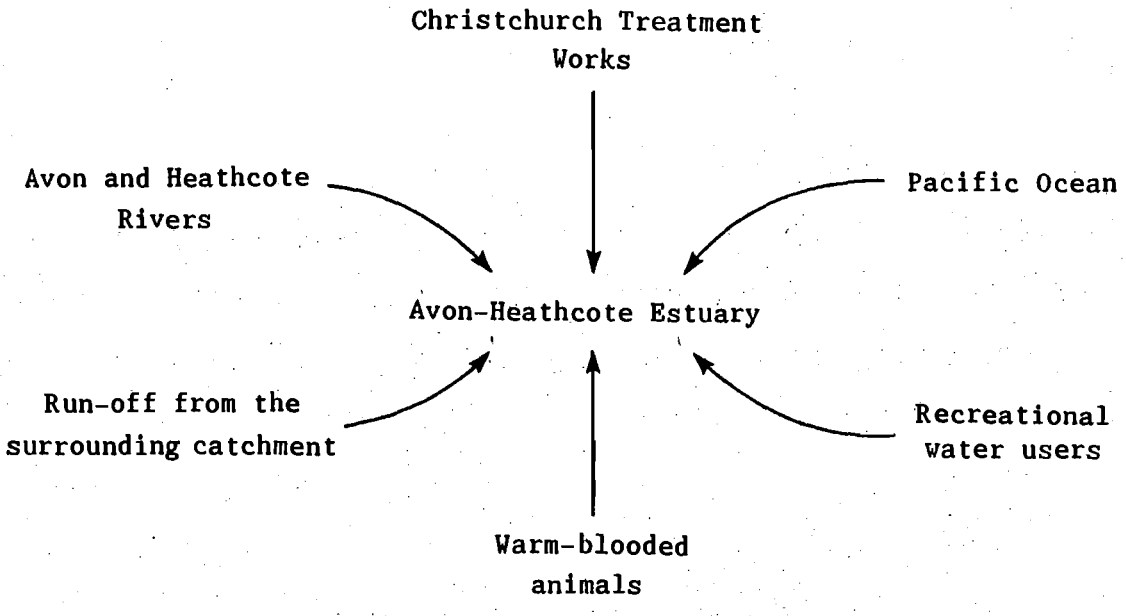


Figure 6.1 Sources of faecal coliforms to the Avon-Heathcote Estuary

100 ml^{-1} in sediment deposited in trays left at the two sites. At one site, trays were left out on two dates, once when there was little or no wind and a second time when there were strong winds at the Estuary. The strong winds induced turbulence in the shallow water of the Estuary which lead to the greater resuspension and relocation of sediment and lower numbers of faecal coliforms in deposited sediment (g^{-1} dry weight). If adsorption of faecal coliforms onto sediment particles is one of the mechanisms which remove faecal coliforms from the water column, then sheer forces created by the turbulent water conditions may have reduced the amount of adsorption between faecal coliforms and caused the release of loosely adsorbed faecal coliforms from resuspended

sediment. Also if deeper sediment containing fewer faecal coliforms was resuspended and then later deposited onto trays, faecal coliform counts would be reduced.

At the second site, trays were also left out when there were strong winds at the Estuary but little sediment was deposited. This reflected the sheltered nature of the site and the type of sediment which was more structured and tightly packed than the sediment at the other site. Numbers of faecal coliforms in deposited sediment (g^{-1} dry weight) from trays left at this site were higher than those of the other site. This could have resulted from the higher densities of faecal coliforms in the water overlying the site and the type of suspended particulate matter in the water which may have influenced the adsorption of faecal coliforms onto suspended particulate matter and consequently enhanced deposition.

The adsorption of faecal coliforms onto sediment consisting largely of sand particles was studied by two methods: (i) enumeration of faecal coliforms in sterile sediment samples after they were mixed with a suspensions containing faecal coliforms; and (ii) enumeration of faecal coliforms in the supernatant liquid of mixtures of sterile sediment and suspensions containing faecal coliforms. The degree of shaking was also varied to establish if this affected the balance between adsorption and desorption.

Numbers of faecal coliforms g^{-1} sediment (dry weight) were only similar to those found in sediment deposited into trays left at the Estuary over one tidal cycle when the sediment was mixed with faecal suspension with higher concentrations of faecal coliforms than would be expected in the water of the Estuary. It appeared that faecal coliforms

were also removed from water by mechanisms other than adsorption and it is concluded that adsorption may only play a small role in the removal of faecal coliforms from the water overlying sediments composed largely of sand.

In Chapter Three, an experiment is described which was undertaken to establish if there was evidence of *in situ* movement of faecal coliforms in sediment. The affect cockles may have on faecal coliform movement in sediment was also investigated. Results of this experiment were not conclusive but appeared to suggest that some of the faecal coliforms which are deposited onto the sediment surfaces do not remain there but infiltrate deeper into the sediment. The activities of cockles did not appear to aid penetration of faecal coliforms to deeper sediment. Experiments are also described which were conducted on the movement of faecal coliforms through columns packed with sandy sediment. Although only a narrow depth of sediment was used (approximately 48 mm), high proportions of faecal coliforms in faecal suspensions (mixtures of estuarine water and bird faeces) applied to sediment profiles appeared to be retained by them, especially when the faecal suspensions contained high levels of suspended faecal material.

In one experiment, faecal suspensions of varying concentration were applied in equal volumes to different sediment profiles. The proportion of faecal coliforms removed from volumes of percolate were estimated by subtracting the numbers of faecal coliforms found in effluent samples from the initial numbers applied. The ratios of faecal coliforms retained by sediment profiles to those applied were similar for all concentrations of faecal suspension.

In another experiment, distilled water was applied in equal volumes to columns of sediment after equal volumes of faecal suspension of varying concentration had been applied. The numbers of faecal coliforms in effluent samples tended to decline, approximately logarithmically, with time. After approximately 210 pore volumes of distilled water had percolated through sediment profiles the decline of faecal coliforms in the percolate was slower which may have been because fewer faecal coliforms were available to be leached from sediment profiles.

In both experiments, numbers of faecal coliforms were enumerated in sections of sediment profiles to determine their distribution. In sediment profiles to which only faecal suspensions were applied, numbers of faecal coliforms declined with depth, however, in sediment profiles to which distilled water was also applied, numbers of faecal coliforms tended to be similar in the top 20 mm of sediment and then declined with depth. As faecal coliforms were flushed from the surface they only travelled a small distance before they were again removed from the percolating water.

A second *in situ* experiment was also undertaken to determine the rate of infiltration of estuarine water and, therefore, enteric organisms if they remained in suspension and were not removed by the sediment. Cylinders were inserted into sediment at the start of a flood tide and were filled with sea water (higher salinity than the estuarine water normally overlying the site) approximating the level of the incoming tide. The idea was to use the higher conductivity of the sea water as a tracer, ie. the possible movement of water through sediment. After the tide had turned and began to drop, water was drained from the cylinders approximating the level of the outgoing tide. After all water had

drained from the cylinders, sediment cores were extracted from areas within and surrounding the cylinders. Cores were sectioned and the sediment mixed with distilled water. The conductivity of the supernatant liquids were measured to determine if sea water was present. Results of this experiment suggested that infiltration rates of estuarine water varied within small areas, probably as a result of different sediment characteristics, and that the rate of infiltration of estuarine water through some sediment could be at least 28 mm h^{-1}, ie. enteric organisms could potentially penetrate to a depth of at least 70 mm during one tidal cycle at the site (submerged for 2.5 h).

In Chapter Four, an experiment is described which was undertaken to determine the extent to which faecal coliforms were released from sediment mixed (to simulate gentle wave action) with estuarine water of varying salinity (conductivity). A linear relationship was used to describe the release of sediment-bound faecal coliforms where the proportion of faecal coliforms released from sediment increased with decreasing conductivity of estuarine water. This reflected an increase in desorption as a result of reduction of the electrolyte concentration of the estuarine water. A proportion of the sediment bound faecal coliforms also appeared to be released by the gentle rotation (physical disturbance) of estuarine sediment water mixtures. This suggests that some faecal coliforms are only loosely adsorbed to sediment or are free living in sediment.

Decrease in the salinity of water in the Estuary would be expected to occur in the vicinity of the Avon and Heathcote River mouths, especially during ebb and neap tides, and the Christchurch Treatment Works oxidation pond outfall when it is discharging. Dilution of

estuarine water and, therefore, a reduction in salinity would also occur as a result of periods of high rainfall which lead to increased freshwater inputs to the Estuary. Resuspension of sediment in the Estuary on the other hand, can be caused by wind-induced turbulence, physical disturbance of sediment by boats (especially jet boats) and water users.

The release of sediment-bound faecal coliforms upon reduction of the salinity of overlying water or as a result of physical disturbance has important public health implications because if the faecal coliforms are released from sediment, pathogens may also be released to the water column, posing a potential health hazard to water users.

In Chapter Five, an experiment is described which demonstrated the extended survival of faecal coliforms in estuarine sediment. Estuarine water and sediment were removed from the same location and incubated in the laboratory at 15°C in the dark. Die-off of faecal coliforms under these conditions was found to be six times faster in estuarine water than in sediment. The values for the T_{90} k of faecal coliforms in estuarine water determined in this experiment compared well with data from other studies.

A survey of the densities of faecal coliforms in the sediment at one intertidal site is also described. Sample cores of sediment were removed from the site and sub-samples analysed for faecal coliforms. Faecal coliforms were found to occur in higher densities in the 0-10 than in the 10-40mm depths of sample cores. Numbers of faecal coliforms, on a volumetric basis, observed in the sediment at this site throughout the survey were higher than those in the overlying water for

similar sites found in a bacteriological survey carried out in 1981. This appears to substantiate the belief that sediments extend the survival of faecal coliforms in aquatic environments. A seasonal variation in faecal coliform counts was also apparent from the survey, however this could not be explained. Calculations of the numbers of faecal coliforms needed to maintain the numbers observed in sediment in the survey, ie. to resupply those which die in the sediment (refer to secion 5.5), suggested that numbers of faecal coliforms introduced to the Estuary by the Avon and Heathcote Rivers and the Christchurch Treatment Works, estimated in the 1981 bacteriological survey, could in some cases, explain the observed numbers alone.

In summary, enteric organisms can be deposited onto sediment of the Estuary. Once deposited, they may remain at the surface, move to deeper sediment, or be resuspended and released to the water column. Survival of enteric organisms may be enhanced in sediment which could explain the relatively high numbers observed in sediment of the Estuary in this study. The findings of this study are significant in terms of public health which alone should warrant further research on enteric organisms in aquatic sediment.

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APPENDIX I Standards for Class SB waters (Water and Soil Conservation Act 1967 and Amendments)

The quality of Class SB waters shall conform to the following requirements:

(a) The natural water temperature shall not be changed by more than 3 degrees Celsius:
(b) The natural pH of the waters shall not be changed by more than 0.1 unit and no time shall be less then 6.7 or greater than 8.5:
(c) There shall be no destruction of the natural aquatic life by reason of a concentration of toxic substances nor shall the waters emit objectionable odours:
(d) The natural colour and clarity of the water shall not be changed to a conspicuous extent:
(e) The dissolved oxygen content in solution in the waters shall not be reduced below 5 milligrams per litre:
(f) Based on not fewer than 5 samples taken over not more than a 30-day period, the median value of the total faecal coliform bacteria content of the waters shall exceed 200 per 100 millilitres.

APPENDIX II Preparation of media

(a) Minerals modified glutimate medium (MMG). Dehydrated powder of MMG (Oxoid) was added to 1,000 ml of water containing 2.5 g of ammonium chloride powder. This was dispensed into test tubes and sterilized at 116°C for 10 minutes.

(b) Brilliant Green Bile Broth (BGBB). Forty g of BGBB (Oxoid) was added to 1,000 ml of water, dispensed in tubes with Durham tubes, and autoclaved at 121°C for 15 minutes.

(c) Peptone water for indole reaction

Casein hydrolysate (Gibco)	20 g
NaCl (Analar)	5 g
Water	1,000 ml

Sterilized in 5 ml aliquots at 121°C for 15 miutes.

(d) Kovacs' Reagent

Paradimethyl amino-benzaldehyde (Analar)	5 g
Amyl alcohol	75 ml
Concentrated hydrochloric acid (Analar)	25 ml

APPENDIX III Computer programme (Fortran) to calculate percent salinity (Christchurch Drainage Board, 1986)

```
      OPEN (21,FILE='CALC.OUT',PAD='YES',STATUS='FRESH')
      WRITE (21,26)
  26  FORMAT(10X,'CONDUCTIVITY',2X,'CHLORINITY',5X,'SALINITY',5X,
     + 'PERCENT',/,52X,'SEAWATER')
      A = 0.0000016177
      B = 0.31147
      STEP = 0.10
      X = 0.0
      DO 1,I=1,550
      Z = NINT(X*10)*100.0
      Y = A*(Z*Z) + B*Z
      RS = 0.03 + 1.805*Y
      PS = RS*100/3500
      WRITE (21,2)Z,Y,RS,PS
   2  FORMAT (14X,F6.0,8X,F8.2,6X,F8.2,4X,F5.1)
   1  X = X + STEP
      CLOSE (21)
      STOP
      END
```

APPENDIX IV Statistical analysis of data for section 2.4.2

(a) Twosample t-tests between faecal coliform counts for sediment and water deposited in trays (TS and TW, respectively) left at the Tern and Bridge Street sites on April 20/21 and May 4/5:

i) Tern Street site, April 20/21

```
TWOSAMPLE T FOR TS VS TW
    N      MEAN      STDEV    SE MEAN
TS  4     23687       8894       4447
TW  4      5775       2074       1037

95 PCT CI FOR MU TS.- MU TW: (3379, 32445)
TTEST MU TS = MU TW (VS NE): T=3.92 P=0.029 DF=3.3
```

ii) Tern Street site, May 4/5

```
TWOSAMPLE T FOR TS VS TW
    N      MEAN      STDEV    SE MEAN
TS  2      5751       3204       2266
TW  2      1095        856        605

95 PCT CI FOR MU TS - MU TW: (-25139, 34450)
TTEST MU TS = MU TW (VS NE): T=1.99 P=0.30 DF=1.1
```

iii) Bridge Street site, May 4/5

```
TWOSAMPLE T FOR C7 VS C8
    N      MEAN      STDEV    SE MEAN
TS  2     45187      17589      12437
TW  2       870        113       80.0

95 PCT CI FOR MU TS - MU TW: (-113713, 202347)
TTEST MU TS = MU TW (VS NE): T=3.56 P=0.17 DF=1.0
```

(b) Twosample *t*-tests between the amounts of sediment deposited in trays left at the Tern and Bridge Street sites on April 20/21 and May 4/5:

i) Tern Street site on April 20/21 (TA) and May 4/5 (TM)

```
TWOSAMPLE T FOR TA VS TM
    N      MEAN     STDEV   SE MEAN
TA  4     157.5      32.9      16.4
TM  2      1875       152       107

95 PCT CI FOR MU TA - MU TM: (-3098, -336.2)
TTEST MU TA = MU TM (VS NE): T=-15.80 P=0.040 DF=1.0
```

ii) Tern Street site on April 20/21 (TA) and Bridge Street site on May 4/5 (BM)

```
TWOSAMPLE T FOR TA VS BM
    N      MEAN     STDEV   SE MEAN
TA  4     157.5      32.9      16.4
BM  2     21.60      1.98      1.40

95 PCT CI FOR MU TA - MU BM: (83.44, 188.4)
TTEST MU TA = MU BM (VS NE): T=8.24 P=0.0037 DF=3.0
```

iii) Tern Street site on May 4/5 (TM) and Bridge Street site May 4/5 (BM)

```
TWOSAMPLE T FOR TM VS BM
    N      MEAN     STDEV   SE MEAN
TM  2      1875       152       107
BM  2     21.60      1.98      1.40

95 PCT CI FOR MU TM - MU BM: (487.9, 3219)
TTEST MU TM = MU BM (VS NE): T=17.25 P=0.037 DF=1.0
```

(c) Twosample *t*-tests between faecal coliform counts (100 ml^{-1}) for sediment deposited in trays left at the Tern and Bridge Street sites on April 20/21 and May 4/5:

i) Tern Street site on April 20/21 (TA) and May 4/5 (TM)

```
TWOSAMPLE T FOR TA VS TM
    N      MEAN     STDEV   SE MEAN
TA  4     23687      8894      4447
TM  2      5751      3204      2266

95 PCT CI FOR MU TA - MU TM: (2053, 33820)
TTEST MU TA = MU TM (VS NE): T=3.59 P=0.037 DF=4.0
```

ii) Tern Street site on April 20/21 (TA) and Bridge Street site on May 4/5 (BM)

```
TWOSAMPLE T FOR TA VS BM
    N      MEAN     STDEV   SE MEAN
TA  4     23687      8894      4447
BM  2     45187     17589     12437

95 PCT CI FOR MU TA - MU BM: (-189326, 146326)
TTEST MU TA = MU BM (VS NE): T=-1.63 P=0.35 DF=1.3
```

iii) Tern Street site on May 4/5 (TM) and Bridge Street site on May 4/5 (BM)

TWOSAMPLE T FOR TM VS BM

	N	MEAN	STDEV	SE MEAN
TM	2	5751	3204	2266
BM	2	45187	17589	12437

95 PCT CI FOR MU TM - MU BM: (-200064, 121191)
TTEST MU TA = MU BM (VS NE): T=-3.12 P=0.20 DF=1.1

(d) Twosample t-tests between faecal coliform counts for water deposited in trays left at Tern and Bridge Street sites on April 20/21 and May 4/5:

i) Tern Street site on April 20/21 (TA) and May 4/5 (TM)

TWOSAMPLE T FOR TA VS TM

	N	MEAN	STDEV	SE MEAN
TA	4	5775	2074	1037
TM	2	1095	856	605

95 PCT CI FOR MU TA - MU TM: (858.9, 8501)
TTEST MU TA = MU TM (VS NE): T=3.90 P=0.030 DF=4.0

ii) Tern Street site on April 20/21 (TA) and Bridge Street site on May 4/5 (BM)

TWOSAMPLE T FOR TA VS BM

	N	MEAN	STDEV	SE MEAN
TA	4	5775	2074	1037
BM	2	870	113	80.0

95 PCT CI FOR MU TA - MU BM: (1595, 8215)
TTEST MU TA = MU BM (VS NE): T=4.72 P=0.018 DF=3.0

iii) Tern Street site on May 4/5 (TM) and Bridge Street site on May 4/5 (BM)

TWOSAMPLE T FOR TM VS BM

	N	MEAN	STDEV	SE MEAN
TM	2	1095	856	605
BM	2	870	113	80.0

95 PCT CI FOR MU TM - MU BM: (-7529, 7979)
TTEST MU TM = MU BM (VS NE): T=0.37 P=0.78 DF=1.0

(e) Twosample t-tests between faecal coliform counts (g^{-1}) for sediment deposited in trays left at the Tern and Bridge Street sites on April 20/21 and May 4/5:

i) Tern Street site on April 20/21 (TA) and May 4/5 (TM)

TWOSAMPLE T FOR TA VS TM

	N	MEAN	STDEV	SE MEAN
TA	4	204.0	76.6	38.3
TM	2	49.5	27.6	19.5

95 PCT CI FOR MU TA - MU TM: (17.73, 291.3)
TTEST MU TA = MU TM (VS NE): T=3.59 P=0.037 DF=4.0

ii) Tern Street site on April 20/21 (TA) and Bridge Street site on May 4/5 (BM)

TWOSAMPLE T FOR TA VS BM

	N	MEAN	STDEV	SE MEAN
TA	4	204.0	76.6	38.3
BM	2	436	170	120

95 PCT CI FOR MU TA - MU BM: (-1833, 1369)
TTEST MU TA = MU BM (VS NE): T=-1.84 P=0.32 DF=1.2

iii) Tern Street site on May 4/5 (TM) and Bridge Street site on May 4/5 (BM)

TWOSAMPLE T FOR TM VS BM

	N	MEAN	STDEV	SE MEAN
TM	2	49.5	27.6	19.5
BM	2	436	170	120

95 PCT CI FOR MU TM - MU BM: (-1931, 1158)
TTEST MU TM = MU BM (VS NE): T=-3.18 P=0.19 DF=1.1

APPENDIX V Statistical analysis of data for section 2.5.2

(a) Regression analysis for experiment I:

The regression equation is $N_a = -9 + 0.00256\ D_f$ where N_a is the number of faecal coliforms adsorbed g^{-1} of sediment and D_f is the density of faecal coliforms 100 ml^{-1} of the faecal suspension

Predictor	Coef	Stdev	t-ratio
Constant	-9.0	143.6	-0.06
D_f	0.0025599	0.0004565	5.61

s = 394.8 R-sq = 79.7% R-sq(adj) = 77.2%

Analysis of Variance

SOURCE	DF	SS	MS	F
Regression	1	4901387	4901387	31.45
Error	8	1246797	155850	
Total	9	6148184		

Unusual Observations

Obs.	D_f	N_a	Fit	Stdev.Fit	Residual	St.Resid
9	700000	1001	1783	278	-782	-2.79R
10	700000	2567	1783	278	784	2.80R

R denotes an obs. with a large st. resid.

(b) Analysis of variance and regression analysis for experiment II:

SOURCE	DF	SS	MS	F
FACTOR	4	1941	485	1.79
ERROR	10	2709	271	
TOTAL	14	4649		

LEVEL	N	MEAN	STDEV	INDIVIDUAL 95 PCT CI'S FOR MEAN BASED ON POOLED STDEV
ST FS	3	159.00	15.39	(----------*---------)
6.20 g	3	129.00	20.66	(----------*---------)
12.40 g	3	139.00	19.70	(----------*---------)
24.79 g	3	138.67	8.08	(----------*---------)
49.59 g	3	156.00	15.39	(----------*---------)

POOLED STDEV = 16.46

120 140 160

The regression equation is $N_B = 6768 + 498\ C_s$ where C_s is the concentration of sediment 100 ml^{-1} of faecal suspension

Predictor	Coef	Stdev	t-ratio
Constant	6768	7924	0.85
g/100ml	497.5	277.3	1.79

s = 15963 R-sq = 24.4% R-sq(adj) = 16.8%

Analysis of Variance

SOURCE	DF	SS	MS	F
Regression	1	820381888	820381888	3.22
Error	10	2548284928	254828496	
Total	11	3368666880		

APPENDIX VI Statistical analysis for section 3.3.2.2

(a) Twosample t-tests between faecal coliform counts for the 0-10 and 10-40 mm depths of plot 1 for March 26 (MT1 and MB1, respectively) and those for April 14 (AT1 and AB1, respectively):

TWOSAMPLE T FOR MT1 VS AT1

	N	MEAN	STDEV	SE MEAN
MT1	2	10215	9125	6453
TA1	2	42560	32409	22917

95 PCT CI FOR MU MT1 - MU AT1: (-334849, 270159)
TTEST MU MT1 = MU AT1 (VS NE): T=-1.36 P=0.40 DF=1.2

TWOSAMPLE T FOR MB1 VS AB1

	N	MEAN	STDEV	SE MEAN
MB1	2	5000	2357	1667
AB1	2	2838	2160	1528

95 PCT CI FOR MU MB1 - MU AB1: (-26566, 30891)
TTEST MU MB1 = MU AB1 (VS NE): T=0.96 P=0.51 DF=2.0

(b) Twosample t-tests between faecal coliform counts for the 0-10 and 10-40 mm depths of plot 2 for March 26 (MT2 and MB2, respectively) and those for April 14 (AT2 and AB2, respectively):

```
TWOSAMPLE T FOR MT2 VS AT2
     N      MEAN     STDEV   SE MEAN
MT2  2     18571     10101      7143
AT2  2    109226    140158     99107

95 PCT CI FOR MU MT2 - MU AT2: (-1353194, 1171885)
TTEST MU MT2 = MU AT2 (VS NE): T=-0.91 P=0.53 DF=1.0

TWOSAMPLE T FOR MB2 VS MT2
     N      MEAN     STDEV   SE MEAN
MB2  2      4381      1885      1333
AB2  2      4941      2553      1806

95 PCT CI FOR MU MB2 - MU AB2: (-29076, 27957)
TTEST MU MB2 = MU AB2 (VS NE): T=-0.25 P=0.84 DF=1.8
```

(c) Twosample t-tests between faecal coliform counts for the 0-10 mm depth and those for the 10-40 mm depth of plot 1 for March 26 (MT1 and MB1, respectively) and April 14 (AT1 and AB1, respectively):

```
TWOSAMPLE T FOR MT1 VS MB1
     N      MEAN     STDEV   SE MEAN
MT1  2     10215      9125      6453
MB1  2      5000      2357      1667

95 PCT CI FOR MU MT1 - MU MB1: (-79464, 89893)
TTEST MU MT1 = MU MB1 (VS NE): T=0.78 P=0.58 DF=1.1

TWOSAMPLE T FOR AT1 VS AB1
     N      MEAN     STDEV   SE MEAN
AT2  2     42560     32409     22917
AB2  2      2838      2160      1528

95 PCT CI FOR MU AT1 - MU AB1: (-252106, 331550)
TTEST MU AT1 = MU AB1 (VS NE): T=1.73 P=0.33 DF=1.0
```

(d) Twosample t-tests between faecal coliform counts for the 0-10 mm depth and those for the 10-40 mm depth of plot 2 for March 26 (MT2 and MB2, respectively) and those for April 14 (AT2 and AB2, respectively):

```
TWOSAMPLE T FOR MT2 VS MB2
     N      MEAN     STDEV   SE MEAN
MT2  2     18571     10101      7143
MB2  2      4381      1885      1333

95 PCT CI FOR MU MT2 - MU MB2: (-78131, 106512)
TTEST MU MT2 = MU MB2 (VS NE): T=1.95 P=0.30 DF=1.1

TWOSAMPLE T FOR AT2 VS AB2
     N      MEAN     STDEV   SE MEAN
AT2  2    109226    140158     99107
AB2  2      4941      2553      1806

95 PCT CI FOR MU AT2 - MU AB2: (-1155197, 1363768)
TTEST MU AT2 = MU AB2 (VS NE): T=1.05 P=0.48 DF=1.0
```

(e) Twosample t-test between faecal coliform counts for the 0-10 mm depth and those for the 10-40 mm depth of plots 1 and 2 (T12 and B12, respectively), excluding highest count:

```
TWOSAMPLE T FOR T12 VS B12
      N      MEAN     STDEV   SE MEAN
T12   7     21830     20507      7751
B12   8      4290      1942       686

95 PCT CI FOR MU T12 - MU B12: (-1505, 36586)
TTEST MU T12 = MU B12 (VS NE): T=2.25 P=0.065 DF=6.1
```

(f) Twosample t-tests between faecal coliform counts for the 0-10 mm depth of plots 1 and 2 for March 26 (MT1 and MT2, respectively) and April 14 (AT1 and AT2, respectively):

```
TWOSAMPLE T FOR MT1 VS MT2
     N      MEAN     STDEV   SE MEAN
MT1  2     10215      9125      6453
MT2  2     18571     10101      7143

95 PCT CI FOR MU MT1 - MU MT2: (-130660, 113946)
TTEST MU MT1 = MU MT2 (VS NE): T=-0.87 P=0.54 DF=2.0

TWOSAMPLE T FOR AT1 VS AT2
     N      MEAN     STDEV   SE MEAN
AT1  2     42560     32409     22917
AT2  2    109226    140158     99107

95 PCT CI FOR MU AT1 - MU AT2: (-1359166, 1225834)
TTEST MU AT1 = MU AT2 (VS NE): T=-0.66 P=0.63 DF=1.1
```

(g) Twosample t-tests between faecal coliform counts for the 10-40 mm depth of plots 1 and 2 for March 26 (MB1 and MB2, respectively) and those for April 14 (AB1 and AB2, respectively):

```
TWOSAMPLE T FOR MB1 VS MB2
     N      MEAN     STDEV   SE MEAN
MB1  2      5000      2357      1667
MB2  2      4381      1885      1333

95 PCT CI FOR MU MB1 - MU MB2: (-26501, 27739)
TTEST MU MB1 = MU MB2 (VS NE): T=0.29 P=0.82 DF=1.9

TWOSAMPLE T FOR AB1 VS AB2
     N      MEAN     STDEV   SE MEAN
AB1  2      2838      2160      1528
AB2  2      4941      2553      1806

95 PCT CI FOR MU AB1 - MU AB2: (-32153, 27947)
TTEST MU AB1 = MU AB2 (VS NE): T=-0.89 P=0.54 DF=1.9
```

APPENDIX VII Statistical analysis for section 3.6.2

(a) Analysis of variance of supernatant liquid conductivities of mixtures of distilled water and sediment from cores taken outside and from inside cylinders, respectively:

SOURCE	DF	SS	MS	F
CORE NO.	5	0.5684	0.1137	2.08
DEPTH	6	1.7106	0.2851	5.21
ERROR	30	1.6412	0.0547	
TOTAL	41	3.9202		

SOURCE	DF	SS	MS	F
CORE NO.	2	1.8262	0.9131	22.33
DEPTH	6	4.2967	0.7161	17.51
INTERACTION	12	0.5635	0.0470	1.15
ERROR	42	1.7196	0.0409	
TOTAL	62	8.4060		

(b) Twosample *t*-tests between conductivities of supernatant liquids of mixtures of distilled water and sediment from the 0-10 mm depth of cores taken from inside and outside cylinders (S1 = outside cylinder, C1, C2 and C3 = inside cylinders 1, 2 and 3, respectively):

TWOSAMPLE T FOR S1 VS C1

	N	MEAN	STDEV	SE MEAN
S1	6	1.835	0.190	0.0776
C1	3	3.5567	0.0569	0.0328

95 PCT CI FOR MU S1 - MU C1: (-1.928, -1.515)
TTEST MU S1 = MU C1 (VS NE): T=-20.44 P=0.0000 DF=6.4

TWOSAMPLE T FOR S1 VS C2

	N	MEAN	STDEV	SE MEAN
S1	6	1.835	0.190	0.0776
C1	3	3.233	0.267	0.154

95 PCT CI FOR MU S1 - MU C2: (-1.947, -0.8499)
TTEST MU S1 = MU C2 (VS NE): T=-8.11 P=0.0039 DF=3.1

TWOSAMPLE T FOR S1 VS C3

	N	MEAN	STDEV	SE MEAN
S1	6	1.835	0.190	0.0776
C3	3	3.383	0.289	0.167

95 PCT CI FOR MU S1 - MU C3: (-2.340, -0.7562)
TTEST MU S1 = MU C3 (VS NE): T=-8.41 P=0.014 DF=2.9

(c) Twosample t-tests between conductivities of supernatant liquids of mixtures of distilled water and sediment from the 10-20 mm depth of cores taken from inside and outside cylinders (S1 = outside cylinder, C1, C2 and C3 = inside cylinders 1, 2 and 3, respectively):

TWOSAMPLE T FOR S1 VS C1

	N	MEAN	STDEV	SE MEAN
S1	6	1.900	0.147	0.0602
C1	3	2.9700	0.0436	0.0252

95 PCT CI FOR MU S1 - MU C1: (-1.230, -0.9104)
TTEST MU S1 = MU C1 (VS NE): T=-16.41 P=0.0000 DF=6.4

TWOSAMPLE T FOR S1 VS C2

	N	MEAN	STDEV	SE MEAN
S1	6	1.900	0.147	0.0602
C2	3	2.563	0.180	0.104

95 PCT CI FOR MU S1 - MU C2: (-1.046, -0.2806)
TTEST MU S1 = MU C2 (VS NE): T=-5.52 P=0.012 DF=3.4

TWOSAMPLE T FOR S1 VS C3

	N	MEAN	STDEV	SE MEAN
S1	6	1.900	0.147	0.0602
C3	3	3.190	0.233	0.134

95 PCT CI FOR MU S1 - MU C3: (-1.923, -0.6569)
TTEST MU S1 = MU C3 (VS NE): T=-8.77 P=0.013 DF=2.8

(d) Twosample t-tests between conductivities of supernatant liquids of mixtures of distilled water and sediment from the 20-30 mm depth of cores taken from inside and outside cylinders (S1 = outside cylinder, C1, C2 and C3 = inside cylinders 1, 2 and 3, respectively):

TWOSAMPLE T FOR S1 VS C1

	N	MEAN	STDEV	SE MEAN
S1	6	1.950	0.119	0.0486
C1	3	2.743	0.180	0.104

95 PCT CI FOR MU S1 - MU C1: (-1.287, -0.2995)
TTEST MU S1 = MU C1 (VS NE): T=-6.91 P=0.020 DF=2.9

TWOSAMPLE T FOR S1 VS C2

	N	MEAN	STDEV	SE MEAN
S1	6	1.950	0.119	0.0486
C2	3	2.233	0.102	0.0590

95 PCT CI FOR MU S1 - MU C2: (-0.4955, -0.07114)
TTEST MU S1 = MU C2 (VS NE): T=-3.71 P=0.021 DF=4.8

TWOSAMPLE T FOR S1 VS C3

	N	MEAN	STDEV	SE MEAN
S1	6	1.950	0.119	0.0486
C3	3	2.793	0.240	0.138

95 PCT CI FOR MU S1 - MU C3: (-1.474, -0.2124)
TTEST MU S1 = MU C3 (VS NE): T=-5.75 P=0.029 DF=2.5

(e) Twosample t-tests between conductivities of supernatant liquids of mixtures of distilled water and sediment from the 30-40 mm depth of cores taken from inside and outside cylinders (S1 = outside cylinder, C1, C2 and C3 = inside cylinders 1, 2 and 3, respectively):

TWOSAMPLE T FOR S1 VS C1

	N	MEAN	STDEV	SE MEAN
S1	6	2.158	0.251	0.103
C1	3	2.853	0.305	0.176

95 PCT CI FOR MU S1 - MU C1: (-1.344, -0.04647)
TTEST MU S1 = MU C1 (VS NE): T=-3.41 P=0.042 DF=3.4

TWOSAMPLE T FOR S1 VS C2

	N	MEAN	STDEV	SE MEAN
S1	6	2.158	0.251	0.103
C2	3	2.467	0.236	0.136

95 PCT CI FOR MU S1 - MU C2: (-0.7823, 0.1657)
TTEST MU S1 = MU C2 (VS NE): T=-1.81 P=0.15 DF=4.3

TWOSAMPLE T FOR S1 VS C3

	N	MEAN	STDEV	SE MEAN
S1	6	2.158	0.251	0.103
C3	3	2.627	0.225	0.130

95 PCT CI FOR MU S1 - MU C3: (-0.9286, -0.008110)
TTEST MU S1= MU C3 (VS NE): T=-2.83 P=0.048 DF=4.6

(f) Twosample t-tests between conductivities of supernatant liquids of mixtures of distilled water and sediment from the 40-50 mm depth of cores taken from inside and outside cylinders (S1 = outside cylinder, C1, C2 and C3 = inside cylinders 1, 2 and 3, respectively):

TWOSAMPLE T FOR S1 VS C1

	N	MEAN	STDEV	SE MEAN
S1	6	2.405	0.410	0.167
C1	3	2.817	0.169	0.0974

95 PCT CI FOR MU S1 - MU C1: (-0.8853, 0.06199)
TTEST MU S1 = MU C1 (VS NE): T=-2.13 P=0.078 DF=7.0

TWOSAMPLE T FOR S1 VS C2

	N	MEAN	STDEV	SE MEAN
S1	6	2.405	0.410	0.167
C2	3	2.4567	0.0643	0.0371

95 PCT CI FOR MU S1 - MU C2: (-0.4922, 0.3888)
TTEST MU S1 = MU C2 (VS NE): T=-0.30 P=0.78 DF=5.5

TWOSAMPLE T FOR S1 VS C3

	N	MEAN	STDEV	SE MEAN
S1	6	2.405	0.410	0.167
C3	3	2.717	0.146	0.0841

95 PCT CI FOR MU S1 - MU C3: (-0.7699, 0.1466)
TTEST MU S1 = MU C3 (VS NE): T=-1.66 P=0.15 DF=6.8

(g) Twosample *t*-tests between conductivities of supernatant liquids of mixtures of distilled water and sediment from the 50-60 mm depth of cores taken from inside and outside cylinders (S1 = outside cylinder, C1, C2 and C3 = inside cylinders 1, 2 and 3, respectively):

TWOSAMPLE T FOR S1 VS C1

	N	MEAN	STDEV	SE MEAN
S1	6	2.218	0.227	0.0927
C1	3	3.013	0.127	0.0736

95 PCT CI FOR MU S1 - MU C1: (-1.085, -0.5053)
TTEST MU S1 = MU C1 (VS NE): T=-6.72 P=0.0005 DF=6.7

TWOSAMPLE T FOR S1 VS C2

	N	MEAN	STDEV	SE MEAN
S1	6	2.218	0.227	0.0927
C2	3	2.457	0.285	0.165

95 PCT CI FOR MU S1 - MU C2: (-0.8394, 0.3628)
TTEST MU S1 = MU C2 (VS NE): T=-1.26 P=0.30 DF=3.3

TWOSAMPLE T FOR S1 VS C3

	N	MEAN	STDEV	SE MEAN
S1	6	2.218	0.227	0.0927
C3	3	2.600	0.233	0.135

95 PCT CI FOR MU S1 - MU C3: (-0.9016, 0.1383)
TTEST MU S1 = MU C3 (VS NE): T=-2.34 P=0.10 DF=4.0

(h) Twosample *t*-tests between conductivities of supernatant liquids of mixtures of distilled water and sediment from the 60-70 mm depth of cores taken from inside and outside cylinders (S1 = outside cylinder, C1, C2 and C3 = inside cylinders 1, 2 and 3, respectively):

TWOSAMPLE T FOR S1 VS C1

	N	MEAN	STDEV	SE MEAN
S1	6	2.307	0.296	0.121
C1	3	2.757	0.181	0.105

95 PCT CI FOR MU S1 - MU C1: (-0.8412, -0.05878)
TTEST MU S1 = MU C1 (VS NE): T=-2.82 P=0.031 DF=6.4

TWOSAMPLE T FOR S1 VS C2

	N	MEAN	STDEV	SE MEAN
S1	6	2.307	0.296	0.121
C2	3	2.503	0.202	0.117

95 PCT CI FOR MU S1 - MU C2: (-0.6283, 0.2350)
TTEST MU S1 = MU C2 (VS NE): T=-1.17 P=0.29 DF=5.9

TWOSAMPLE T FOR S1 VS C3

	N	MEAN	STDEV	SE MEAN
S1	6	2.307	0.296	0.121
C3	3	2.727	0.184	0.107

95 PCT CI FOR MU S1 - MU C3: (-0.8140, -0.02598)
TTEST MU S1 = MU C3 (VS NE): T=-2.61 P=0.040 DF=6.3

APPENDIX VIII Regression analysis for section 4.3.2

The regression equation is PR = 92.5 - 1.12 C where PR is the percent release of faecal coliforms from the sediment and C is the conductivity of the estuarine water/sediment mixture

Predictor	Coef	Stdev	t-ratio
Constant	92.471	6.469	14.29
C	-1.1205	0.2349	-4.77

s = 17.71 R-sq = 58.7% R-sq(adj) = 56.1%

Analysis of Variance

SOURCE	DF	SS	MS	F
Regression	1	7132.0	7132.0	22.75
Error	16	5015.8	313.5	
Total	17	12147.8		

Unusual Observations

Obs.	C	PR	Fit	Stdev.Fit	Residual	St.Resid
12	17.6	116.30	72.74	4.25	43.56	2.53R

R denotes an obs. with a large st. resid.

Omitting the observation with a large standard residual, the regression equation is PR = 89.3 - 1.09 C

Predictor	Coef	Stdev	t-ratio
Constant	89.316	5.264	16.97
C	-1.0926	0.1879	-5.81

s = 14.15 R-sq = 69.3% R-sq(adj) = 67.2%

Analysis of Variance

SOURCE	DF	SS	MS	F
Regression	1	6766.1	6766.1	33.81
Error	15	3002.2	200.1	
Total	16	9768.3		

APPENDIX IX Regression analysis for section 5.3.2

(a) Faecal coliform die-off in estuarine water incubated in the dark at 15°C

The regression equation is N_s = 2.80 - 0.698 T where N_s is the number of surviving faecal coliforms and T is the time (d)

Predictor	Coef	Stdev	t-ratio
Constant	2.80369	0.08696	32.24
T	-0.69793	0.03550	-19.66

s = 0.1945 R-sq = 96.7% R-sq(adj) = 96.5%

Analysis of Variance

SOURCE	DF	SS	MS	F
Regression	1	14.613	14.613	384.6
Error	13	0.492	0.038	
Total	14	15.105		

Unusual Observations

Obs.	T	N_s	Fit	Stdev.Fit	Residual	St.Resid
11	3.00	1.2788	0.7099	0.0615	0.5689	3.08R

R denotes an obs. with a large st. resid.

Omitting the observation with the large standard residual, the regression equation is $N_s = 2.80 - 0.719\ T$

Predictor	Coef	Stdev	t-ratio
Constant	2.80369	0.04691	59.77
T	-0.71900	0.01950	-36.87

s = 0.1049 R-sq = 99.1% R-sq(adj) = 99.1%

Analysis of Variance

SOURCE	DF	SS	MS	F
Regression	1	14.955	14.955	1360
Error	12	0.132	0.011	
Total	13	15.087		

(b) Faecal coliform die-off in estuarine sediment incubated in the dark at 15°C

The regression equation is $N_s = 3.54 - 0.124\ T$

Predictor	Coef	Stdev	t-ratio
Constant	3.5369	0.1817	19.46
T	-0.12445	0.01465	-8.50

s = 0.6432 R-sq = 76.6% R-sq(adj) = 75.6%

Analysis of Variance

SOURCE	DF	SS	MS	F
Regression	1	29.853	29.853	72.1
Error	22	9.101	0.414	
Total	23	38.954		

Unusual Observations

Obs.	T	N_s	Fit	Stdev.Fit	Residual	St.Resid
17	11.0	0.000	2.170	0.136	-2.170	-3.45R

R denotes an obs. with a large st. resid.

Omitting the observation with the large standard residual, the regression equation is $N_s = 3.61 - 0.122\ T$

Predictor	Coef	Stdev	t-ratio
Constant	3.6072	0.1267	28.47
T	-0.12162	0.01017	-11.96

s = 0.4456 R-sq = 87.2% R-sq(adj) = 86.6%

Analysis of Variance

SOURCE	DF	SS	MS
Regression	1	28.421	28.421
Error	21	4.170	0.199
Total	22	32.591	

Unusual Observations

Obs.	T	N_s	Fit	Stdev.Fit	Residual	St.Resid
19	18.0	2.3263	1.4205	0.1341	0.9058	2.13R

R denotes an obs. with a large st. resid.

APPENDIX X Statistical analysis for section 5.4.2

(a) Analyses of variance of faecal coliform counts for the 0-10 and 10-40 mm depths for autumn and spring, respectively:

SOURCE	DF	SS	MS	F
FACTOR	1	97535	97535	12.23
ERROR	16	127640	7977	
TOTAL	17	225174		

LEVEL	N	MEAN	STDEV	INDIVIDUAL 95 PCT CI'S FOR MEAN BASED ON POOLED STDEV
0-10	9	208.22	119.37	(-------*-------)
10-40	9	61.00	41.30	(-------*-------)
POOLED STDEV =		89.32		0 75 150 225

SOURCE	DF	SS	MS	F
FACTOR	1	65764128	65764128	2.53
ERROR	12	311756640	25979720	
TOTAL	13	377520768		

LEVEL	N	MEAN	STDEV	INDIVIDUAL 95 PCT CI'S FOR MEAN BASED ON POOLED STDEV
0-10	7	7407	6264	(----------*---------)
10-40	7	3072	3567	(----------*---------)
POOLED STDEV =		5097		0 4000 8000 12000

(b) Twosample t-tests between counts of faecal coliforms for the 0-10 and 10-40 mm depths for autumn (AT and AB, respectively) and spring (ST and SB, respectively):

TWOSAMPLE T FOR AT VS ST

	N	MEAN	STDEV	SE MEAN
AT	7	7407	6264	2368
ST	9	208	119	39.8

95 PCT CI FOR MU AT - MU ST: (1403, 12995)
TTEST MU AT = MU ST (VS NE): T=3.04 P=0.023 DF=6.0

TWOSAMPLE T FOR AB VS SB

	N	MEAN	STDEV	SE MEAN
AB	7	3072	3567	1348
SB	9	61.0	41.3	13.8

95 PCT CI FOR MU AB - MU SB: (-288.2, 6311)
TTEST MU AB = MU SB (VS NE): T=2.23 P=0.067 DF=6.0