MICROBIOLOGICAL CHARACTERISTICS IN URBAN STORMWATER RUNOFF AND SEDIMENTS

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ABSTRACT

This study was undertaken to investigate the microbiological composition of urban surface water runoff and sediments in both North London and Valencia. The survey focused primarily on the relationships between indicator microorganisms (total coliforms, faecal coliforms and faecal streptococci) and water quality, and between indicator microorganisms and pathogens (*Pseudomonas aeruginosa* and *Salmonella*) which cause waterborne disease.

High levels of microorganisms were found in dry weather flow sanitary wastewater, combined sewage, urban receiving stream, beach outfall and storm water runoff as well as sediments. Pathogenic bacteria were consistently isolated from the urban water courses. *Pseudomonas aeruginosa* were recovered at high concentration and *Salmonella* required concentration for enumeration.

The results indicated that storm water runoff from urban areas contain high levels of indicator microorganisms and pathogenic bacteria. The pollution appears to be predominantly of non-human origin and is mainly derived from animal wastes. In general, the runoff resembled dilute raw sewage in microbiological composition and obversely represents a public health risk.

Although some connection between bacteria densities and selected environmental factors is apparent, the relationship is not simple. Factors such as BOD_5, flow, temperature, pH, faecal deposit age and hydrologic proximity of pollution sources all affect bacterial densities in runoff and according to different water sources. However, the parameter of bacterial loading may be used in survey of aquatic environment to estimate probable bacteria density ranges.

The characteristics of bacterial concentration in different layers of sediments and bacterial release from sediment in a continuous flow tank have also been investigated. Indicator and pathogenic bacteria occur at the highest concentration in the upper layers of sediment. *Pseudomonas aeruginosa* was more easy released than indicator microorganisms. The microbial release peak was detected at 6 hours. The ratio of faecal coliform to faecal streptococcus densities can be used to help identify particular sources of faecal pollution and had relatively small fluctuation rate after faeces deposited in water under 4°C than 20°C.

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Chapter 1: Introduction

1.1. The Research Context

During recent years there has been a growing recognition of the importance of urban sewage discharges and stormwater runoff as major sources of microbiological pollution in urban natural surface water and sediment. Several studies have been conducted to determine the qualitative and quantitative characteristics of microorganisms in urban sewage, stormwater and sediments as well as their impact on receiving waters (Geldreich *et al.*, 1968; Field *et al.*, 1976; Olivieri *et al.*, 1978; Rhodes and Kator, 1988; Marino and Gannon, 1991). The striking emphasis of these studies has been to identify the similarity between stormwater runoff and sewage effluent in term of their inherent potential hazards to public health.

This thesis describes research carried out over two years into aspects of microbiological contaminants in urban surface water runoff in both North London (The U.K.) and Valencia (Spain). The studies were designed to collect baseline data and information on the current topics with the following major objectives in mind.

a). To determine the levels of indicator microorganisms (total coliforms, faecal coliforms and faecal streptococci) and pathogenic bacteria (*Pseudomonas aeruginosa* and *Salmonella*) in domestic wastewater, combined sewage, receiving stream and beach outfall and to utilise bacterial indices to enable a comparison of temperate and Mediterranean situations.

b). To determine the incidence and distributions of pollutant indicator microorganisms and pathogenic bacteria in areas having different urban land uses.

c). To determine whether the ratio of faecal coliform to faecal streptococcus has significance as a pollution index that may be used in urban surface runoff studies under different climatic conditions.

d). To determine and compare the survival and release characteristics of indicator

microorganisms and pathogenic bacteria in different sediments of an urban water discharge system and in different sediment layers.

e). To determine diurnal patterns of indicator microorganisms and pathogenic bacteria in dry weather flow sanitary wastewater flows.

f). To determine the relationship between microorganisms and environmental factors in order to evaluate the potential influence of flow to bacteria pollution.

1.2. Thesis Content and Organization

The structure of this thesis and a fuller description of the research programme is outlined below:

This is the introduction and context to the research constitutes the first of seven Chapters. The contents of the remainder are briefly summarised in the following descriptions.

Chapter 2 provides a review of the literature which gives background information of general relevance to the subjects considered in later sections. The sources and characteristics of microorganisms and pathogenic bacteria in urban surface waters and sediments are described.

Chapter 3 provides details of sampling locations, sampling methods and microbiological analytical methods as well as physio - chemical analysis techniques which have been employed in the research; details of the microbiological media are also included.

Chapter 4 describes the main investigations carried out to determine the microbiological characteristics and levels of both indicator microorganisms and pathogenic bacteria found in urban surface runoff and sediment. A comparison of the levels of indicator microorganisms and pathogenic bacteria between an urban catchment in North London and Valencia is made. The relationships between indicator microorganisms, pathogenic

bacteria and selected environmental factors are developed.

Chapter 5 describes an investigation undertaken to determine the occurrence and distribution of pollution indicator microorganisms and pathogenic bacteria found in storm water samples collected during several storm events in both North London and Valencia.

Chapter 6 discusses laboratory experiments conducted to determine changing ratios of faecal coliforms and faecal streptococci in various kind of sewage under different conditions and bacterial release rates from sediment.

Chapter 7 provides a general overview of the main conclusions and recommendations for future work.

Chapter 2: A Review of Microbiological Characteristics in Urban Surface Water Runoff and Sediments

2.1. Background/Introduction

Water quality is a measure of the suitability of a water for specific uses and is defined in terms of the physical, chemical, and biological parameters which are pertinence to the use. Historically, the "microbial" quality of water has meant some assessment of the safety of the water in respect to the possible transmission of infectious waterborne diseases. However, water borne disease is only of concern where there is a possibility of direct or indirect human contact with the water and thus microbial quality has been traditionally determined and applied in respect of public water supplies, swimming pools, direct contact recreational waters, and waters used for crop irrigation.

There are many microbial parameters which could be used to assess the quality of raw water. For example the total numbers of viruses, bacteria, bacteriophages, fungi, protozoa, or number of particular groups (e.g. enteric viruses, coliforms, *Salmonella etc.*) could be determined. However the relative simplicity, speed and specificity of bacterial tests in particular has enabled most use to be made of these tests in water examination. Hence, most standards and guidelines of raw water quality are framed in terms of maximum numbers of bacterial indicators for faecal pollution (coliforms, faecal coliforms (*Escherichia coli*), faecal streptococci and *Clostridium perfringens*).

In early urban drainage developments, sanitary and storm water flows were traditionally combined. More recently, efforts have been made to 'correct' the perceived problems of combined sewer systems by installing partially or completely separate sewer systems. Many large older cities such as metropolitan London still retain predominantly combined systems. In many cases however, when the combined flow exceeds three times the average dry weather flow, the overall transporting efficiency is impaired (Dumbar and Henry, 1966) and at 5 or 6 times dry weather flow the sewage treatment capacity is usually exceeded. Therefore, following heavy and prolonged rainfall, sewage will by-pass treatment completely and discharge directly to adjacent water courses, as

freely discharging or uncontrolled overflows (Greeley and Langdon, 1961; Shuttleworth, 1986; Fiddes, 1989). Urban stormwater discharged through these types of storm sewer systems is comparable with other forms of sewage effluent and should arguably be treated to remove pathogenic bacteria and viruses that may be discharged to recreation areas, shellfish growing waters or source waters that may be used for water abstraction.

In areas where surface water quality has been degraded below established river quality objectives or set standards, field investigations should attempt to identify the types and sources of pollution through sanitary surveys and appropriate laboratory analysis. Since a major concern of water pollution relates to the occurrence of intestinal pathogens that could create a human health hazard, bacteriological tests must be directed toward establishing the magnitude of faecal pollution, including the locations and contributions of both point and non-point discharges of any polluted inputs.

Some studies of the survival and increased numbers of both indicators and pathogens in sediments have been done. However the knowledge of the detailed microbial characteristics of urban sewer/stream sediments may need to be extended to provide valuable information which relates to the structural characteristics of the sediments; in environmental microorganism terms, it is the indicator bacteria which are the main concern.

2.2. Microorganism Sources and Occurrence

2.2.1. The Microbial Flora in Faeces

Since the major microbiological health hazard associated with water consumption originates from faecal contamination, the search for an adequate indicator has logically been associated with organisms occurring in the microbial flora of human and animal faeces. The most consistent microorganisms and groups of microorganisms found in human faeces are *Bacteroides fragiles* (an anaerobic Gram negative bacilli), total coliforms, *Escherichia coli*, faecal streptococci and enterococci.

Of the microorganisms having a high frequency of isolation, *B. fragiles* is the most numerous followed by members of the faecal streptococci. Leclerc *et al.* (1977) have summarised the basic microbial flora found in normal human faeces (Tables 2.1 and 2.2). They noted the occurrence and density of *Citrobacter* which had previously been considered to occur infrequently and in low density; they further ranked the microorganisms found in human faeces. The primary microorganisms found, as well as the observed densities, are for the most part similar to those reported by Geldreich (1978).

Many of the microorganisms observed in these early surveys are not confined to the human intestinal tract. Numerous reports regularly found many of the aforementioned microorganisms in other warm - blooded animals and frequently in cold-blooded animals. The average density of selected microorganisms for several domesticated and wild animals is shown in Table 2.3. The average density per gram of faecal streptococci for pets, farm and wild animals is consistently higher than faecal coliforms, whereas the average density per gram associated with human faeces of faecal coliforms is more than four times higher than streptococci. It should be noted that the anaerobic bacilli reported were conspicuous by their absence from certain warm-blooded animals but were consistently found in human faeces.

Whilst the initial concern is for the presence of human faeces in water, the level of some animal pathogens cannot be ignored. Geldreich (1978) has summarized the available information on the percentage of individuals excreting pathogenic microorganisms. Animals provide a significant reservoir for *Salmonella*, *Leptospira*, and enteric pathogenic *E.coli*. More information (Pipes, 1982) suggests that *Giardia lamblia* occurs frequently in animals found in the wild, particularly beavers. *Shigella*, *Vibrio cholerae*, *Mycobacterium tuberculosis* and the enteric viruses appear to be associated solely with humans.

Table 2.1: Human faeces flora: qualitative survey on 30 adults

(from Leclerc *et al.*, 1977)

	Number Positive		
Species / Genus	Samples	Presence(%)	Frequency
Aerobic Bacteria (gram-negative)			
Escherichia coli	30	100	constant
Citrobacter-Levinea	20	66	high
Klebsiella	15	50	medium
Enterobacter	3	10	rare
Aerobic bacteria (gram-positive)			
Staphylococcus	15	50	medium
Enterococcus	30	100	constant
Bacillus	28	93	constant
Anaerobic Bacteria (gram-negative)			
Lactobacillus	30	100	constant
Bacteroides	30	100	constant
Anaerobic Bacteria (gram-positive)			
Clostridium	23	76	high

Table 2.2: Human faeces flora: quantitative survey on 30 adults

(from Leclerc *et al.*, 1977)

Species	Average number of samples where (cfu/g^3) the species is found/30	
Total bacteria	$1.5x10^{11}$	24
Total aerobic bacteria	$7.0x10^8$	30
Aerobic bacteria (gram - negative)		
Escherichia coli	$4.0x10^8$	30
Citrobacter-levinea	$1.0x10^6$	20
Klebsiella	$5.0x10^4$	14
Enterobacter	$1.0x10^5$	3
Aerobic bacteria (gram - positive)		
Enterococcus	$2.0x10^5$	30
Staphylococcus	$8.0x10^6$	15
Bacillus	$3.0x10^4$	28
Anaerobic bacteria (gram - negative)		
Bocteroides	$1.0x10^{10}$	30
Lactobacillus	$1.0x10^9$	30
Anaerobic bacteria (gram - Positive)		
Clostridium	$4.0x10^6$	23

[a]cfu = colony forming units.

Table 2.3: Estimates of microbial flora of animal faeces

(from Geldreich, 1978)

Average Density/g

	FC	FS	CP	Bac	Lac
FARM ANIMALS					
Cow	$2.3x10^4$	$1.3x10^6$	$2.0x10^2$	<1	$2.5x10^2$
Pig	$3.3x10^6$	$8.4x10^7$	$3.9x10^3$	$5.0x10^5$	$2.51x10^8$
Sheep	$1.6x10^7$	$3.8x10^7$	$1.9x10^5$	<1	$7.9x10^4$
Horse	$1.3x10^4$	$6.3x10^6$	<1	<1	$1.0x10^7$
Duck	$3.3x10^7$	$5.4x10^7$	-	-	-
Chicken	$1.3x10^6$	$3.4x10^6$	$2.5x10^2$	<1	$3.2x10^8$
Turkey	$7.9x10^6$	$2.8x10^6$	-	-	-
ANIMAL PETS					
Cat	$7.9x10^6$	$2.7x10^7$	$2.5x10^7$	$7.9x10^8$	$6.3x10^8$
Dog	$2.3x10^7$	$9.8x10^8$	$2.51x10^8$	$5.0x10^8$	$3.9x10^4$
WILD ANIMALS					
Mice	$3.3x10^5$	$7.7x10^6$	<1	$7.9x10^8$	$1.3x10^9$
Rabbits	20	$4.7x10^4$	<1	$3.9x10^7$	<1
Chipmunk	$1.5x10^5$	$6.0x10^6$	-	-	0
HUMAN	$1.3x10^7$	$3x10^6$	$1.6x10^3$	$5.0x10^9$	$6.3x10^8$

FC: Faecal coliforms. FS: Faecal streptococci.

Cp: *Clostridium perfringens*. Bac: Bacteroides.

Lac: Lactobacilli.

2.2.2. Pollution Indicator Microorganisms and Pathogenic Bacteria

The most microorganisms are common microbial flora of faeces including total coliforms, faecal coliforms, faecal streptococci, *Clostridium perfringens* and probably to other anaerobic groups, such as *Bifidobacterium bifidus*, *Bacteroides* and *Lactobacilli* (Berg, 1978, Olivieri, 1982). In previous studies, total coliforms, faecal coliforms and faecal streptococci have been widely accepted and selected as indicator microorganisms for research into the water environment. *Pseudomonas aeruginosa* and *Salmonella spp* have also been chosen as representative for water pollution research, and have recognised significance for public health risk associated with recreational waters.

Total coliforms

Total coliforms have long been recognized as suitable microbiological indicators of water quality largely because they are easy to detect and quantify in water. Although the name of the indicator was changed several times over the years, the current coliform group is essentially the some group of microorganisms that has served since the late 19th century as an indicator. The total coliform group is currently defined in the 16th edition of 'Standard Methods for the Examination of Water and Wastewater' as "aerobic and facultative anaerobic, gram-negative, non-spore-forming, rod-shaped bacteria that ferment lactose with gas formation with 48 hours at 35°C" (APHA, 1985).

Faecal coliforms

Much of the conflict in accurately assessing the microbiological quality of natural waters can be eliminated by using faecal coliforms as indicators of faecal pollution. The presence of this subgroup of the total coliform population in surface waters a more accurate allows correlation with warm - blooded animal faecal discharges than the total coliform group.

Faecal streptococci

The faecal streptococcus group includes a wide spectrum of strains that have specific faecal origins and diverse survival rates and includes several biotypes of limited sanitary significance (Mundt and Graham, 1968; Geldreich and Kenner, 1969). Within the faecal

streptococcus group, *S. bovis* and *S. equinus* are specific indicators of nonhuman warm - blooded animal pollution. In addition, *S. bovis* and *S. equinus* are faecal streptococci that die off most rapidly outside the animal intestinal tract. By contrast, the ubiquitous *S. faecalis* may affect the precision of this indicator system at counts below 100 faecal streptococci per 100 ml because at these low population levels, this biotype generally predominates.

Pseudomonas aeruginosa

Pseudomonas aeruginosa (P. aeruginosa) is frequently considered to be an ubiquitous microorganism which is easily isolated from surface waters and soils. Sanitary wastewater is regarded as a major source of *P. aeruginosa* in surface waters, although it is occasionally isolated from non - faecal polluted waters, especially from waters leaching agricultural soils or urban runoff (Hoadley, 1968a and 1977; Wheater *et al.*, 1980; Dutka, 1981) and may be associated with high densities of bacteria (Cabelli *et al*, 1976; Cabelli, 1977). The presence of *P. aeruginosa* in urban runoff must be considered because it is an opportunistic pathogenic microorganism which may be spread by water. It is know to cause infections of the skin, ears, eyes and other mucous membranes of bathers swimming in waters polluted by organisms (Hoadley, 1977 and Dutka, 1981).

Salmonella

The increase of pollution in natural waters has intensified the detection frequency and persistence of other pathogenic microorganisms, mainly *Salmonella spp.*, in urban areas affected by sewage discharges with subsequent potential hazard to public health.

2.2.3. Relationships between Indicators

While the criteria for the utility of an indicator microorganism appear to be simple and straightforward, no one microorganism or group of microorganisms adequately satisfies all of them. The utilization of water by man is an important factor to be considered when the indicator criteria are evaluated. Indicator species by their presence, indicate pollution, but their absence will not absolutely guarantee a clean environment and

quantitative relationships to other factors are not presupposed.

Olivieri *et al.* (1978) have reported a positive correlation between the bacterial indicators and the bacterial pathogens. As the levels of total coliforms, faecal coliforms and faecal streptococci increase, the levels of *Salmonella spp. P. aeruginosa* and *Staphylococcus aureus* also increase. However, small differences in the levels of indicator bacteria may not reflect changes in the levels of pathogens. Conversely, small differences in the levels of pathogens may not be reflected in the levels of indicators. Correlations between levels of total coliform, faecal coliform and faecal streptococcus and bacterial pathogens were found by Olivieri (1982) to be highly significant at the 1 % level when outfall samples were considered. However, little or no correlation was found between indicator and pathogenic bacteria in storm and stream samples. Morinigo *et al.* (1990) also reported that close correlation between indicator bacteria and *Salmonella* in highly polluted freshwater was observed. However, *Salmonella* was occasionally detected when faecal streptococci levels were $\leq$ 50/100ml. Generally, the percentage of samples in which *Salmonella* were detected increased with the pollution level. 95% of storm samples with faecal coliform levels greater than 2,000/100ml were positive for *Salmonella* (Olivieri *et al.*, 1977).

2.2.4. Indicator Ratios

Many ratios of indicator microorganisms have been employed to provide some insight into the possible source of microbial contamination such as: The ratios of faecal coliforms to total coliforms (Geldreich 1970), *Escherichia coli* to faecal coliforms, Enterococci to faecal coliforms (Dufour, 1984; Cabelli, 1983; Gannon and Busse, 1989), faecal coliforms to total coliphages (Kenard and Valentine 1974; Bell, 1976; Kott, 1976; Borrego *et al.*, 1987 and O'Keefe and Green, 1989), *Streptococcus faecalis* to *Staphylococcus faecium* (Wheater *et al,* 1979), *Pseudomonas aeruginosa* to faecal coliforms (Cabelli *et al,* 1976; Wheather *et al*, 1980; Vicente *et al.*, 1991) and faecal coliforms to faecal streptococci (Geldreich and Kenner, 1969; Geldreich *et al.*, 1968; Feachem, 1974; Skinner *et al.*, 1974).

The ratio of faecal coliforms to faecal streptococci (FC/FS) has been utilized more frequently than others to determine whether the pollution is of human or animal origin. Many workers have demonstrated that faecal streptococci are present in greater numbers than coliform bacteria in the faeces of animals (Bartley and Slanctz, 1960; Kenner *et al.*, 1960; Mundt, 1963; Roges and Sarles, 1964). In human faeces however, faecal coliforms are found in greater numbers than faecal streptococci. According to Geldreich (1976) and Geldreich and Kenner (1969), ratios above 4.0 are indicative of human faecal pollution whilst ratios below 0.7 are indicative of animal pollution. Ratios between 0.7 and 4.0 are usually taken to indicate waste of mixed human and animal sources. However, Wheater *et al.* (1979) have reported that FC/FS ratios are not always relevant in waters containing treated sewage effluent. McFeters *et al.* (1973) has pointed out that die-away ratios depend upon differential die-away rates of faecal coliforms and faecal streptococci. Olivieri *et al.* (1977) in stormwater studies found FC/FS difficult to interpret, even within the initial 24 hour period in urban streams within Baltimore. The applicability of the FC/FS ratio to the determination of sources of pollution in urban runoff is therefore questionable (Olivieri *et al.*, 1989). Hussong *et al.* (1979) found the FC/FS ratios in faeces of waterfowls to be similar to that of human faeces and thus the ratio may not even be useful to separate animal from human faecal contamination.

FC/FS ratios must be applied carefully. The correlations are most meaningful when developed from bacterial densities of samples taken at the point of wastewater outfall into a receiving stream, together with the value of the FC/FS ratios for the initial 24 hours of downstream travel from the point of discharge. Once organisms are diffused into the receiving stream, factors such as water temperature, available organic nutrients, toxic metal ions such as copper, zinc, silver, unfavourable pH below 4.0 or above 9.0 as well as other ecological forces may fundamentally alter the interrelationship between these indicator systems during flow-time downstream (Geldreich and Kenner, 1969). To minimize misinterpretation of ratios the following precautions have been recommended by APHA (1985):

"(a) Measure sample pH because faecal streptococci densities can be altered significantly if water pH is above 9.0 or below 4.0.

(b) sample as close as possible to the pollution source because faecal streptococci have relatively short life times outside the animal host. Sampling points downstream, where travel time from pollution sources exceeds 24 hours, will provide erroneous ratios.
(c) inspect source(s) of pollution when various pollution sources are present because ratios may yield deceptive assessments.
(d) careful use must be made of ratios for samples taken from marine waters, bays and estuaries, because ratios may be of little value in differentiating between human and non-human sources.
(e) do not calculate the ratios when faecal streptococci are below 100/100ml as too many factors will influence the densities of faecal coliforms and faecal streptococci".

The magnitude of these densities, along with the volume of water which carries the contamination, combined with the numerous environmental factors that will affect the levels of these microorganisms, make the useful application of the FC/FS ratio difficult and contentious in the urban environment. The calculation of the FC/FS ratio for a storm outfall or a stream must be recognized to be the nett result of the effects of many different localized environmental conditions that are likely to alter the microbial populations and species distribution.

2.2.5. Microbial Characteristics of Rain and Snow

Geldreich *et al.*(1968) have showed that pollution indicator bacteria counts for rainfall are generally less than 1 per 100 ml as demonstrated by the medium values for 49 samples. In their analysis individual storms during two summers of record that contained total coliform densities between 1 and 92 per 100 ml. One of these rain samples had 1 faecal coliform per 100 ml and two samples contained 1 and 2 faecal streptococci per 100 ml respectively. The origin of these positive samples was associated with material acquired in dust, storms, insects, vegetation fragments and soil particles, insect or vegetation fragments found on microscopic examination of debris trapped by the membrane filtration method used in the bacteriological examination of these rainwater samples.

Since rainwater contains 'insignificant' levels of bacteria, its major contamination must occur on contact with the land environment, creating a potential pollution problem in the resulting runoff (Geldreich *et al.*, 1968). A variety of surface materials from diverse sources contaminate rainwater runoff and can find their way into stormwater discharges (Van Donsel *et al.*, 1967; Sartor *et al.*, 1974).

2.3. Microorganisms in Urban Stormwater and Sewage Effluent

2.3.1. The Levels and Characteristics of Microorganism in Stormwater Runoff

Historically, the major concern about stormwater in rural areas has been related to erosion control or diversion to storage impoundments for potable water supplies, livestock feeding waters, irrigation releases, and flow control to prevent river flooding or to maintain navigable rivers during otherwise low flow periods during the year. However, the problems of stormwater runoff in metropolitan areas have resulted in the development of an extensive collection networks of drains from street, shopping areas, building foundations and roofs for transport of the runoff to more convenient areas for release. Intermittent water collection systems are thus either diverted to nearby creeks, rivers, lakes and coastal waters or combined with the sanitary wastewater for treatment before discharge to receiving waters. Although the bacterial quality of stormwater runoff has been recognized for many years, the health hazard implications of these discharges into bathing areas, urban reservoirs and shellfish growing waters have largely been ignored until fairly recently.

A major source of microbial contamination within the urban catchment are derived from faecal material deposited on soil, asphalt and cement surfaces by cats, dogs and rodents (Geldreich *et al.*, 1968; Faust, 1982). Storm water runoff from impervious surfaces within urban areas is known to be associated with a wide range of pollutants and is undoubtedly of poor quality (Ellis, 1979). There is now a considerable evidence from United States as well as other studies that urban storm runoff water contains high bacterial levels, including a variety of pathogenic strains (Geldreich *et al.*, 1962; Qureshi and Dutka, 1974; Olivieri *et al.*, 1979; Canada Ontario Agreement, 1979;

Cowan *et al*, 1989).

Benzie and Courchaine (1966) have reported on the densities of microorganisms found in both separate and combined sewer systems in the Detroit area. Considerably larger quantities of total coliforms (mean value 9.4 x 10^6/100ml), faecal coliforms (mean value 2.7 x 10^6/100ml) and faecal streptococci (mean value 5.8 x 10^5/100ml) were found in the discharge from a combined system comparison to a separate storm sewer system. During the period of their study, the average median total coliform density for the combined system was about eight times that of the separate system whilst faecal coliform and faecal streptococci were approximately 3 times and 4 times larger respectively, in the discharges of the combined system. Similar results have been reported by Burm and Vaughan (1966). Ellis (1985b) has also reported on the high levels of bacteria and selected pathogens obtained from separate and combined sewers during a seven year survey conducted between 1975 - 1982 in the Silk Stream catchment, N London.

Previous studies on the sources of bacteria found in stormwater runoff from residential and light commercial areas have indicated that bacteria are predominantly of non-human origin (Weibel *et al.*, 1964; Benzie and Courchain, 1966; Geldreich and Kenner, 1969). Geldreich *et al.* (1968) has investigated levels of bacteria found in stormwater from various residential streets, suburban business districts and wooded hill-sides adjacent to a city park. Total coliform peak densities for urban locations (wooded hill-side, street gutters, and suburban business district) occurred in autumn. This was also noted for faecal coliform and faecal streptococcus densities occurring in urban street gutters and business district stormwater runoff. In drainage from rural stormwater runoff, the possible existence of summer and winter peaks in bacterial indicator densities was demonstrated. These peaks may be related, in part, to the existence of more lateral, sub-surface drainage conditions. In the spring and autumn however, land cultivation results in a greater downward migration of water, and thus by association, bacteria into both soil and ground waters. Faecal streptococci densities were consistently higher than faecal coliform levels for all four different sources of stormwater runoff. The highest median value for faecal streptococci (7.9x10^5/100 ml) occurred in the rural runoff during winter. A median value of 4.7x10^4/100 ml

represented the highest faecal coliform densities and this occurred in stormwater discharges coming from street gutters during autumn. Total coliform and faecal coliform densities have also been show by other studies to demonstrate significant increases in magnitude during the summer months (Geldreich, 1968).

Qureshi and Dutka (1979) also have reported on the characteristics of stormwater runoffs obtained from residential and commercial areas in the nearly Burlington area of Toronto. It is interesting to note that the distribution was similar throughout various periods of the storm and that, among coliforms found in the runoff water, approximately 45% of the isolates were *E.coli* with the remainder belonging to *Klebsiella* (23 -31%), Enterobacter (16 - 22%) and *Citrobacter* (6 - 10%). A larger density of *E.coli* was found in association with infiltration samples as compared with runoff waters. Coliform isolates from one of these infiltration samples showed the following distribution pattern: *Aeromonas sp.* 50%, *Klebsiella sp.*45%, and *E. coli* 5%. In both the infiltration and runoff samples, the faecal coliforms belonged to the *E. coli* group.

A number of studies have indicated that the proportion of faecal coliform to faecal streptococcus bacteria is consistent for samples of similar origin. In sewage, both total and faecal coliforms are predictably more numerous than faecal streptococci (Burm and Vaughan, 1966; Geldreich and Kenner, 1969; Cohen and Shuval, 1973). In stormwater runoff this relationship is reversed and faecal streptococci exceed faecal coliform bacteria (Geldreich and Kenner, 1969; Evans *et al.*, 1968; Geldreich *et al.*, 1968). Actuarial densities of these bacteria are shown in Table 2.4. In addition to faecal bacteria, the presence of pathogenic bacteria in stormwater has frequently been reported. Evans *et al.* (1968) demonstrated the existence of a potential health hazard by isolating *Salmonella thompson* (at a level of 4500/100ml) from a stormwater sample taken from an urban business district separate storm sewer. Olivieri *et al.*(1978) have also reported that a high recovery of pathogens is from separate and stormwater sewers. The levels of *Salmonella spp.*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* in stormwater were several fold higher than found in local urban streams, but significantly lower than found in raw sewage.

Table 2.4: Densities of faecal coliform and faecal streptococci bacteria in sewage and stormwater (from Geldreich and Kenner, 1969).

Sources	Organisms/100ml
Sewage	
Faecal coliforms	$3.7x10^5$ - $4.9x10^7$
Faecal streptococci	$6.4x10^4$ - $4.5x10^6$
Stormwater	
Faecal coliforms	$2.7x10^3$ - $1.4x10^4$
Faecal streptococci	$5.8x10^4$ - $1.5x10^5$

The most abundantly occurring pathogen in urban water is *P.aeruginosa* which is found in the range of 20 to $3.1x10^4$ MPN/100 ml (Ellis, 1985a). Although foul sewage discharges represent the major potential sources of *P.aeruginosa* in the environment, storm drainage from municipal areas contributes a continuous inoculum to surface waters and farm drainage can also contain small numbers of the bacterium under certain conditions. Relatively heavy populations found in streams below sewage outfalls decrease rapidly as they progress are traced further downstream. On the other hand, population levels of less than about 100 MPN/100ml consistently occur in streams contiguous to human habitation and activity. In this sense, the presence of *P. aeruginosa* in river waters at population levels at about 100 MPN/100 ml and below reflects the influence of drainage from inhabited areas and sewage discharges respectively. Populations in excess of $1.0x10^3$ MPN/100 ml would suggest very recent sewage contamination (Hoadley, 1968b).

In term of pathogen incidence, the relative order appears to be *P. aeruginosa* > *Staphylococcus* > *Salmonella* and there is always a better than 70 per cent recovery of these pathogenic bacteria; the recovery efficiency of animal viruses has been reported

to be even higher at 80 to 90 per cent (Ellis, 1985b).

2.3.2. Microorganisms in Raw Sewage

Raw sewage is a collection of diverse wastes created by man. To a large extent, raw urban sewage is principally composed of human wastes, kitchen wastes from food preparation and home laundry wastes. Wastes from hospitals, schools, restaurants, hotels, businesses also contributes to the total composition of raw sewage found in the sewer pipes.

Microbial populations in raw sewage vary greatly, depending on the size of the population served, the common prevalence of diseases in the community, dilution, impact of stormwater, physical and chemical characteristics of the mixture, degree of diffusion of the micro-faecal pellets, retention time in the sewage pipe network, season of the year and hour of sampling (Geldreich, 1978). The discharge of certain industrial wastes, including phenols, acids, alkaline substances and heavy metal ions, can give rise to very low bacterial counts but the introduction of wastes rich in organic nutrients may promote rapid multiplication of substantial portions of the bacteria flora.

2.3.3. Microorganisms Survival and Die-off

Once microorganisms enter a receiving water they may multiply, remain static in number or die-off. The survival of a microorganism in an environment in which it is not indigenous is dependent upon its ability to withstand the physical, chemical and biological conditions which are different from those encountered in its natural habitat.

Numerous reports have compared the survival rate of faecal streptococci with that of faecal and coliform bacteria. These survival studies have been carried out in a variety of natural and polluted test waters including sewage (Cohen and Shuval, 1973; Chamberlin and Mitchell, 1978), stormwater (Geldreich *et al.*, 1968), marine water (Alonso *et al.*, 1987) and well water (McFeters *et al.*, 1973; Rhodes and Kator, 1988;

Lim and Flint, 1989). Geldreich *et al* (1968) carried out experiments in stormwater studies at 10°C and 20°C; incubation temperatures chosen to represent the average winter and summer stormwater temperatures respectively. The resulting data indicate organisms persisted at higher levels at 10°C during the 14 days of storage as compared with 20°C. *S. faecalis* persisted longer at both temperatures and at higher levels than *Aerobacter aerogenes*, faecal coliform or the *S. typhimurium* strains used. *S. typhimurium* persistence at 10°C was slightly higher than that of *Aerobacter aerogenes* but less than that of the faecal coliforms for the first 11 days of storage. These data indicate a die-away pattern between faecal coliforms and *S. typhimurium* than between faecal streptococci and the Salmonella strain.

Mancini (1978) has suggested that temperature is the dominant influence on survival of coliforms. Flint (1987) has also shown the *E. coli.* was survive for longer than 260 days at temperature between 4°C and 25°C in the absence of other microorganisms, although in the presence of the natural microbial flora, temperature was again the main factor which influenced its survival. He suggested that temperature and competition for nutrients were the major factors influencing the survival of *E. coli* in freshwater.

McFeters *et al.* (1973) have also reported a study on the survival of enteric pathogenic cultures found in well water. The survival of the organisms tested under their experimental conditions showed the following orders: *Aeromonas sp. > Shigella sp. (S. flexneri, S. sonnei and S. dysenteria) > faecal streptococci > coliforms and Salmonella (S. enteritidis ser., paratyphi A and S. enteriditis ser. typhimurium) > Streptococcus equinus > Vibrio cholerae > Salmonella typhi > Streptococcus bovis > Salmonella enteritidis. paratyphi B.* They also reported different die-off rates of indicator microorganisms from bovine faecal material. Human and faecal material yielded results similar to pure cultures tested. Andre *et al.* (1967) reported that *Salmonella* (*S. typhosa, S.newport, S. worthington,* and *S. manhattan*) survived for 16 days and *Shigella (S. flexneri I, S. flexneri II,* and *S. sonnei*) for 12 days in farm pond water at 21°C to 29°C. These studies however, provide little information on the natural survival of microorganisms in mixed populations under variable environment conditions. The mechanisms of natural inactivation, the effect of natural predators on the microbial population and the effect of daily change in the normal aquatic environment are not well

understood.

It seems that the faecal bacteria which have been used as indicators of faecal contamination are not, in general, good indicators of pollution. Their densities in water do not correlate very well with the typical chemical parameters used to measure the deterioration of water quality resulting from discharge of waste waters (Pipes, 1982). A number of studies have been undertaken by several investigations on the relationship between bacterial and physical parameters (Jannasch, 1968; Zobell, 1968; Meaon *et al.*, 1972; Qureshi and Dutk, 1974; El Shaarawi *et al.*,1978). The consensus of these studies is that bacterial populations are related to nutrient levels and temperature and that the higher the total nutrient levels the greater in the effluent, the population will be (Flint, 1978).

The importance of light to the mortality of microorganisms in an aquatic system was first demonstrated by Gameson and Saxon (1969) and by other studies (Chamberlin and Mitchell, 1978; Jujioka *et al.*, 1981; McCambridge and McMeekin, 1981). Most of these studies were carried out in marine and estuary systems and all strongly suggested the dominant influence of light on bacterial die - off. Cavari and Berostein (1986) showed that light was also a dominant factor on the survival of the number of *E. coli* in a freshwater system and that the decrease in cell number is directly related to light intensity.

There is very little information on how chemical changes affect bacterial populations in surface waters. Some studies have been made to assess the correlation of chemical factors with various bacterial populations that are used as faecal pollution indicators. Jana and Bhattacharga (1988) have reported that a gradual decline in growth population of the faecal coliforms with increase of exposure time and concentration of metals. Nuttall (1982) indicated that bacterial populations were positively correlated to discharges and variables representing articulated and organic matter increased in concentration with increased river flow; conversely, the ionic chemical parameters decreased in concentration.

Dutka and Kwan (1980) have also reported a bacterial die-off study of closed water

bodies in Lake Ontario and Hamilton Bay, Canada. The three bacteria, *E. coli*, *Streptococcus faecalis* and *Salmonella thompson* used in the membrane filter diffusion chamber studies were all isolated from obtained water samples. The die-off patterns produced by these three organisms are very similar (Fig 2.1, 2.2 and 2.3), with bottom chamber populations tending to decrease at a slightly slower rate than surface chamber populations. The data also indicate that organisms suspended in the less polluted waters of Lake Ontario have a tendency to die-off faster than organisms suspended in the more polluted waters of Hamilton Bay. These observations may be due to differences in nutrient levels between the lake and land runoff, as well as between sewage and industrial discharges which reflect the higher concentration of nutrients from in addition to the influence of vertical mixing. It is also very clear that it is extremely difficult to establish consistent relationships between specific biological, physical, or chemical parameters and bacterial densities. Population decreases after 3 days of immersion varied from 0.1 to 3.1 logarithms for *E. coli*, 0.2 to 3.7 logarithms for *S. faecalis* and 0.4 to 3.8 logarithms for *S. thompson.*

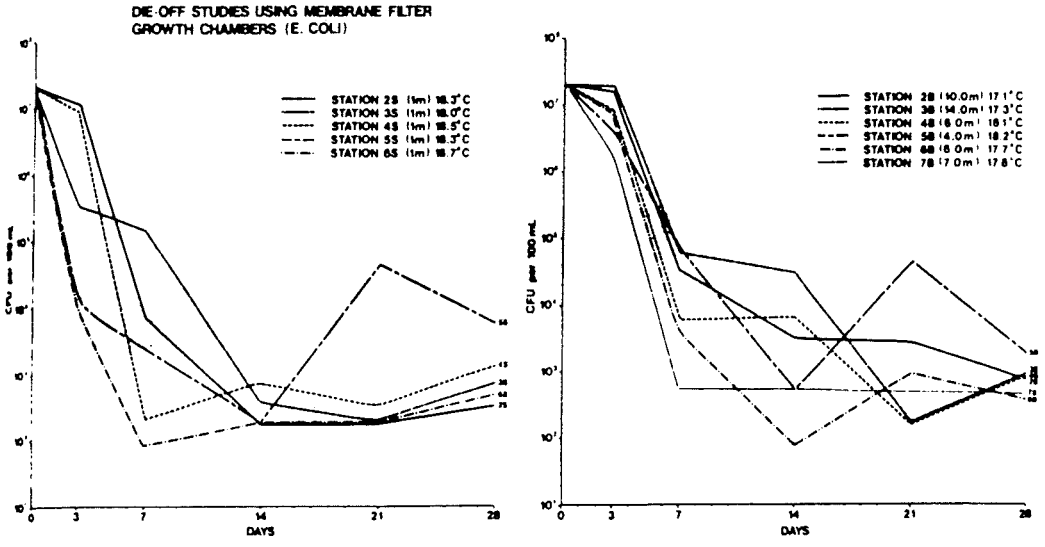


Figure 2.1: *E. coli* survival in membrane filter chambers suspended in Lake Ontario and Hamilton Bay (from Dutka and Kwan, 1980).

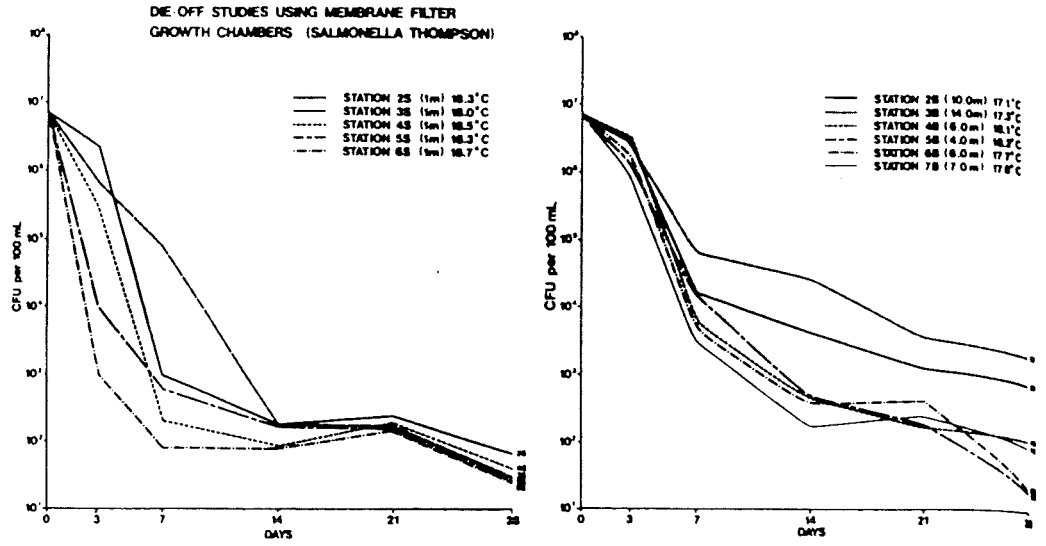


Figure 2.2: *Streptococcus faecalis* survival in membrane filter chambers suspended in Lake Ontario and Hamilton Bay (from Dutka and Kwan, 1980).

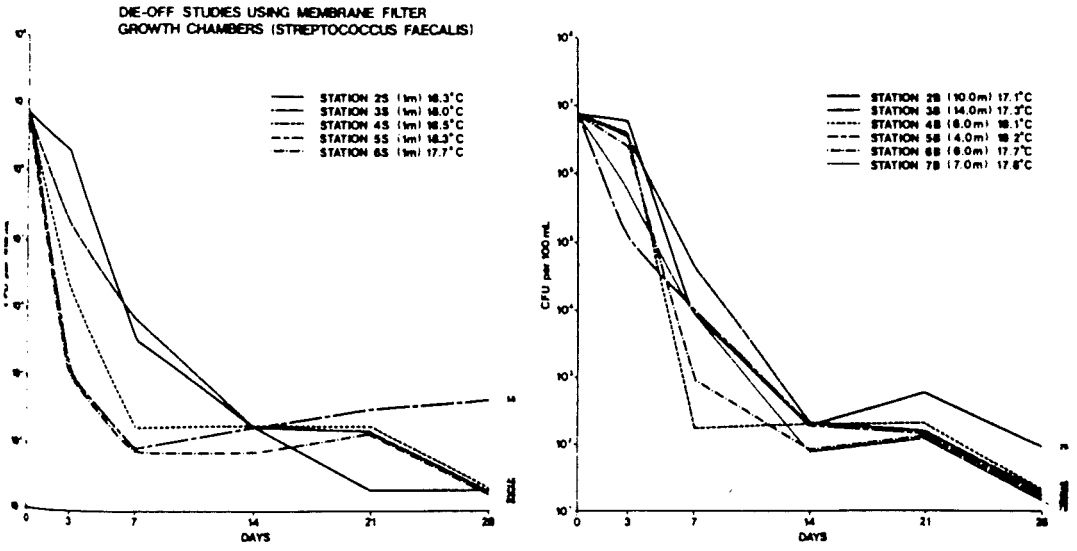


Figure 2.3: *Salmonella thompson* survival in membrane filter chambers suspended in Lake Ontario and Hamilton Bay (from Dutka and Kwan, 1980).

These die-off rates are a little slower than those reported by McFeters *et al.* (1973) for organisms suspended in well water, but were equivalent to those noted by Vasconcelos and Suartz (1976) in sea-water studies and faster than those noted by Slanctz and Bartley (1965) for sea-water.

2.3.4. Diurnal Variations of Bacterial Concentration

Many workers have agreed that microbial concentrations of sewage effluent input be correlated with flow rate and time of samples (Gameson, 1978; Ruiter and Fujioka, 1978; Jefferies *et al.*, 1989). Jefferies *et al.* (1989) demonstrated that bacterial counts tend to be low in combined sewer systems during the early morning in the case of total coliform, faecal coliform and faecal streptococcus under dry weather conditions. A sharp rise in the number of faecal coliforms and faecal streptococci occurs around 07.00 hours with peak recovery of both species being at approximately 10.00 hours . The average number of total coliforms also show a dramatic increase at this time but the peak value is obtained much later in the day at about 14.00 hours. A second smaller peak was noted for both total and faecal coliforms at around 20.00 hours. A further small peak was noted for both total and faecal coliforms at around 20.00 hours. This Dundee data is consistent with Gameson's Northampton results (1978) and show typical daily variations in bacterial counts encountered during dry weather flows. Jacobs and Ellis (1991) have also demonstrated that only 36% of the total bacterial mess discharged in the first 65% of mass of flow volume in Silk stream. The studies also indicate the difficulty of undertaking accurate bacteria studies in urban effluent; errors of the order of a factor of 3 appear almost inevitable. The data suggest that under dry weather flow patterns, bacteria deposited with the same pattern as suspended solid with an observable 'first flush' effect. Different bacteria predominate at different times of day with total coliforms peaking in the afternoon and faecal coliforms in the morning. Total coliforms are likely to be associated with wash out of older deposits lying on or near the sewer invert. Despite the peak values, there is evidence that total coliforms increase during storms whereas faecal coliforms and faecal coliforms decrease. This may be due to storm dilution of the foul input and resuspension of older bed deposits with the release of a matured bacterial population reflected in the increased total coliforms

counts.

Ruiter and Fujioka (1978) reported that enteroviruses were discharged with sewage into Mamala Bay, Hawaii. 24 hour grab samples, as well as 12 two-hour composite samples of sewage were obtained from the outfall pipes and analyzed for the virus. Both sampling methods resulted in similar results, with peaks of virus discharge observed between 11.00 and 14.00 hours and again between 20.00 and 23.00 hours probably reflecting the daily morning (breakfast) and evening (dinner) activities of the city with the time differential accounting for the flow of sewage to the outfall pipe.

2.4. Sediment Microbiology

2.4.1. Microbiological Characteristics in Sediment

Several authors have suggested that sediment is a bacterial store (Van Donsel and Geldreich, 1971; Grimes, 1975; Matson *et al.*, 1978). No conclusions have been made however about the mechanisms by which bacteria are stored and released. The possible explanations include simple deposition of bacteria clumps, adsorption to particular grain sizes, slower rates of death in sediment or growth of the *E. coli* population in sediment by metabolism of hexose and protein of an organic origin (Hendricks, 1970 and Gerba and Mclead, 1976). The bacterial species present in urban sediments will be heavily dependent on oxygen availability with the sediment regime and thus on the nature of liquid flows immediately above and within the upper sediment layers.

It is now generally agreed that sediment bacteria from stormwater runoff constitutes a major source of stream pollution within urban rivers and indeed in terms of average storm event characteristics, suspended solids can be viewed as being of "sewage" quality (Ellis, 1985a). There is sufficient evidence available to confirm that many bacteria can be taken up by solids and become concentrated in the benthal sediments and interstitial waters of urban rivers (Ellis, 1985b). Hendricks (1970) found approximately 90 percent of *Salmonella* downstream of a wastewater effluent and demonstrated a higher recovery rate from sediments than from water. Van Donsel and

Geldreich (1971) recovered 100 to 1000 times more faecal coliforms in river mud than in the overlying water. Grimes (1975) showed increased numbers of faecal coliforms in the Mississippi River during and after channel dredging and concluded that the resuspended sediments had released coliforms into the water.

The importance of benthic microorganisms is recognized in shallow water systems, since the benthic community accounts for most of the system's biomass and metabolic activity (Hynes, 1960; Matson *et al.*, 1978). It is clear that sediment analysis for indicators can provide additional water quality information.

Ellis (1985a) reported the bacterial enumeration of sediments obtained from an urban stream bed; be showed the sediment - water bacterial ratio to be 45:1. Scanning electron microscopy showed that over 65 per cent of bacteria cells were associated with particulate less than 36 μm in size with 80 and 92 per cent of faecal coliforms and *P. aeruginosa* respectively being associated with this size fraction. It was noticeable that there was a consistent correlation of organic particulate with bacterial attachments, and the high organic content of storm runoff solids is well documented (Ellis, 1976). Therefore, high numbers of opportunistic pathogens are always likely to be present in the thick benthal organic sludge typical of urban receiving waters (Ellis, 1985b). More investigations (Hendricks, 1971; Van Donsel and Geldreich, 1971; Gary and Adems, 1985) have found that coliforms, faecal coliforms and *Salmonella* tend to concentrate in sediment and that they are almost totally located in the upper layers of sediment.

Reports of survival and multiplication of indicators and pathogens in sediments are significant. Enteric organisms have been shown to metabolize freshwater sediment elutes (Hendricks, 1971). Thus, rapid die-off from the water column may result in increased sediment population due to settlement and accumulation. The bacteria which exist in sediment will depend to a very large extent on the nature of the dissolve oxygen regime in the sediment. The water flow patterns above the sediment and temperature of the water will have an effect on the microbial population.

2.4.2. Bacterial Release from Sediment

The importance of aquatic sediment as reservoirs of potential health hazard indicators is determined by at least two factors:
(1) the possibility of extended survival or growth of indicators in sediment (thus altering temporal concepts of wastewater pollution).
(2) the potential for resuspension of the sediment into the water column, thus exposing water users to sediment-bound indicators and pathogens (Matson *et al.*, 1978).

The fate of indicators microorganisms which do attach to the sediment is regulated by several factors, including the ability to metabolize benthic nutrients (Hendricks, 1970; Gerba and Mclead, 1976), withstand predatory pressure and metabolically compete with other microbes (Gerba and Mclead, 1976). Temperature and velocity are also important factors in the sediment - water equilibrium. There is a clear evidence to demonstrate the concentration of indicators and their extended survival in aquatic sediment relative to the overlying water and survival in a variety of real and simulated lentic, fresh, estuarine, and salt water systems.

Grimes (1975) demonstrated that faecal coliform densities were significantly higher during dredging than before or after. Data indicated that disturbance and relocation of bottom sediments by dredging results in a concomitant release of sediment-bound faecal coliform. Matson *et al.* (1978) reported that sediments harboured sufficient indicators in shallow water systems to provide increased health hazard information. The question remains however, as to whether these populations present any real potential threat to water users.

Certainly, physical resuspension of shallow water sediment can be accomplished through (1) increased river discharge, waves, and tides, (2) wind - induced turbulence, (3) dredging, (4) motor boats, (5) swimming, walking, wading, and (6) normal loading, roosting and other related activities of aquatic animals. Whether these mechanisms result in the actual release of indicators from the resuspended sediment is more difficult to answer. Past and present research on the subject involves developing mechanisms to desorb attached sediment bacteria for enumeration procedures. However, since the

resuspended sediment would be included in any water samples taken at the time of resuspension, it may not matter whether the organisms are released in terms of indicator detection.

Matson *et al.* (1976) have constructed a simple model which describes phenomena observed during several storms in the Shetucket River basin. The relative concentrations of microorganisms in sediments and water resulting from a rapid increase in river discharge are illustrated in Fig 2.4.

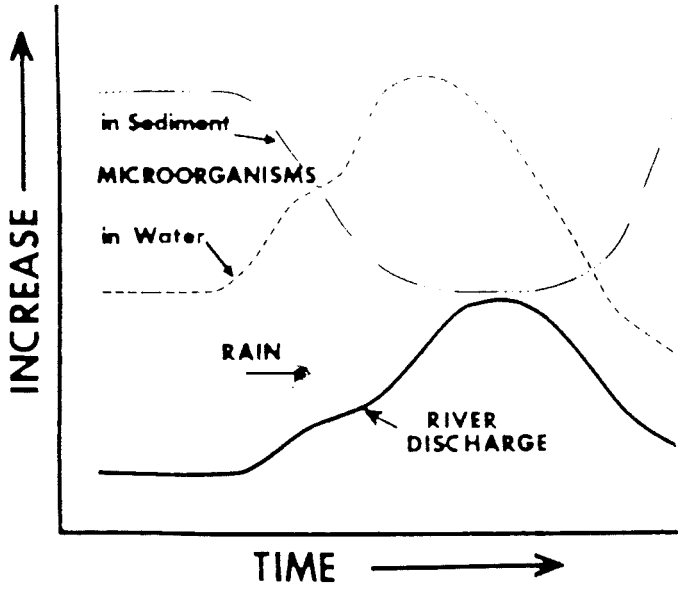


Figure 2.4: Model of the relative changes in numbers of microorganisms in sediment and water during changing river discharge rates (From Matson *et al.*, 1978).

During stable flow conditions, sediment and water populations achieve a relatively "steady - state" level. During high runoff when river discharge increases, sediment organisms are scoured from the benthic surface and mobilised into the water column. This results from the abrasion of attachment surfaces and a release of given particle sizes. Simultaneously, populations in the ambient water increase due to runoff from

terrestrial sources as well as from release by sediment. Both of these events reach a maximum at or just before the slope of the river hydrography reaches zero, since no more resuspension will occur. During peak discharge, water numbers decrease through dilution since all resuspension and runoff mechanisms have ceased. Thus water populations become reduced to pre-storm values. After a system-specific time period, the populations achieve their pre-storm "steady-state" values.

2.5. Summary

The search for an adequate indicator of faecal contamination in water has logically been associated with total coliforms, faecal coliforms and faecal streptococci, *Clostridum perfringens* and possibly to other anaerobic groups such as Bacteroides, lactobacilli and to Bifidobacterium bifidus. Of these indicator groups, total coliforms, faecal coliforms and faecal streptococci are most frequently used because they correlate best with faecal occurrence, are relatively easy to test for and because their survival patterns are similar to those of the most persistent waterborne pathogens. Human wastes and runoff from urban area are probably major sources of *P. aeruginosa* reaching most surface waters (Hoadley, 1977).

Previous studies have demonstrated that urban stormwater runoff must be considered as making a significant contribution to the microbial load in a sewage system and that sewer discharges are associated with a variety of bacteria which can exert acute impacts on the receiving water regime as well as large term deleterious effects due to bacterial mobilization. However the mechanisms of bacterial growth, removal and remobilisation are very unclear as are the temporal variations. Bacterial species require further analysis, as do the mechanisms and functions of aerobic bacteria in sediments associated with urban discharges.

Microorganisms population in aquatic environment are very related to nutrient levels and temperatures. Microorganisms can survival longer in lower nutrient and temperature levels than higher. Many workers have demonstrated that bacterial levels of sewage effluent are correlated with flow and sampling time. Peak bacterial levels are

always delayed behind the peak of flow. Many ratios of indicator microorganisms have been employed, but FC/FS ratio has been utilized more frequently to determine whether the pollution is of human and animal origin.

Sedimentation leads to the formation of high population of microorganisms and pathogens in bottom sediments, which is of special health concern in recreational waters. Microbial populations in sediment can be resuspended relatively easily. High flow regimes will cause dispersion of any sediment within the sewer/river and this will result in the transport of sediment solids, including bacteria, along the sewer/river. The studies of survival of indicator bacteria and pathogens have demonstrated that growth survival depends on redox condition in sediment.

Chapter 3: Methods

3.1. Introduction

All of the experimental data on which the discussion of chapters 4 - 7 is based were obtained at various field sites in North London and Eastern Spain, Valencia. Simulation experiments were carried out at the Microbiology Laboratory of the Urban Pollution Research Centre at Middlesex Polytechnic, U.K. and at the Instituto de Hidrologia y Medio Natural in the Universidad Politecnica, Valencia. Details of each of the major field sites are given in this chapter. An outline of the investigations carried out at each site is included with forward reference to subsequent chapters where the work will be reported in further detail. Descriptions of microbiological and physico - chemical methods are also included.

3.2. Sampling Locations

Twenty one sampling sites were selected for investigation in North London (U.K.) and Valencia (Spain), varying from dry weather flow sanitary wastewaters, urban receiving stream waters within heavily urbanised areas, combined sewers, a beach discharge point as well as for storm water runoff within typical urban areas. Most of the samples were obtained during the period October 1990 to July 1991 under different weather conditions and storm water samples were obtained during the summer of 1990 in Valencia. The study sites are individually listed in Table 3.1. Sampling sites located in North London are given with a initial capital L and sites in Valencia, a capital V.

Table 3.1: Locations of sampling sites of investigations

Sources	Site	City	Site code
Combined Sewage	New Barnet	London	LA
Combined Sewage	Waterfall Walk Overflow	London	LB
Sanitary Wastewater	Osidge Road	London	LC
Combined Sewage	Arnos Park (twin pipe)	London	LD
Combined Sewage	Arnos Park (under the bridge)	London	LE
Upper Pymmes Brook	K.G. Playing Field	London	LF
Down Pymmes Brook	Inside Arnos Park	London	LG
Sanitary Wastewater	Las Fuentes	Valencia	VH
Combined Sewage	South Politecnica	Valencia	VI
Beach Outfall	Malvarrosa	Valencia	VJ
Commercial Area	Enfield Town Shopping Centre	London	LK
Open Market	Enfield Town Open Market	London	LL
Car Parking	Enfield Town Car Park	London	LM
Urban Main Road	Ponders End High Street	London	LN
Residential Area	Garfield Road	London	LO
Commercial Area	Plaza del la Virgen	Valencia	VP
Open Market	Plaza del Mercado Market	Valencia	VQ
Car Parking	Nuevo Centro	Valencia	VR
Urban Main Road	Fernando Calle	Valencia	VS
Residential Area	Calle de Doctor Zomenhoff	Valencia	VT
Valencia Suburban	Valencia Politecnica Campus	Valencia	VU

3.2.1. Sampling Sites in North London

3.2.1.1. Urban Sewage Discharges and Receiving Stream Sites

The principal field work was undertaken within the catchment of the Pymmes Brook in North London during the period of October 1990 to February 1991. Pymmes Brook stream is a major tributary of the River Lea in North London. Some eighty per cent of the catchment is in urbanized areas and contains a total population of about 300,000. Over half of the catchment carries a high urban density populations particularly in the areas of Barnet and Southgate. Sixty per cent of the total storm flow is derived from impervious surface runoff and many outfalls and overflows from separated and combined sewer systems discharge to the main stream. There are a considerable number of parks, open spaces and playgrounds located immediately adjacent to the stream which act as natural wash and flood plains for over bank discharges as well as being a focus for recreational and local amenity activities. The main area of sampling interest within the catchment is shown in Fig. 3.1. All sites were on the receiving stream except site LC which is the trunk sanitary sewer discharging to the Edmonton Sewage Treatment Works.

The New Barnet site (LA) is situated in one of the tributaries of the upper Pymmes Brook stream. At this point about $0.027m^3/s$ of sewage effluent is discharged into the stream from 3 major sources during periods dry weather. About $0.29m^3/s$ sewage effluent is derived from 9 sources (plus effluent from 5 storm water pipes during wet weather at this site (Plate. 3.1). The Waterfall Walk site (LB) further down stream is at an overflow point from the main trunk sewer. The sewage overflow enters a $1.2m^3/s$ (1.2m x 1m x 1m) storage tank before passing into the river which is located at the edge of Brunswick Park (Plate. 3.2).

The Osidge site (LC) is at the main entry to the Barnet - Edmonton trunk sewer from the North London region (Plate. 3.3) and is located inside Brunswick Park. During dry weather conditions, the average flow rate is about $0.13m^3/s$ with flows of $0.26m^3/s$ measured in periods of wet weather.

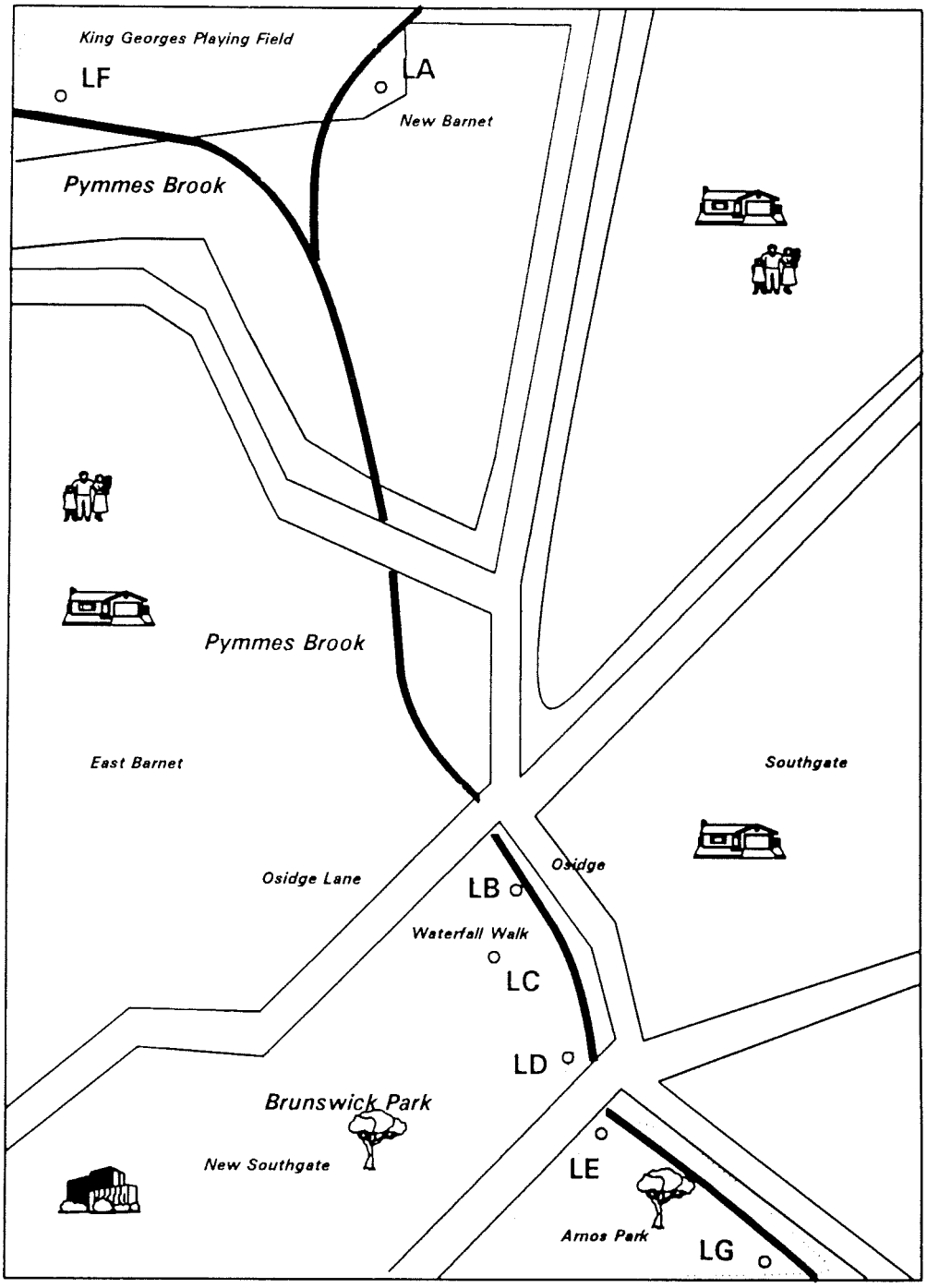


Figure 3.1: Sewage and receiving stream locations in North london.

Plate 3.1: Site LA, the combined sewers at New Barnet.

Plate 3.2: Site LB, the combined sewer at Waterfall Walk.

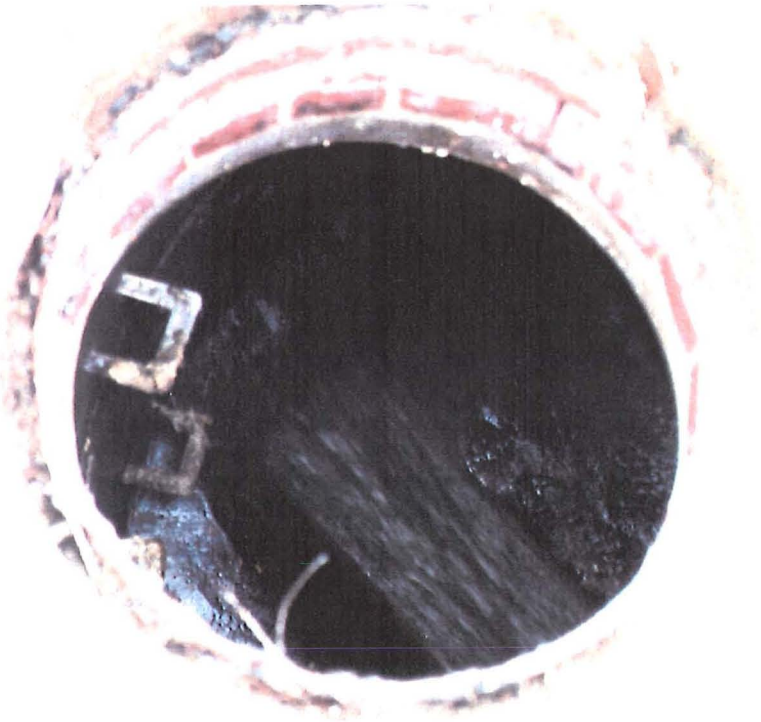


Plate 3.3: Site LC, sanitary wastewater sewer at Brunswick Park.

Site LD is at a twin pipe discharge situated at northern edge of Arnos Park (Plate 3.4). The pipes are of 1.2m diameter and discharge combined sewage into the stream at a average rate varying between 0.015m^3/s and 0.08m^3/s during periods of dry weather and wet weather respectively. The stream receives a further 0.002m^3/s of sewage effluent during periods of dry weather and 0.005m^3/s in wet weather at site LE further down stream in Arnos Park (Plate. 3.5).

The King George Playing Field site (LF) is situated in the upper Pymmes Brook and is located near the regulated source of the stream (Plate 3.6); this was chosen as the background control site as there was no further contributing effluent above this site. The stream at this point is 0.7m wide and approximately 0.4m deep and has average flow rates of 0.001m^3/s in periods of dry weather and 0.003m^3/s in periods of wet weather.

The down stream site (LG) is situated in the lower reaches of the Pymmes Brook which passes through Arnos Park to its confluence with the River Lea. The meandering stream is about 8m wide and has a depth of about 0.8m. During dry weather the average flow is 0.113m^3/s rising to 0.599m^3/s during rainfall (Plate 3.7).

Plate 3.4: Site LD, two combined sewage discharge pipes at Arnos park.

Plate 3.5: Site LE, under the bridge sewer at Arnos Park.

Plate 3.6: Site LF, upper Pymmes Brook stream at King Playing Field.

Plate 3.7: Site LG, in the lower reaches of Pymmer Brook stream.

3.2.1.2. Urban Surface Runoff

Enfield area is one of the largest commercial and residential centres in Northeast London with a population of over 100,000 and is comprised of many individual neighbourhood communities as well as major business and industrial zones. The sampling areas for road surface runoff were selected from within the centre of Enfield Town and Ponders End which is one of the main residential area of Enfield. Figure 3.2 shows the sampling locations within Enfield Town and Ponders End where impermeable surface runoff was collected during the winter period from October 1990 to February 1991.

Sites LK, LL and LM are located in Enfield Town shopping centre, an open market and a main car park respectively. The area is nearly 100% impervious area with side walks, a paved car park surface and commercial lands. The shopping centre contains many principal large chain stores as well as small consumer shops. The open market area contains multiple business including hardware, clothes, food and so on. The Car Park site is located in the largest ground car park available in the centre of the town. The main trunk road site (LN) is located in the High Street (A 1010) at Ponders End. The residential site (LO) is also located in Ponders End at Garfield road. It is totally residential in character and is generally clean and free of litter although characterised by continuous on street car parking.

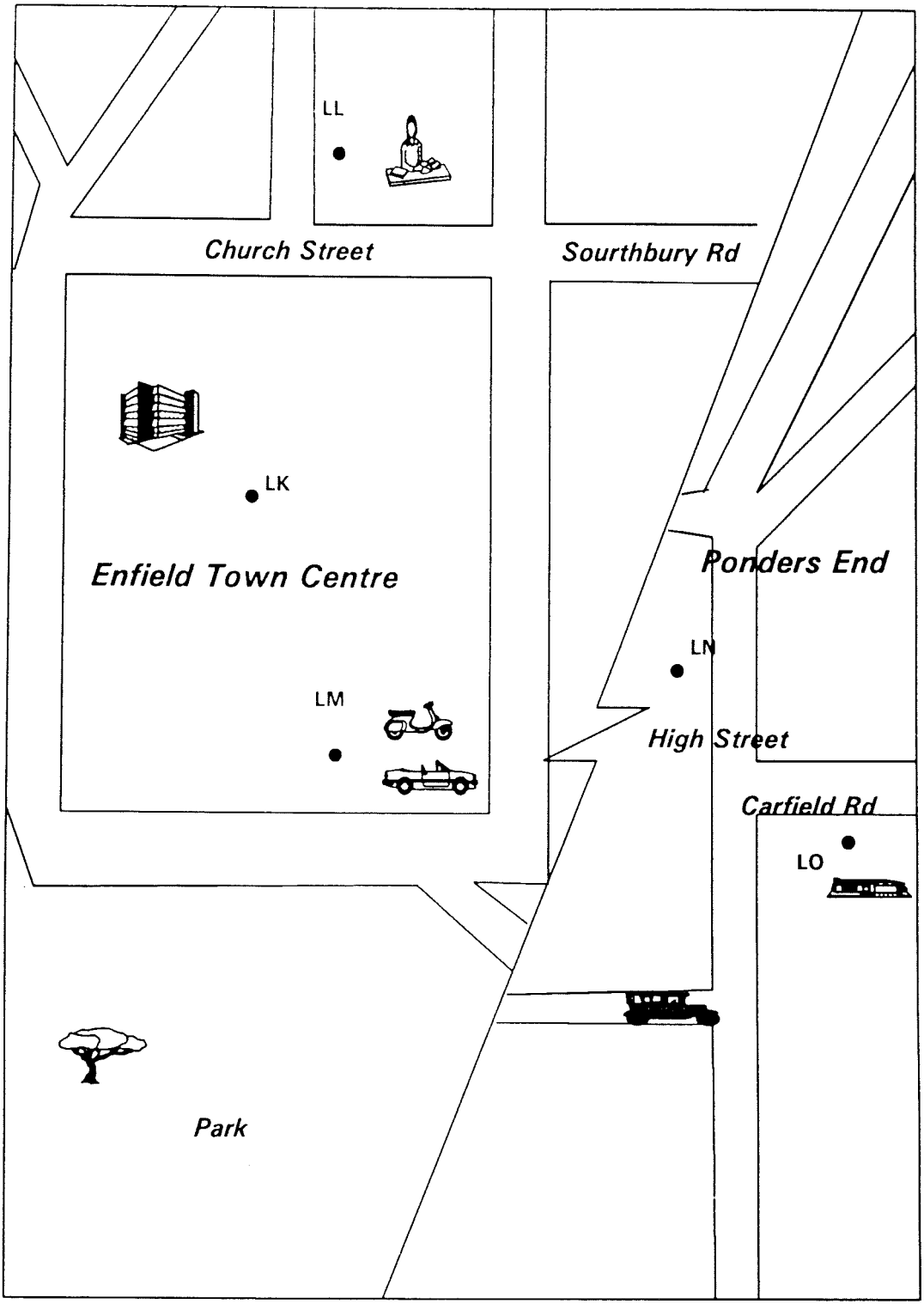


Figure 3.2: Road surface runoff sampling sites in Northeast London.

3.2.2. Sampling Sites in Valencia

3.2.2.1. Urban Sewers and Outfall within Valencia Bay

Valencia is situated in Eastern Spain, and is a popular Spanish tourist city on the Mediterranean seaboard with a total population of 800,000, increasing to over 1,000,000 during the summer. The principal field work was undertaken within the Benimaclet catchment in the north suburbs of the city during March 1991 to July 1991.

The Benimaclet catchment (population 120,000) is a major residential area in the north suburbs of Valencia. Two main sewers (VH and VI) or open ditches discharge the untreated sewage directly from the catchment to Valencia Bay (Fig. 3.3).

The sites at Las Fuentes (VH) and South Politecnica (VI) are outside the Benimaclet catchment. Site VH is an open shallow sanitary wastewater ditch with slow-flowing effluent. During dry weather the average flow rate was about $0.028m^3m/s$ increasing to $0.14m^3/s$ during wet weather (Plate. 3.8).

Site VI is located within the grounds of south Politecnica (Plate. 3.9). Where it passes through an agricultural area and is a deeper and faster-flowing than at site VH. This is a combined system used for both irrigation and effluent transport. The flow rates were very different between the dry and wet weather conditions, being mainly dependent on local agricultural activity. During the time of study average dry weather flow was about $0.177m^3/s$ and wet weather flow was $0.53m^3/s$.

The Malvarrosa site (VJ) is located at the combined outfall of the two main combined sewers which drain the Benimaclet catchment to Valencia Bay (Plate. 3.10). Sewage effluent, representing the contribution from the entire Benimaclet catchment was obtained by sampling from the outfall just before prior to entering the ocean. The discharge point at this location was about 12m wide with a depth of 0.4m and an average flow rate of about $2.6m^3/s$ during periods of dry weather.

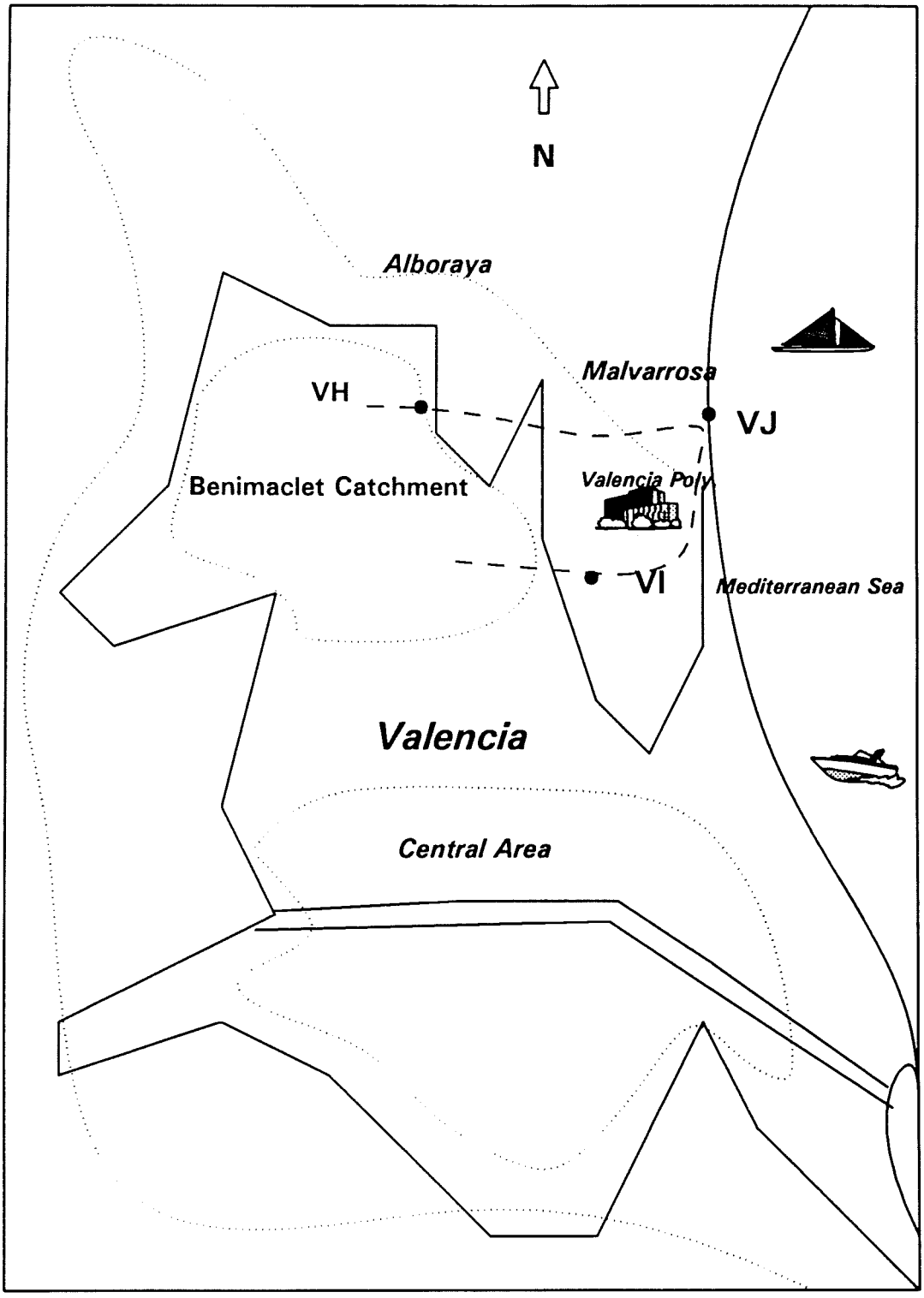


Figure 3.3: Sewage locations in Valencia

Plate 3.8: Site VH, sanitary wastewater sewer at Las Fuentes, Valencia.

Plate 3.9: Site LI, combined sewage sampling site at South Valencia Politecnica.

Plate 3.10: Site VJ, Malvarrosa beach outfall in Valencia.

3.2.2.2. Urban Surface Runoff

Figure 3.4 shows the six urban surface runoff sampling sites within Valencia city. Plaza del la Virgen (VP) is located within the old commercial area in the city centre. Land use here is 85% commercial and 15% residential. Plaza del Mercado (VQ) is the biggest open market in Valencia. It is about 12000 m^2 in area with almost 100% of the commercial land used. Nuevo Centro (VR) is located in the Northwest part of the city and provides the main car parking for the central shopping area of the city. Fernando Street (LS) is one of the main roads within the city whilst the residential site (LT), named Doctor Zomenhoff, is located in the western part of Valencia. This street is 300m long and 10m wide and is 100% residential. Three six floors residential blocks border the alley on each side with some trees and ground vegetation. The alley is in generally good sanitary condition, but like Garfield road in Ponders End is used for car parking.

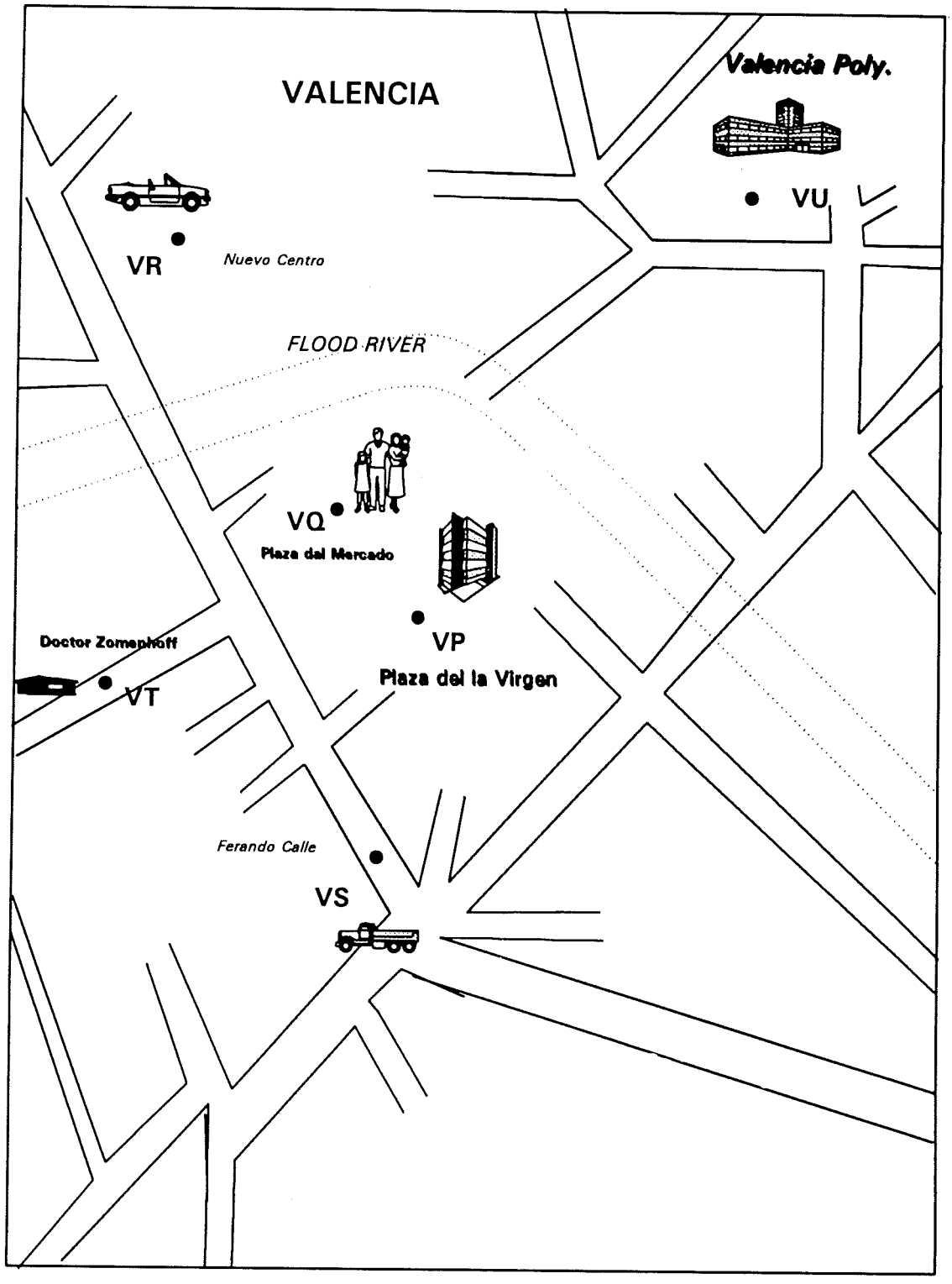


Figure 3.4: Surface runoff sampling sites in Valencia

Site (VU) is located within the open campus of the Valencia Politecnica in the Northeast suburbs of Valencia. It has 60 % cultivated land (mainly horticulture), 5% is undeveloped, 5% is paved road and 30% is comprised of Polytechnic buildings with inter-grassed space.

3.3. Sampling Methods

3.3.1. Sewage and Stream Water Samples

Samples were taken at two week intervals over the period October 1990 to February 1991 in North London and over the period March to July of 1991 in Valencia. All samples were taken by hand with wide-mouthed sterile glass bottles (500ml). Water samples were taken by holding the bottle near its base and plunging it, neck downward, some 3cm below the water surface. The bottle was than turned until the neck pointed slightly upward and its mouth was directed toward the current. Samples were transported back to the laboratory on ice in a dark container. All analyses were usually performed within 4 hours of sampling. Flow, temperature and pH of the effluent were recorded concurrently with sampling.

3.3.2. Road Surface Runoff Samples

All road surface runoff samples were collected during days of heavy rain in sterile wide mouthed bottles. For these samples, a technician was dispatched to the sampling site as soon as possible after the rainfall began and the sample was collected from a gully pot on the street within about 20 min of the initiation of a storm event on the street surface. All samples were immediately placed after collection in a dark insulated chest containing ice packs and transported to the laboratory within 2 hours for analysis.

3.3.3. Continuous Samples

An automatic vacuum sampler (Model MK.4B) was installed to obtain continuous data throughout a 24 hour period at site LC in North London and at site VH in Valencia respectively during periods of dry weather. The inside of the sampler, the rubber tubes and the outside of the plastic vacuum tubes were immersed in 1000 mg/l bleach for 1 hour, then triple washed with sterilized distilled water. Ice packs were placed around the sample bottles during the period of continuous sampling. Samples were drawn into pre - sterilised bottles every 3 hours.

3.3.4. Sediment Samples

Sediment samples were also taken at sites LE and LG (North London) as well as VH and VI (Valencia). The sediment was retrieved with a sterilized cylindrical sampler and subsamples were aseptically removed with a sterile spoon at different depths from the surface of three successive layers at (1) at 0 - 0.3cm depth, (2) at 3.0cm - 5.0cm depth and (3) at 5.0cm - 7.0cm. All samples were transferred to separated sterile 100ml glass bottles which were placed on ice and returned to the laboratory within 2 hours and enumeration procedures were completed within 4 hours. 1.0g of wet sediment was suspended in 99 ml of sterile dilution water. This slurry was vigorously mixed on a platform shaker for 0.5 h (SBSS Shaking Machine, Model AD 0 - 1) with a frequency of 100 revs/min.

3.4. Materials

3.4.1. The Methods Used in the Investigation

The experimental method used to determine the selected microorganisms (total coliforms, faecal coliforms, faecal streptococci and *P. aeruginosa*) was the multiple tube fermentation technique base on "Standard Methods for the Examination of Water and Wastewater" (APHA, 1985). The results, expressed in terms of the most probable

number (MPN) based on certain probability formula, provide an estimate of the mean density/100ml of test bacteria in the water samples and mean density/g in sediment. As most samples possessed a high turbidity, the membrane filter technique could not be used for enumeration purposes.

The water samples were concentrated by membrane filter for the detection of *Salmonella* in water to qualitatively assess the presence or absence of the organism.

3.4.2. Media and Broth

All media were prepared according to the manufacturer's instructions and sterilized at 121°C for 15 mins unless stated. All agar plates were dried at room temperature and stored at 4°C until used.

a. Lauryl Tryptose Broth (OXOID). 35.6g/litre distilled water.
3 ml Phenol Red solution (0.003 g/l) was added to 1 litre Lauryl Tryptose Broth as an indicator. Lauryl Tryptose Broth was dispensed in 10 ml qualities in test tubes with an inverted Durhan's tube. Double strength of Lauryl Tryptose Broth was prepared for 10 ml volume samples. The pH of the medium was adjusted to 6.8 $\pm$ 0.2 before sterilization.

b. EC Broth (OXOID). 37g/litre distilled water.
The EC broth was dispensed in 10 ml quantities in test tubes with an inverted Durhan's tube. Double strength of the broth was prepared for 10 ml volume samples. The pH of the broth was adjusted to 6.9 $\pm$ 0.2 before sterilization.

c. Azide Dextrose Broth (DIFCO). 34.7g/litre distilled water.
Azide Dextrose broth was dispensed in 10 ml test tubes. Double strength of the medium was prepared for 10ml volume samples. The pH of the medium was adjusted to 7.2 before sterilization.

d. Ethyl Violet Azide Broth

Ethyl Violet Azide broth (Litsky *et al*, 1953) was prepared in the laboratory. the ingredients were as follows:

NaCl	20.0 g
K_2HPO_4	2.7 g
KH_2PO_4	2.7 g
NaN_3	0.4 g
Glucose	7.5 g
Ethyl violet	0.00083 g
Distilled water	1000 ml

The medium was dispensed in 10 ml test tubes. The pH was adjusted to 7.0 $\pm$ 0.2 before sterilization. Double strength of this medium was also prepared for 10ml volume samples.

e. Asparagine Broth

Asparagine broth (APHA, 1985) was prepared in the laboratory as follows:

Asparagine	3.0 g
K_2HPO_4	1.0 g
MgSO4.$7H_2O$	0.5 g
Distilled water	1000 ml

The pH was adjusted to between 6.9 - 7.2. The medium was filtered through 0.45 μm pore size membrane (millipore) to sterilize and then 10 ml volumes of broth were dispensed into sterilized test tube. Double strength of this medium was also prepared for 10 ml volume samples.

f. Cetrimide Agar (DIFCO). 45.4g/litre distilled water.

Cetrimide agar was dispensed into 10 ml test tube. After sterilization the tubes were inclined during cooling to provide a large slant surface. The pH of the agar was adjusted to 7.2 $\pm$ 0.2 before sterilization.

g. NR 10/43 Enrichment Broth

NR10/43 (Alcaide *et al.*, 1982) was prepared in the laboratory. The ingredients were as follows:

(1)	Tryptone	5.0 g
	Sodium chloride	8.0 g
	K_2HPO_4	1.2 g
	Distilled water	1000 ml
(2)	$MgCl_26H_2O$	40.0 g
	Distilled water	100 ml
(3)	Malachite Green-oxalate	4.0 mg
	Distilled water	10 ml

NR 10/43 broth: 1000 ml(1) + 100 ml(2) + 10 ml(3) = 1110 ml

100 ml NR 10/43 broth was dispensed into conical flasks and sterilised as stated previously. 1 ml Sodium Novobiocin (40mg/l) was added before use (Alcaide, *et al*, 1982). The pH of the broth adjusted to about 7.4 before sterilization.

h. Rambach Agar (Rambach, 1990). 33.1g/litre distilled water.

The pH of this agar was adjusted to 7.2 $\pm$ 0.2 before sterilization. The agar was made up and sterilised in the conventional manner. Rambach agar contains the following ingredients:

Propylene glycol	10.0 g
Peptone	5.0 g
Yeast extract	2.0 g
Sodium desoxycholate	1.0 g
Neutral red	0.003 g
5-bromo-4-chloro-3-indoclyl.βD	
-galactopyranoside	0.1 g
Agar	15.0 g
Distilled water	1000 ml

i. Buffer

3.0 g of K_2HPO_4 and 1.0 g of KH_2PO_4 was suspended in 1 litre distilled water (APHA, 1985) which was then dispensed in 9 ml quantities in test tubes and sterilized as stated previously. The pH of the buffer adjusted to 7.4 $\pm$ 0.5 with 1N sodium hydroxide (NaOH) before sterilization.

3.4.3. Microorganisms Selected and Enumeration for Water and Sediment Samples

The indicators chosen for this investigation are total coliforms, faecal coliforms and faecal streptococci all of which have been widely accepted and selected for research into the water environment. *Pseudomonas aeruginosa* and *Salmonella spp* were also chosen as representative for the study, and have recognised significance for public health risks associated with recreational waters.

a. Total Coliforms by MPN Technique

For total coliforms, a series of five test tubes (5 x 10ml) of Lauryl Typtose broth (OXOID) were inoculated with appropriate decimal quantities to determine the most probable number (MPN) of total coliforms (APHA, 1987). The tubes were incubated at 37°C for 48h. Formation of gas in any amount within the inverted Durhan's tubes and acid production in 48h was considered a positive reaction.

b. Faecal coliforms by MPN Technique

All positive presumptive tubes from the total coliform MPN test were used to determine levels of faecal coliforms. 3 loops of each presumptive tube were transferred to EC broth (OXOID). The inoculated tubes were incubated in a water bath at 44.5 $\pm$ 0.2°C for 24 $\pm$ 2 hours. Gas production in EC broth within 24h or less was a positive reaction indicating coliforms of faecal origin.

c. Faecal streptococci by MPN Technique

Water samples were inoculated into a series of five tubes (5 x 10ml) containing Azide Dextrose broth (DIFCO) using 10 ml of single strength broth to inoculate 1 ml samples and 10 ml of double - strength broth for 10 ml samples (APHA, 1985). The inoculated

tubes were incubated at 35°C ± 0.5. Each tube was examined for turbidity after 48h. 3 loops of culture from all tubes showing turbidity were transferred to 10ml of Ethyl Violet broth . The inoculated tubes were incubated at 37°C ± 0.5 for 24 ± 2h. Tubes with turbidity and purple deposition on the bottom confirm the presence of faecal streptococci (APHA, 1985).

d. *Pseudomonas aeruninosa* by MPN Technique

Asparagine broth was used throughout this study for the presumptive enumeration by the MPN technique (APHA, 1985). Appropriate dilutions of water samples were inoculated into a five tube series (5 x 10ml) and incubated at 41.5°C for 48 h (Alonso, et. al.,1989). The tubes showing pigment production, superficial pellicle formation and/or turbidity after 48h of incubation were interpreted as presumptively positive.

The confirmatory test was carried out by transferring inocula from positive asparagine broth to cetriminde agar slants (Brodsky and Ciebin, 1978) and incubated at 41.5°C for 48h. Tubes that developed a yellow - green colour were considered positive and used to establish confirmed MPN counts.

e. Qualitative Test for *Salmonella* by Filtration Technique

The standard membrane filtration technique was used for the qualitative assessment of *Salmonella* (APHA, 1985). 200 ml samples from sanitary wastewater sewer (LC and LV) and 500 ml samples from other combined sewage samples was pre-filtered through sterile sartorius membrane filters (pore size 12 μm). The filtrate was further filtered through a sterile Millipore membrane (0.45 μm pore size) and the membranes introduced respectively into flasks containing 100ml NR10/43 enrichment broth. Enrichment media were incubated at 43°C for 24h All flasks which contained 100 ml NR10/43 were streaked on to duplicate plates of Rambach agar, one heavily and one lightly and incubated at 37°C for 24h. Typical colonies with a bright red colour were considered positive (Rambach, 1990).

3.4.4. Physico - Chemical Methods

Temperature, pH and flow were concurrently recorded during sampling. Temperature was measured by a fine centigrade thermometer (scale, -10 - 30°C) and pH by universal pH paper. Flow was measured using a flow meter (Armeied, No. 1178 - 1020). BOD_5 (Biological oxygen Demand) was determined using standard WTW (Wissenschaftlich-Techn-Werkstatten) test equipment (Model 1002). For the laboratory bacterial release experiment, pH was measured using a Micro-pH meter (Crison Ltd Model 2000). DO (Dissolved Oxygen) was measured by YSI Oxygen Meter (Yellow Springs, Model 51A). Organic matter was measured by heating the dried sediment to 550°C in a Muffle Furnace Overnight (Block, 1965) and then determining the average weight loss of three replicates.

3.5. Investigation of Changes in FC/FS Ratio

Two types of effluent samples of dry weather flow sanitary wastewater and combined sewage water were sampled from sites LC and LA in January 1991 in North London. Samples were transported back to the laboratory on ice in a dark container. Samples were divided and stored in the dark at both 20°C and 4°C and total coliforms and faecal coliforms enumerated every 24 h for 5 days.

3.6. Bacterial Release Experiments

This experiment was carried out in order to determine the rate of release of bacterial from sediments.

3.6.1. Experimental Design

Experiments were conducted in plastic tanks under continuous flow conditions. The

size of tank 1 (water feeder tank) was 40 cm x 30 cm x 18 cm and tank 2 (water - sediment system tank) was 40 cm x 30 cm x 15cm. Both tanks were initially sterilized by immersing in bleach at 500 mg/l for 2 hours, followed by triple washing with sterilized distilled water. All connecting tubes, inlet and outlet tubes were sterilized by the same method. At sampling site VI, a section of sediment of similar size to tank 2, and about 4 cm thick was removed without water, and was placed into the bottom of tank using a sterilized sampling spade, then transported to the laboratory within 20 min. The sediment was washed continuously with sterilised sewage. This sewage was removed from site VI in about 20 litre quantities and sterilized in the autoclave at 121°C for 15 mins, cooled and poured into tank 1. New sterilized sewage water was added to water tank 1 every day to keep the system in operation for 9 days. Water was flowed into tank 2 from tank 1 through a connective tube as tank 1 was higher than tank 2. The overflow from tank 2 was located at the water surface, 12 cm above the bottom of the tank. The flow rate was 15 ml/min ($2.5x10^{7}$ m^3/s).

Figure 3.5 shows the experimental tanks devised for the water - sediment system to determine bacterial release.

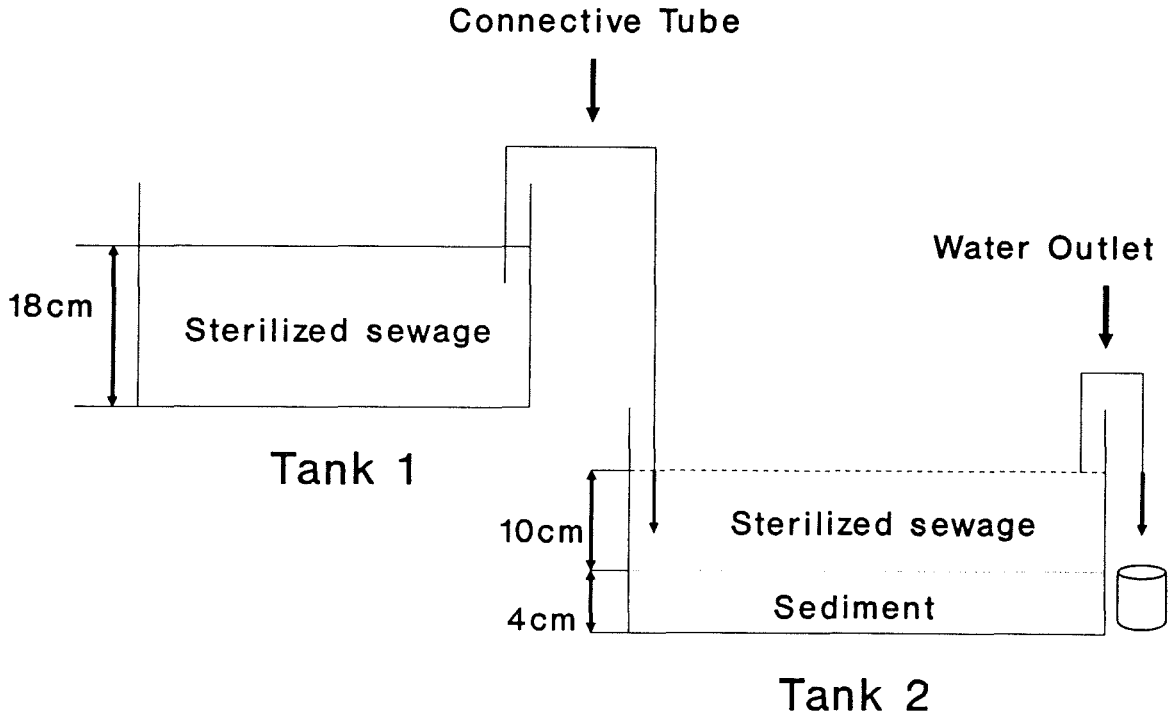


Figure 3.5: Water - Sediment System

3.6.2. Microbiological Assays

Water samples were collected at time zero (started from the first drip) and then after at 1h, 3h, 6h, 12h, 24h, 3 days, 5 days, 7 days and 9 days successively. All water samples were collected using a sterile 100 ml flask at the outlet of tank 2. Sediment samples were taken using a sterile spoon removed from both the edges and centre of the tank, resulting in a total of 3g of wet sediment. 1 g of this wet sediment was taken from the mixed sediments for finial analysis. The first sample of sediment was taken at day zero before the experiment began and the second sample taken on the 9th (the last day), after the last water sampling. Each sample was assayed for levels of total coliform, faecal coliform, faecal streptococci and *Pseudomonas aeruginosa* as well as for pH, temperature and DO (Dissolved oxygen).

3.6.3. Test Sediment

Sediment texture was determined by the hydrometer method (Day, 1956). Approximate particle sizes ranges were as follows; sand $>$ 70 μm; silt, 4 - 62 μm; and clay, $<$ 4 μm. Organic matter was also estimated.

3.7. Statistical Analysis

Statistical analysis of the parameter variables measured at each sampling site has been carried out using the MINITAB programme. The multiple correlation matrices and principle component analysis (Krazanowski, 1988) of the data are reported in the relative chapter. Only correlations significant at the 95%, 99% and 99.9% level of probability are reported. The log normal distribution has been widely applied to interpret microbiological data in environmental microbiological studies (Alonso *et al.*, 1984; Meynard *et al.*, 1989; Mujeriego, 1990 and Vicent *et al.*, 1991). In this study, the log normal distribution (Log10 transformation) was also found to best describe the variation of the bacteria indicator organisms according to the test of normality. All

microbiological data were transformed to natural logarithms in graphs and tests for statistical significance. However, the normal distribution was found to best describe the variation of the physico - chemical data, and it is this format which has been used to summarize and test these parameters.

Chapter 4: The Levels and Characteristics of Microorganisms in Urban Sewage, Stormwater, Receiving Stream and Sediments.

4.1. Introduction

As with most aquatic microbiology studies, the determination of faecal indicator bacteria distributions for both urban surface waters and sediments as well as the relationships between different indicator microorganisms and pathogens are of great importance. Many studies have shown that the increase of pollution in natural surface waters has intensified the detection frequency and persistence of coliform and pathogenic microorganisms in areas affected by urban sewage discharges (Bonde, 1963; Braga and Pagano, 1970; Olivieri *et al.*, 1978; Geldreich, 1982; Morinigo *et al.*, 1990). This current study represents investigations of indicator microorganisms and pathogenic bacteria at the various field sites to give indications concerning the nature of faecal indicator bacterial distributions and relationships between different indicator microorganisms and pathogens, in order to provide baseline information on bacterial indicators in urban sewage discharges, receiving streams and associated coastal outfalls.

Several studies of faecal bacterial survival and characteristics have been completed for sediment systems (Grimes, 1975; Gerba and McLead, 1976; Roper and Marshall, 1978; Chan *et al*, 1979; Hood and Ness, 1982; Laliberti and Grimes, 1982; Goyal and Adams, 1984), but most of this freshwater sediment microbiology has been confined to studies of the deep water sediment. This chapter will therefore include details of further investigations undertaken in the urban stormwater and sediments. The indicators and pathogens as well as their essential characteristics under different weather conditions for London and Valencia will also be compared.

4.2. Results

4.2.1. The Levels of Indicator Microorganisms, Pathogens and Physico - Chemical Parameters in Combined Sewage and Receiving Stream in North London.

The levels and geometric mean densities of total coliform (TC), faecal coliform (FC), faecal streptococci (TS), *Pseudomonas aeruginosa* (PA) and *Salmonella* obtained at combined sewage sites LA, LB, LD, LE, dry weather flow (DWF) sanitary wastewater site LC, receiving stream sites LF and LG are presented in Tables 4.1, 4.2, 4.3, 4.4, 4.5, 4.6 and 4.7, respectively for the period October 1990 to February 1991. The figures reflect variable conditions although in general they can be said to reflect dry, cold temperate weather and low flow conditions. The tables also include wet weather combined sewage, receiving stream water in order to provide a basis for comparison of levels recovered in these different systems under differing conditions.

The geometric mean densities of indicator microorganisms and pathogenic bacteria at all combined sewage sites (LA, LB, LD, and LE) were similar. Total coliforms were found in the ranges of 1.0×10^5 - 2.8×10^5 MPN/100 ml, faecal coliforms in the ranges of 1.8×10^4 - 5.9×10^4 MPN/100 ml, faecal streptococci in the ranges of 4.7×10^3 - 2.3×10^4 MPN/100 ml and *P. aeruginosa* in the range of 2.7×10^2 - 4.2×10^2 MPN/100 ml.

Site LF is located in the head water of the Pymmes Brook. There is no sewage discharged in to the stream above this site. Results for this site are presented in Table 4.6. Low levels of total coliforms, faecal coliforms and faecal streptococci (geometric mean range was from 5.0×10^2 to 4.5×10^3 MPN/100 ml) and pathogenic bacteria (geometric mean of *P. aeruginosa* was 2.4 MPN/100 ml) were recovered. *Salmonella* was also absent at this site confirming the "good" quality of this background site. Indicator microorganisms at this site may be derived from soil particles, insects, vegetation (Geldreich *et al.*, 1964) and pets which local residents exercise in the surrounding space adjacent to the river channel.

Table 4.1: Levels of Microorganisms Recovered at Site LA (MPN/100ml).

Date	TC	FC	FS	PA	Sal.*
3/10/90	$1.3x10^5$	$3.0x10^4$	$2.0x10^4$	$9.5x10^3$	+
24/10	$3.0x10^6$	$8.0x10^5$	$4.5x10^3$	$4.5x10^3$	+
29/10	$7.0x10^5$	$3.0x10^5$	$7.5x10^3$	4.5x10	+
26/11#	$1.3x10^5$	$8.0x10^4$	$1.5x10^3$	$9.5x10^3$	-
4/12	$5.0x10^3$	$1.3x10^3$	$4.5x10^4$	7.5x10	-
17/12	$3.0x10^3$	$8.0x10^2$	$9.5x10^3$	9.5	-
9/1/91#	$3.0x10^5$	$2.3x10^5$	$4.0x10^4$	$6.5x10^2$	NT
21/1	$8.0x10^4$	$2.3x10^4$	$2.5x10^3$	$9.5x10^2$	-
GM[a]	$1.0x10^5$	$3.7x10^4$	$8.1x10^3$	$3.5x10^2$	
MIN[b]	$3.0x10^3$	$8.0x10^2$	$1.5x10^3$	7.5	
MAX[c]	$3.6x10^6$	$8.0x10^5$	$4.5x10^4$	$9.5x10^3$	

*: *Salmonella*. a: Geometric mean. b: Minimum. c: Maximum. NT: Not tested.
#: Rainfall date.

Table 4.2: Levels of Microorganisms Recovered at Site LB (MPN/100ml).

Date	TC	FC	FS	PA	Sal.*
3/10/90	$2.3x10^5$	$8.0x10^4$	$1.5x10^4$	$9.5x10^2$	-
24/10	$1.2x10^6$	$5.0x10^5$	$7.5x10^3$	$9.5x10^3$	+
29/10	$8.0x10^5$	$3.0x10^5$	$7.5x10^3$	$4.5x10^3$	-
26/11#	$8.0x10^5$	$5.0x10^4$	$2.5x10^4$	7.5x10	-
4/12	$3.0x10^4$	$2.3x10^4$	$4.5x10^3$	4.0x10	-
17/12	$4.0x10^3$	$5.0x10^3$	$9.5x10^3$	2.5x10	-
9/1/91#	$8.0x10^5$	$3.0x10^4$	$2.5x10^2$	$7.5x10^2$	NT
21/1	$1.4x10^5$	$7.0x10^4$	$4.5x10^4$	$1.5x10^2$	-
GM[a]	$1.9x10^5$	$5.9x10^4$	$7.5x10^3$	$2.7x10^2$	
MIN[b]	$4.0x10^3$	$5.0x10^3$	$2.5x10^2$	2.5x10	
MAX[c]	$1.2x10^6$	$5.0x10^5$	$4.5x10^4$	$9.5x10^3$	

*: *Salmonella*. a: Geometric mean. b: Minimum. c: Maximum. NT: Not tested.
#: Rainfall date.

Table 4.3: Levels of Microorganisms Recovered at Site LD (MPN/100ml).

Date	TC	FC	FS	PA	Sal.*
3/10/90	$5.0x10^5$	$2.3x10^5$	$4.5x10^4$	$1.5x10^2$	-
24/10	$5.0x10^5$	$2.3x10^5$	$1.5x10^4$	$7.5x10^2$	+
29/10	$1.7x10^5$	$5.0x10^4$	$4.5x10^4$	$4.5x10^3$	-
26/11#	$1.3x10^6$	$2.8x10^5$	$4.5x10^5$	$4.5x10^2$	-
4/12	$5.0x10^5$	$8.0x10^4$	$9.5x10^3$	$4.5x10^2$	-
17/12	$5.0x10^4$	$1.3x10^3$	$2.5x10^3$	4.0x10	-
9/1/91#	$7.0x10^4$	$2.3x10^4$	$4.5x10^4$	1.5x10	NT
21/1	$1.1x10^5$	$5.0x10^4$	$2.5x10^3$	4.5x10	-
2/2	$9.0x10^5$	$7.0x10^4$	$4.5x10^4$	$1.5x10^2$	-
GM[a]	$2.8x10^5$	$5.7x10^4$	$2.3x10^4$	$4.2x10^2$	
MIN[b]	$5.0x10^4$	$1.3x10^3$	$2.5x10^3$	1.5x10	
MAX[c]	$1.3x10^6$	$2.8x10^5$	$4.5x10^5$	$7.5x10^3$	

*: *Salmonella*. a: Geometric mean. b: Minimum. c: Maximum. NT: Not tested.
#: Rainfall date.

Table 4.4: Levels of Microorganisms Recovered at Site LE (MPN/100ml).

Date	TC	FC	FS	PA	Sal.*
3/10/90	$3.0x10^5$	$5.0x10^4$	$4.5x10^4$	$9.5x10^2$	-
24/10	$1.1x10^6$	$3.0x10^5$	$1.5x10^4$	$4.5x10^2$	+
29/10	$7.0x10^5$	$3.0x10^5$	$7.5x10^3$	$4.5x10^3$	-
26/11#	$5.0x10^4$	$2.3x10^4$	$4.5x10^3$	7.5x10	-
4/12	$1.3x10^4$	$3.0x10^3$	$7.5x10^2$	9.5	-
17/12	$5.0x10^3$	$2.3x10^3$	4.0x10	9.5	-
9/1/91#	$3.0x10^4$	$1.1x10^4$	$2.5x10^2$	9.5x10	NT
21/1	$9.0x10^5$	$3.0x10^5$	$4.2x10^3$	$4.5x10^3$	-
2/2	$3.3x10^5$	$7.9x10^4$	7.5x104	$4.5x10^3$	-
GM[a]	$1.3x10^5$	$1.8x10^4$	$4.7x10^3$	$4.0x10^2$	-
MIN[b]	$5.0x10^3$	$2.3x10^3$	4.0x10	9.5	
MAX[c]	$9.0x10^5$	$3.0x10^5$	$9.3x10^4$	$4.3x10^4$	

*: *Salmonella*. a: Geometric mean. b: Minimum. c: Maximum. NT: Not tested.
#: Rainfall date.

Table 4.5: Levels of Microorganisms Recovered at Site LC (MPN/100ml).

Date	TC	FC	FS	PA	Sal.*
3/10/90	$2.3x10^7$	$1.3x10^7$	$1.5x10^4$	$2.5x10^3$	+
24/10	$8.0x10^7$	$5.0x10^7$	$7.5x10^5$	$4.5x10^5$	+
29/10	$1.7x10^8$	$8.0x10^7$	$4.5x10^5$	$1.5x10^5$	+
26/11#	$1.1x10^7$	$8.0x10^6$	$7.5x10^5$	$1.5x10^5$	+
4/12/90	$1.3x10^7$	$3.0x10^6$	$2.5x10^4$	$2.5x10^3$	+
17/12/90	$7.0x10^6$	$5.0x10^6$	$4.5x10^5$	$4.5x10^3$	-
9/1/91#	$1.3x10^7$	$8.0x10^6$	$4.5x10^5$	$4.5x10^4$	NT
21/1	$3.0x10^6$	$8.0x10^5$	$4.0x10^4$	$2.0x10^3$	+
2/2	$4.9x10^7$	$1.1x10^7$	$1.5x10^5$	$2.0x10^4$	NT
GM[a]	$2.0x10^7$	$9.1x10^6$	$1.3x10^5$	$2.0x10^4$	
MIN[b]	$3.0x10^6$	$8.0x10^5$	$1.5x10^4$	$2.0x10^3$	
MAX[c]	$1.7x10^8$	$8.0x10^7$	$7.5x10^5$	$4.5x10^5$	

*: *Salmonella*. a: Geometric mean. b: Minimum. c: Maximum. NT: Not tested.
#: Rainfall date.

Table 4.6: Levels of Microorganisms Recovered at Site LF (MPN/100ml).

Date	TC	FC	FS	PA	Sal.*
3/10/90	$2.3x10^4$	$2.3x10^4$	$2.0x10^4$	$9.5x10^2$	-
24/10	$3.0x10^3$	$2.3x10^3$	9.5x10	NF	-
29/10	$1.3x10^4$	$3.0x10^3$	$2.5x10^2$	2.5	-
26/11#	$3.0x10^3$	$4.0x10^2$	$1.5x10^2$	NF	-
4/12	$3.0x10^3$	$1.1x10^3$	7.5x10	NF	-
17/12	$2.2x10^3$	$1.1x10^3$	$2.3x10^3$	NF	-
9/1/91#	$3.0x10^3$	$5.0x10^2$	$1.5x10^2$	NF	NT
21/1	$1.3x10^4$	$5.0x10^3$	$4.5x10^2$	NF	-
2/2	$2.2x10^3$	$1.1x10^3$	$2.3x10^3$	NF	-
GM[a]	$4.9x10^3$	$1.8x10^3$	$5.0x10^2$	2.4	
MIN[b]	$2.2x10^3$	$4.0x10^2$	9.5x10	NF	
MAX[c]	$2.3x10^4$	$2.3x10^4$	$2.0x10^4$	$9.5x10^2$	

*: *Salmonella*. a: Geometric mean. b: Minimum. c: Maximum. NF: Not found.
#: Rainfall date. NT: Not tested.

Table 4.7: Levels of Microorganisms Recovered at Site LG (MPN/100ml).

Date	TC	FC	FS	PA	Sal.*
3/10/90	$2.3x10^4$	$2.3x10^4$	$2.5x10^4$	2.5x10	-
24/10	$1.7x10^5$	$1.7x10^4$	4.5x10	4.0	-
29/10	$5.0x10^4$	$3.0x10^4$	9.5x10	$1.2x10^2$	-
26/11#	$1.3x10^3$	$5.0x10^2$	$4.5x10^2$	9.5x10	-
4/12	$4.0x10^3$	$1.3x10^2$	$6.5x10^3$	7.5x10	-
17/12	$1.1x10^4$	$5.0x10^3$	$2.5x10^3$	9.5x10	-
9/1/91#	$5.0x10^5$	$1.7x10^5$	$9.5x10^3$	$9.5x10^2$	-
21/1	$1.1x10^5$	$2.3x10^4$	$4.5x10^4$	7.5	-
GM[a]	$3.0x10^4$	$7.9x10^3$	$2.1x10^3$	7.0x10	
MIN[b]	$1.3x10^3$	$5.0x10^2$	4.5x10	4.0	
MAX[c]	$5.0x10^5$	$1.7x10^5$	$4.5x10^4$	$9.5x10^2$	

*: *Salmonella*. a: Geometric mean. b: Minimum. c: Maximum. #: Rainfall date.

The levels of bacteria recovered at receiving stream site LG were generally lower than at the combined sewage sites. The levels of total coliform, faecal coliform, faecal streptococcus and *P. aeruginosa* were 3.0 x 10^4 MPN/100 ml, 7.9 x 10^3 MPN/100 ml, 2.1 x 10^3 MPN/100 ml and 7.0 x 10 MPN/100ml, respectively. However, the levels of bacteria at site LC were generally higher than those recorded at any of the combined sites. The geometric mean of total coliforms recorded was 2.0 x 10^7 MPN/100 ml, faecal coliforms was 9.1 x 10^6 MPN/100 ml, faecal streptococci was 1.3 x 10^5 MPN/100 ml and *P. aeruginosa* was 2.0 x 10^4 MPN/100 ml.

The levels of organisms recovered showed a descending order as follows: total coliform > faecal coliform > faecal streptococcus > *P. aeruginosa* for all sampling sites (Fig. 4.1). There is a general trend towards maximum numbers in the earlier (October) and late winter (February), but decreasing to a minimum in the middle winter (December), for all indicator microorganisms at all sites. Figures 4.2, 4.3 and 4.4 are the examples

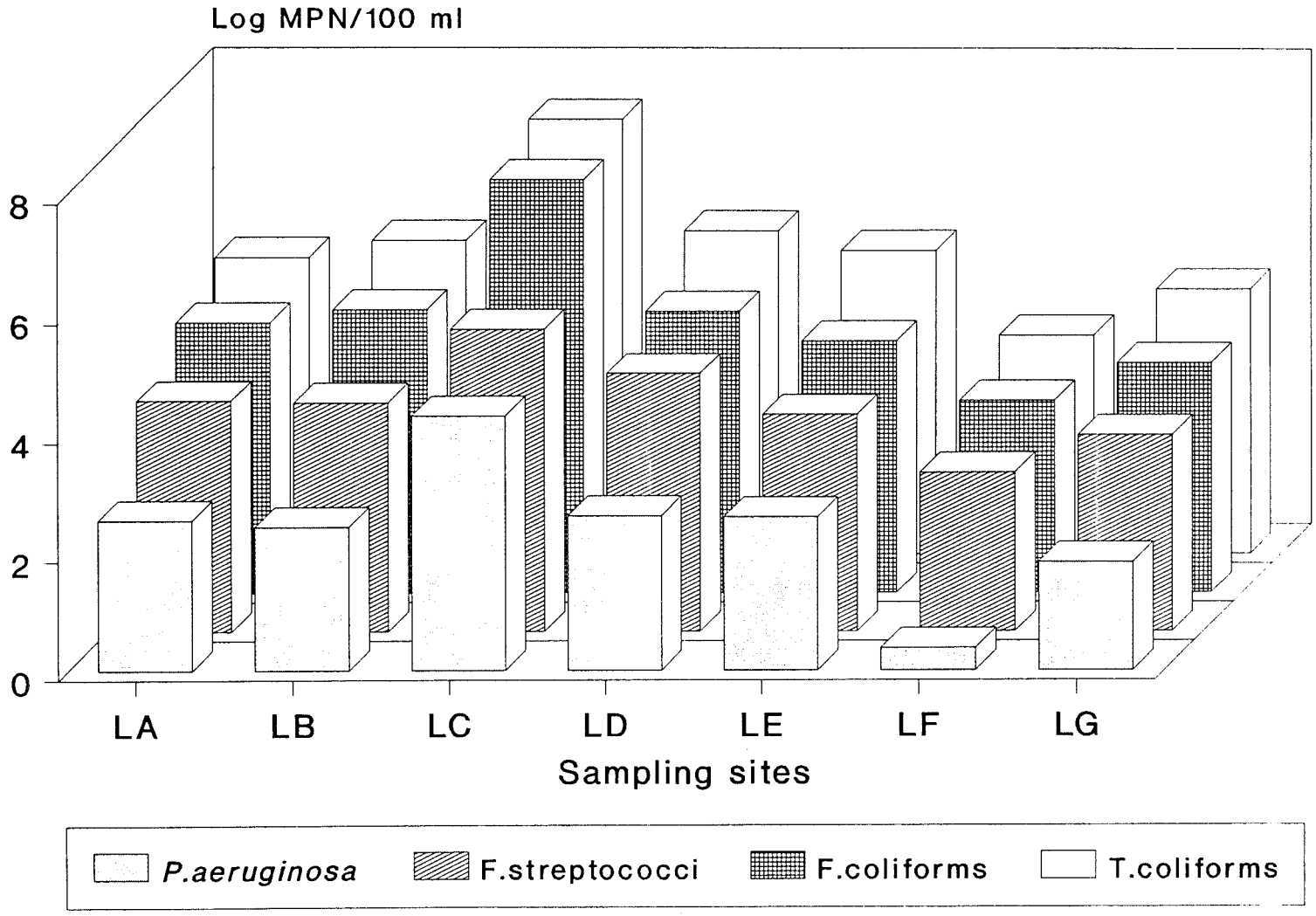


Figure 4.1: Geometric mean of bacteria in combined sewage and receiving stream sites in North London.

of bacterial variations at sites LE, LG and LC respectively.

The occurrence of *Salmonella* was found in 42.9%, 14.2% and 14.2% of samples at sites LA, LB, and LD, respectively. At site LC, *Salmonella* was found in 85.7% of samples. No *Salmonella* was recovered from sites LE, LF and LG.

Geometric mean densities of indicator microorganisms and pathogenic bacteria recovered appear to reflect the quality and sources of sewage and can be grouped accordingly. The first group contains sites LA, LB, LD and LE which are all combined sewage. The second group includes sites LF and LG. Site LF is located at the upper end of receiving stream and site LG at the lower reaches. The values were lower than those as sites LA, LB, LD and LE. Finally site LC presented about 100 - fold higher values than sites LA, LB, LD and LE.

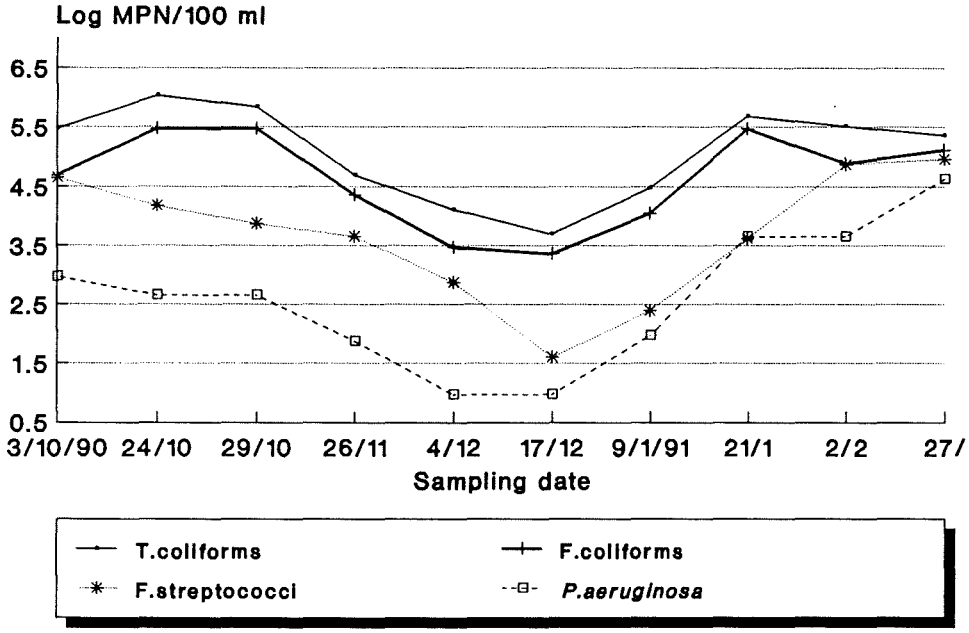


Figure 4.2: Variation of bacteria in combined sewage at site LE.

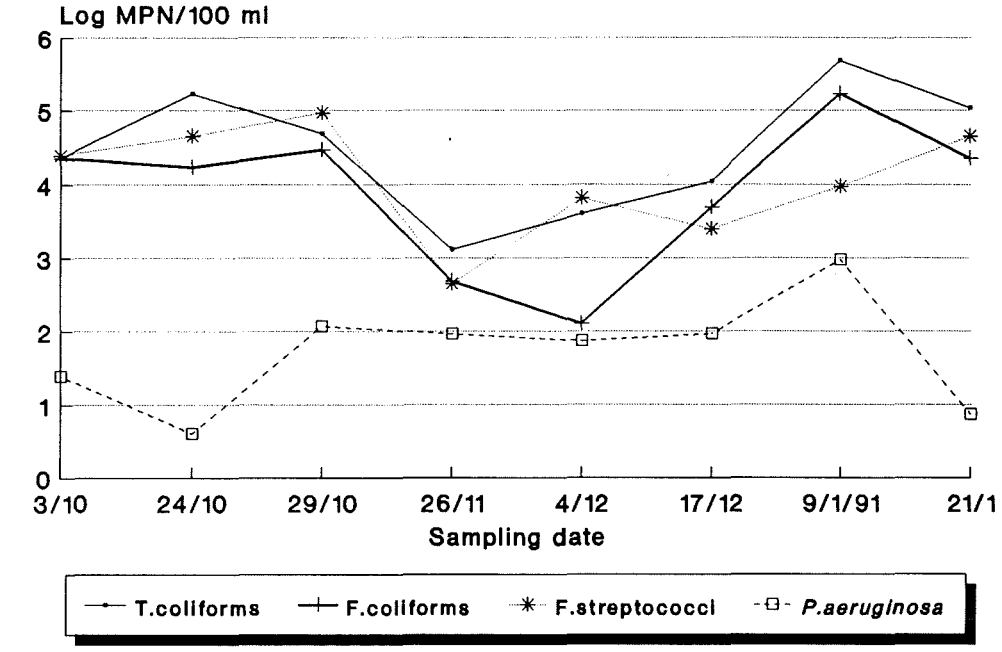


Figure 4.3: Variations of bacteria in receiving stream at site LG.

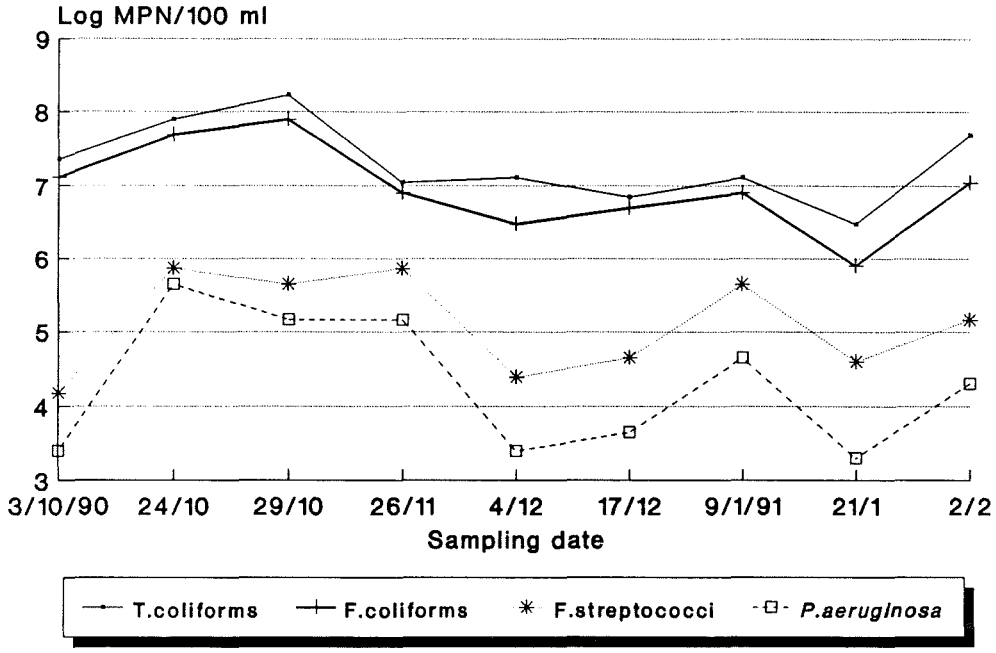


Figure 4.4: Variations of bacteria in combined sewage at site LC.

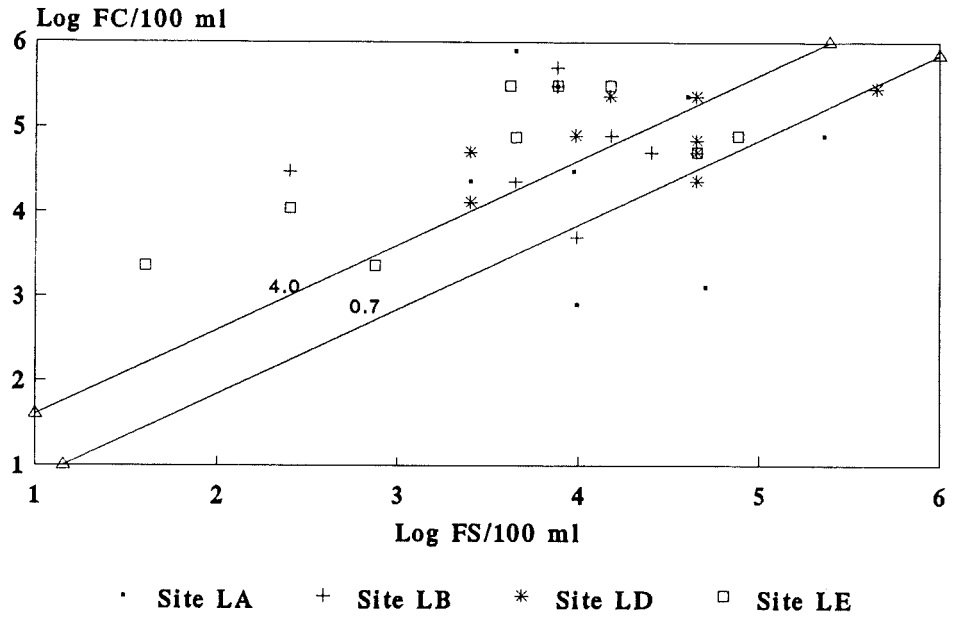


Figure 4.5: FC/FS ratios in combined sewage at sites LA, LB, LD and LE.

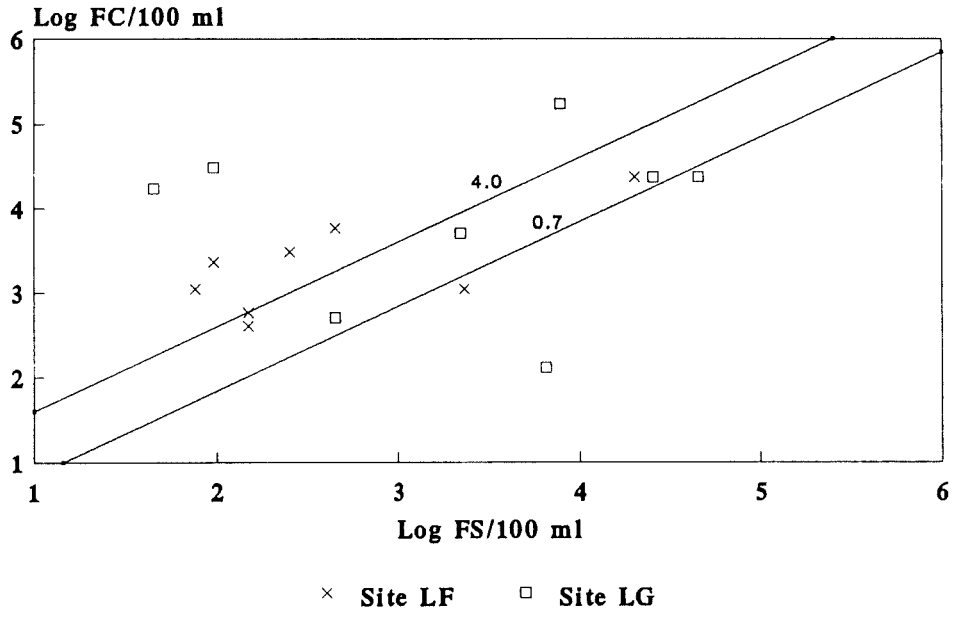


Figure 4.6: FC/FS ratios in receiving stream at sites LF and LG.

The logarithm of faecal coliform densities has been plotted against the logarithm of the density of faecal streptococci for all sites. 60% of all samples from the combined sewage sites LA, LB, LD and LE had a ratio of FC/FS of more than 4.0, 23% of samples had a ratio of between 4.0 and 0.7, and 17% of samples had a ratios of less than 0.7 (Fig. 4.5). At the upper stream site LF, 50% samples showed a FC/FS ratio of more than 4.0 and some 13% of samples less than 0.7. At site LG, 25% of samples had a ratio of less than 0.7 and 37.5% of samples had a ratio of more than 4.0 (Fig. 4.6). At site LC, 44.4% samples showed a FC/FS ratio of more than 4.0 and total 88.8% of samples had a ratio of more than 0.7 (Fig. 4.7).

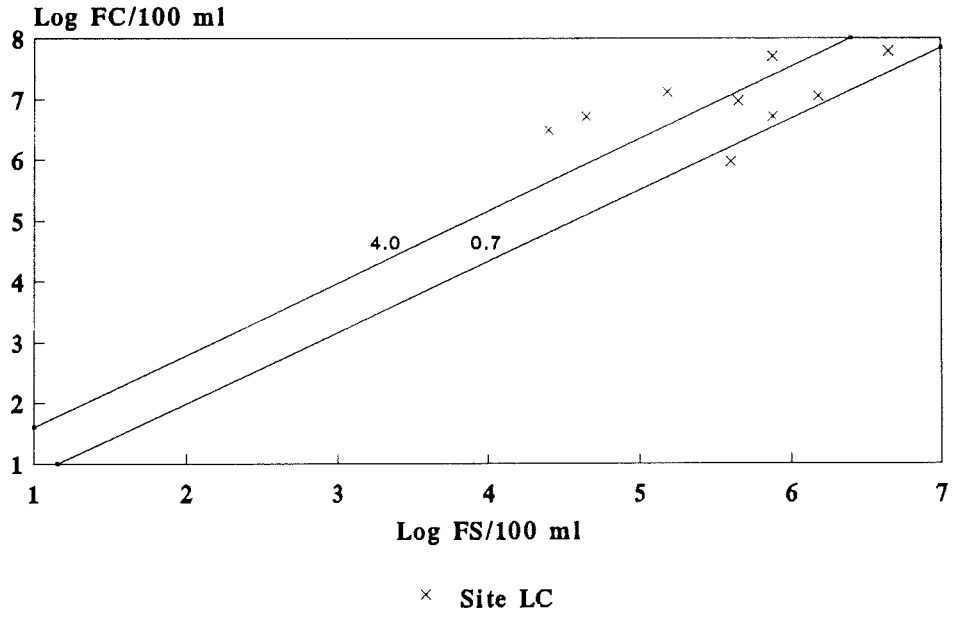


Figure 4.7: FC/FS ratios in DWF sanitary wastewater at site LC.

Correlation coefficients for total coliform, faecal coliform, faecal streptococcus and *P. aeruginosa* levels observed in the North London sampling sites are summarized in Table 4.8. Significant correlations between total and faecal coliforms were found for all sites. This is presumably due to the fact that the decrease of concentrations of total coliform

and faecal coliform is nearly paralleled in sewage and faeces. No significant correlations were obtained between faecal coliforms and faecal streptococci for sites LA, LB, LC, LD, LF and LG. This may be due to the fact that the origins of faecal coliforms and faecal streptococci were from different sources in the combined sewage. At site LE, there are significant relationships between *P. aeruginosa* and indicator bacteria. At site LC, the correlation coefficients between *P. aeruginosa* and indicator bacteria were 0.710 ($p < 0.05$) for faecal coliforms and 0.960 ($p < 0.001$) for faecal streptococci. The correlation coefficient between *P. aeruginosa* and total coliform was 0.758 ($p < 0.05$) and 0.862 ($p < 0.01$) between *P. aeruginosa* and faecal coliform at site LB. No correlations between *P. aeruginosa* and indicators were found at sites LA, LD and LG.

Physico - chemical observations, summarized in Tables 4.9 and 4.10, reflect the type and sources of effluent and receiving water runoff. Generally, the temperatures are at a maximum during early winter (October) with minimums occurring in mid-winter (December - January). pH values obtained tended to fluctuate considerably over the study period. However, the pH values were relatively stable at site LC probably due to its below ground location, which is less affected by atmospheric environmental factors. There were notable changes at site LG, where the values ranged between 5.8 to 7.1, reflecting the effects of variable types of sewage effluent.

The changes in pH values recorded at the combined sewer sites LA, LB, LD and LE were effected by many factors, but mainly by prevailing environmental and sanitary conditions. At site LD, for example, there were strong detergent smells and visible bubbles. There are possibly from car washing or other surfactant sources which have a inevitable impact on pH. Flow data are presented in Table 4.10. Two typical winter rainfall events occurred during the study period. The first rainy day was on 26/11/90 with rainfall of 8.6mm and the second on 9/1/91 with a rainfall total of 16.3mm.

Table 4.8: Correlation coefficients between bacteria for all sampling sites in North London

Site		TC	FC	FS
LA	FC	0.985[a]		
	FS	-0.300	-0.291	
	PA	0.561	0.546	-0.342
LB	FC	0.789[c]		
	FS	-0.160	0.208	
	PA	0.758[c]	0.862[b]	-0.197
LC	FC	0.915[a]		
	FS	0.482	0.525	
	PA	0.651	0.710c[c]	0.960[a]
LD	FC	0.817[b]		
	FS	0.633	0.598	
	PA	0.471	0.476	0.394
LE	FC	0.984[a]		
	FS	0.807[b]	0.731[c]	
	PA	0.909[a]	0.902[a]	0.767[c]
LF	FC	0.854[b]		
	FS	0.396	0.604	
	PA	- *	- *	- *
LG	FC	0.886[b]		
	FS	0.048	0.071	
	PA	-0.059	0.062	0.117

TC: Total coliform. FC: faecal coliform. FS: Faecal streptococcus.

PA: *Pasedumonas aeruginosa.* **a: $p < 0.001$. b: $p < 0.01$. c: $p < 0.05$.**

*: *Pasedumonas aeruginosa* recovered on only 2 occasions thus no correlation coefficient calculated

Table 4.9: The pH and temperature data from the sampling sites in North London.

Date	Sampling sites													
	LA		LB		LC		LD		LE		LF		LG	
	pH	T°C	pH	T°C	pH	T°C	pH	T°C	pH	T°C	pH	T°C	pH	T°C
3/10/90	6.0	14.0	6.0	15.5	6.8	17.0	5.5	15.0	6.4	15.0	6.7	10.5	7.1	14.0
24/10	6.5	14.5	6.4	14.0	6.8	19.0	6.4	15.0	5.8	14.0	6.7	11.5	6.2	10.5
29/10	6.0	12.5	6.5	15.0	7.0	17.0	6.0	12.5	6.7	14.0	6.7	9.5	6.7	0.0
26/11	6.4	10.0	6.5	11.0	7.0	15.0	6.1	10.0	6.4	12.0	6.7	7.0	5.8	9.0
4/12	6.4	10.0	6.4	12.0	6.8	15.0	6.1	10.0	6.4	12.0	7.0	6.0	6.5	9.0
17/12	6.5	8.0	6.4	9.0	6.8	15.0	6.0	9.0	5.8	9.0	7.0	4.0	6.7	6.5
9/1/91	5.8	7.0	6.4	6.0	6.8	13.0	6.3	7.0	6.2	9.0	7.0	2.0	6.5	6.0
21/1/91	6.5	10.5	5.9	10.0	7.1	15.5	6.1	8.5	5.9	10.0	6.5	6.5	5.8	7.0
MAX	6.5	14.5	6.5	15.5	7.1	19.0	6.4	15.0	6.7	15.0	7.0	11.5	7.1	14.0
MIN	5.8	7.0	5.9	6.0	6.8	13.0	5.5	7.0	5.8	9.0	6.5	2.0	5.8	6.0
AM	6.2	11.05	6.3	12.06	7.1	15.9	6.0	11.27	6.2	12.7	6.8	7.3	6.4	8.7

MAX: Maximum MIN: Minimum AM: Arithmetic mean

Table 4.10: Flow data from sampling sites in North London (m^3/s)

Date	Sampling sites						
	LA	LB	LC	LD	LE	LF	LG
3/10/90	0.029	-	0.190	0.002	0.002	0.001	0.262
24/10/90	0.029	-	0.103	0.022	0.002	0.002	0.034
29/10/90	0.029	-	0.095	0.018	0.002	0.001	0.028
26/11/90*	0.044	-	0.229	0.033	0.003	0.002	0.853
4/12/90	0.023	-	0.123	0.009	0.002	0.001	0.169
17/12/90	0.016	-	0.124	0.010	0.002	0.001	0.159
9/1/91*	0.540	-	0.284	0.130	0.007	0.004	0.345
21/1/91	0.037	-	0.184	0.033	0.002	0.001	0.026
MAX	0.539	-	0.284	0.130	0.007	0.004	0.853
MIN	0.012	-	0.090	0.002	0.002	0.001	0.026
AM	0.084	-	0.158	0.029	0.003	0.001	0.223

MAX: Maximum MIN: Minimum AM: Arithmetic mean *: Rainfall.

4.2.2. The Levels of Indicator Microorganisms and Pathogens in Combined Sewer and Receiving Stream Sediments in North London.

The data in Table 4.11 show geometric mean densities of selected indicator microorganisms and pathogenic bacteria for sediment samples obtained at combined sewage sites LE and LG. The comparative ratios of the geometric mean of indicator microorganisms and pathogenic bacteria between water and sediment column (sediment / water) are also included. At site LE, levels of bacteria found in sediment were generally much lower than those recovered in sewage. Sediment / water ratios of total coliform, faecal coliform, faecal streptococcus and *P. aeruginosa* are 0.17, 0.16, 0.30 and 0.17 respectively. At site LG, sediment / water ratios are higher than at site LE with the ratios of total coliform, faecal coliform, faecal streptococcus and *P. aeruginosa*

Table 4.11: Levels of microorganisms recovered from sediments and sediment/water (S/W) ratios at sites LE and LG (MPN/g).

		Total	Faecal	Faecal	*Pseudomona*
LE	3/10/90	$3.0x10^5$	$3.0x10^4$	$4.5x10^2$	$4.5x10^2$
	24/10	$7.0x10^5$	$5.0x10^4$	$9.5x10^3$	$1.5x10^2$
	29/10	$5.0x10^4$	$3.0x10^3$	$9.5x10^1$	$1.2x10^2$
	26/11	$8.0x10^3$	$7.0x10^2$	9.0	$1.5x10^1$
	4/12	$6.0x10^3$	$2.3x10^2$	$2.5x10^1$	$4.5x10^1$
	17/12	$3.0x10^3$	$8.0x10^2$	$7.5x10^2$	$4.5x10^1$
	9/1/91	$5.0x10^3$	$2.3x10^3$	$9.5x10^2$	$4.5x10^1$
	21/1	$8.0x10^3$	$3.0x10^3$	2.5	$4.5x10^1$
	GM	$2.2x10^4$	$2.8x10^3$	$1.4x10^3$	$6.9x10^1$
	MAX	$7.0X10^5$	$5.0X10^4$	$4.5X10^4$	$4.5X10^2$
	MIN	$5.0X10^3$	$2.3X10^2$	9.0	$1.5X10^1$
	S/W	0.17	0.16	0.30	0.17
LG	3/10/90	$5.0x10^4$	$4.5x10^4$	$3.0x10^4$	$2.3x10^2$
	24/10	$4.5x10^4$	$2.5x10^4$	$8.0x10^3$	$1.2x10^2$
	29/10	$6.5x10^4$	$2.5x10^4$	$3.0x10^3$	$2.3x10^1$
	26/11	$9.5x10^2$	$4.5x10^2$	$1.7x10^2$	$1.7x10^1$
	4/12	$1.4x10^4$	$5.0x10^3$	$4.5x10^3$	$4.5x10^3$
	17/12	$1.1x10^4$	$5.0x10^3$	$2.5x10^3$	$2.5x10^3$
	9/1/91	$5.0x10^4$	$1.3x10^4$	$1.5x10^3$	$1.5x10^3$
	21/1	$1.7x10^4$	$2.3x10^3$	$2.5x10^3$	$2.5x10^3$
	GM	$1.9x10^4$	$7.4x10^3$	$2.9x10^3$	$3.8x10^2$
	MAX	$6.5x10^4$	$4.5x10^4$	$1.5x10^4$	$4.5x10^3$
	MIN	$1.1x10^4$	$2.3x10^2$	$1.7x10^2$	$1.7x10^1$
	S/W	0.63	0.94	1.38	5.42

GM: Geometric mean. MAX: Maximum. MIN: Minimum.

being 0.63, 0.94, 1.38 and 5.42 respectively.

The levels of indicator organisms and pathogenic bacteria in different layers of sediment at site LE are presented in Fig. 4.8. The results show that as the depth of sediment increases from the top layer (L1) to the second layer (L2) and than the third layer (L3), the levels of total coliform, faecal coliform and *P. aeruginosa* are reduced gradually. Faecal streptococci, however, showed a slightly higher level in layer 2 (3.9 x 10^3 MPN/g) than in 1 (3.2 x 10^3 MPN/g), but then decreased again in layer 3.

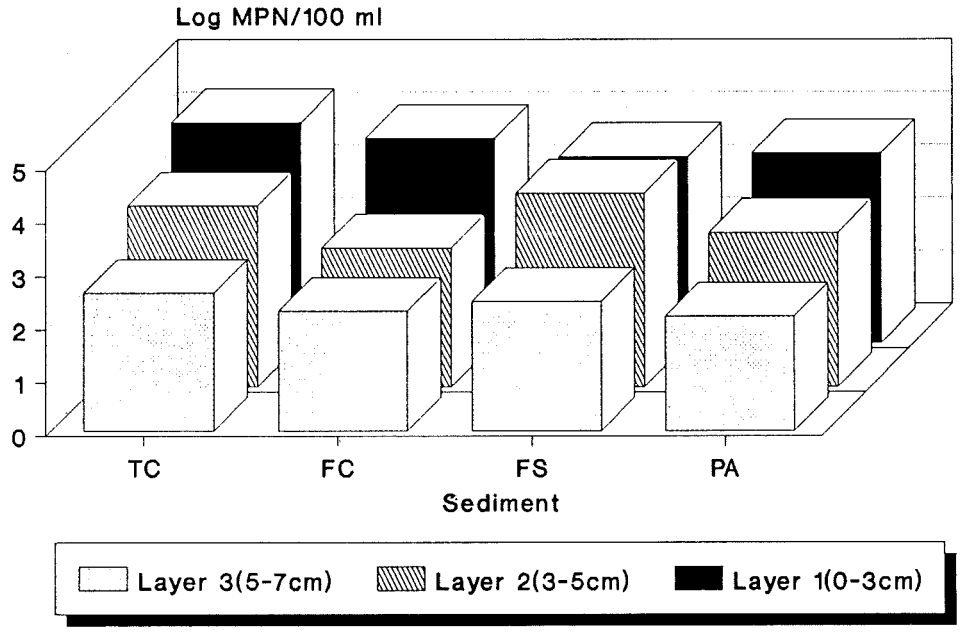


Figure 4.8: The levels of bacteria from different sediment layers at site LE.

4.2.3. The Levels of Indicator Microorganisms, Pathogens and Physico - Chemical Parameters in Combined Sewage and Beach Outfall in Valencia.

The occurrence of selected microorganisms at combined sewage sites VH (DWF sanitary wastewater), VI and beach outfall VJ in Valencia are summarised in Tables 4.12, 4.13 and 4.14. They provide an indication of the relative microbial quality. One rain day sample is also included in Table 12 in order to compare different weather conditions.

Table 4.12: Levels of microorganisms recovered at site VH.

Date	Total coliforms	Faecal coliforms	Faecal streptococci	*Pseudomonas aeruginosa*	*Salmonella*
22/3/9	$7.9x10^7$	$3.3x10^7$	$9.3x10^5$	$2.3x10^5$	+
23/3	$2.9x10^6$	$3.3x10^6$	$4.3x10^5$	$4.3x10^3$	+
10/4	$3.0x10^7$	$2.3x10^6$	$9.3x10^6$	$9.3x10^4$	+
15/4	$8.0x10^7$	$3.5x10^7$	$4.3x10^4$	$1.5x10^5$	+
30/4	$7.9x10^7$	$7.9x10^6$	$4.3x10^4$	$2.1x10^4$	+
6/5	$7.9x10^7$	$7.9x10^6$	$2.3x10^4$	$2.3x10^5$	NT
7/5	$4.9x10^7$	$4.9x10^7$	$2.3x10^5$	$2.1x10^4$	+
13/5	$3.3x10^8$	$2.3x10^8$	$2.3x10^5$	$2.3x10^4$	+
14/5	$7.9x10^7$	$1.8x10^7$	$7.5x10^3$	$2.5x10^3$	+
20/5	$1.1x10^8$	$3.5x10^7$	$2.3x10^3$	$9.3x10^4$	+
27/5	$3.6x10^7$	$1.3x10^6$	$4.3x10^5$	$1.5x10^5$	+
11/6	$3.3x10^7$	$7.9x10^6$	$4.3x10^5$	$4.3x10^4$	+
MAX	$3.3x10^8$	$2.3x10^8$	$9.3x10^6$	$2.3x10^5$	
MIN	$2.9x10^6$	$2.3x10^6$	$2.3x10^3$	$2.5x10^3$	
GM	$5.4x10^7$	$1.4x10^7$	$1.3x10^5$	$4.4x10^4$	

MAX: Maximum MIN: Minimum GM: Geometric mean NT: Not tested

Table 4.13: Levels of microorganisms recovered at site VI.

Date	Total coliforms	Faecal coliforms	Faecal streptococci	Pseudomonas aeruginosa	Salmonell
22/3/91	$4.9x10^7$	$1.1x10^7$	$1.5x10^5$	$2.4x10^4$	+
23/3*	$3.3x10^7$	$7.0x10^6$	$9.3x10^4$	$4.3x10^3$	+
10/4	$1.3x10^6$	$1.3x10^6$	$2.4x10^4$	$9.3x10^3$	+
15/4	$5.0x10^7$	$2.5x10^7$	$4.3x10^4$	$2.3x10^4$	+
30/4	$7.9x10^6$	$2.3x10^6$	$4.3x10^5$	$1.5x10^4$	+
7/5	$5.0x10^6$	$2.5x10^6$	$2.3x10^5$	$1.5x10^4$	+
13/5	$3.3x10^6$	$1.3x10^6$	$2.3x10^5$	$4.3x10^4$	+
14/5	$1.7x10^7$	$4.9x10^6$	$2.1x10^4$	$4.3x10^4$	+
20/5	$3.5x10^7$	$8.0x10^6$	$2.3x10^5$	$1.1x10^4$	+
27/5	$7.9x10^7$	$1.3x10^6$	$2.3x10^4$	$9.3x10^4$	+
11/6	$7.9x10^7$	$2.2x10^6$	$2.3x10^5$	$9.3x10^4$	+
MAX	$7.9x10^7$	$2.5x10^7$	$4.3x10^5$	$9.3x10^4$	
MIN	$3.3x10^6$	$1.3x10^6$	$2.1x10^4$	$4.3x10^3$	
GM	$1.7x10^7$	$3.3x10^6$	$9.8x10^4$	$1.8x10^4$	

MAX: Maximum MIN: Minimum GM: Geometric mean. *: rainfall day.

Table 4.14: Levels of microorganisms recovered at site VJ.

Date	Total coliforms	Faecal coliforms	Faecal streptococci	Pseudomonas aeruginosa	Salmonella
22/3	$8.0x10^5$	$1.7x10^5$	$1.5x10^5$	$4.3x10^3$	+
10/4	$8.0x10^5$	$2.5x10^5$	$4.3x10^5$	$4.3x10^3$	+
15/4	$7.9x10^3$	$4.9x10^3$	$2.3x10^2$	$2.3x10^2$	-
20/5	$1.3x10^6$	$2.3x10^5$	$2.3x10^3$	$4.3x10^3$	+
27/5	$1.7x10^6$	$1.3x10^6$	$4.3x10^4$	$4.3x10^3$	+
11/6	$7.9x10^5$	$3.3x10^5$	$4.3x10^4$	$1.5x10^3$	+
MA	$1.7x10^6$	$1.3x10^6$	$4.3x10^5$	$4.3x10^3$	
MIN	$7.9x10^3$	$4.9x10^3$	$2.3x10^2$	$2.3x10^2$	
GM	$4.5x10^5$	$1.7x10^5$	$2.0x10^4$	$2.2x10^3$	

MAX: Maximum MIN: Minimum GM: Geometric mean

Generally, densities of indicator microorganisms and pathogenic bacteria found at site VH were higher than site VI which in turn is higher than site VJ (Fig. 4.9). The geometric mean of total coliform isolate was 5.4 x 10^7 MPN/100 ml at site VH and 1.7 x 10^7 MPN/100 ml at site VI; similarly, the geometric mean of faecal coliforms was 1.4 x10^7 MPN/100 ml at site VH and 3.3 x 10^6 MPN/100 ml at site VI. Levels of faecal streptococci recovered at sites VH and VI were in the ranges of 1.3 x 10^5 and 9.8 x 10^4 MPN/100 ml respectively. *P. aeruginosa* levels of 4.4 x 10^4 MPN/100 ml and 1.8 x 10^4 MPN/100 ml were recovered at sites VH and VI respectively. However, the levels of organisms recovered at site VJ were lower than those at sites VH and VI. The levels of total coliforms and faecal coliforms at site VJ were in the range of 4.5 x 10^5 MPN/100 ml and 1.7 x 10^5 MPN/100 ml, faecal streptococci were found at level of 2.0 x 10^4 MPN/100 ml and *P. aeruginosa* at the level of 2.2 x 10^3 MPN/100 ml. The geometric mean of organisms for all sites showed the following order: total coliform > faecal coliform > faecal streptococcus > *P.aeruginosa* for all sites (Fig. 4.9). *Salmonella* was recovered from all samples at sites VH and VI and with an 83 %

recovery from the samples at the beach site (VJ).

The physico - chemical characters of the various sewage effluent are shown in Table 4.15. During the sampling periods, mean water temperature was 19.5°C, 19.5°C and 18.2°C at sites VH, VI and VJ respectively. Generally, there were gradual increases from 19°C to 24° at sites VH and VI during the study except on the rainy day (23/3). At site VJ, temperatures ranged from 15.0°C to 23.0°C.

pH values of the samples obtained were relatively stable for all sites. The mean of pH was 7.3, 7.1 and 7.2 for sites VH, VI and VJ respectively. BOD_5 concentration at sites VH, VI and VJ show a noticeable parallel with levels of bacteria. The flow changed significantly on the rainy day (23/3/91) when rainfall of 9.5mm was recorded (Table 4.16).

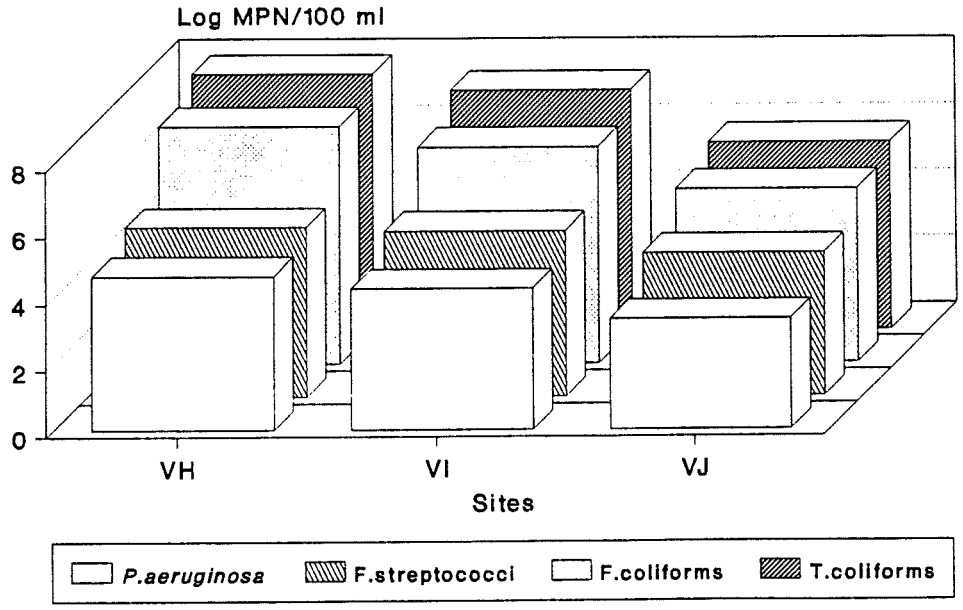


Figure 4.9: Geometric mean of bacteria recover at sites VH, VI and VJ.

The logarithm of faecal coliform levels has been plotted against the logarithm of faecal streptococci levels for sites VH, VI and VJ (Fig. 4.10). The samples from sites VH and VI all had FC/FS ratios of greater than 7.0. At site VJ, most of samples are greater

than 4.0. 80% of samples had a ratio of more than 4.0 at site VH and some 60% of samples had a ratio of more than 4.0 at site VI.

Table 4.15: pH and temperature data at sites VH, VI and VJ.

Date	Sampling sites					
	VH		VI		VJ	
	pH	T°C	pH	T°C	pH	T°C
22/3/91	7.0	19.0	6.9	19.0	7.2	18
23/3*	7.1	16.0	7.0	16.0		#
10/4	7.1	19.0	7.0	19.0	7.0	16
15/4	7.0	18.0	7.0	18.0	7.0	15
30/4	7.0	18.0	7.0	18.0	#	#
6/5	7.7	19.0	#	#	#	#
7/5	7.8	19.0	7.6	19.0	#	#
13/5	7.3	20.0	7.0	19.0	#	#
14/5	7.6	20.0	7.0	20.0	#	#
20/5	7.4	20.0	7.2	20.0	7.4	18
27/5	7.2	24.0	7.1	24.0	7.1	23
11/6	7.0	22.0	7.1	22.0	7.3	19
MAX	7.8	24.0	7.6	24.0	7.4	23
MIN	7.0	16.0	6.9	16.0	7.0	15
AM	7.3	19.5	7.1	19.5	7.2	18.2

AM: Arithmetic mean MAX: Maximum MIN: Minimum. *: rainfall day.

#: Not tested.

Table 4.16: DO, BOD_5 and flow (M^3/s) data at sites VH, VI and VJ.

Date	Sampling sites								
	VH			VI			VJ		
	DO	BOD_5	Flow	DO	BOD_5	Flow	DO	BOD_5	Flow
22/3/	1.29	488.7	0.02	2.04	377.9	0.15	7.1	248.9	2.5
23/3*	2.8	317.2	0.14	4.4	265.6	0.53	#	#	#
10/4	2.7	497.3	0.03	4.75	265.3	0.24	6.8	283.2	1.65
15/4	6.3	483.7	0.005	4.5	435.5	0.38	6.4	249.6	4.8
30/4	2.4	437.6	0.01	6.8	353.2	0.13	#	#	#
6/5	5.0	470	0.03	#	#	#	#	#	#
7/5	3.5	416.5	0.04	6.5	333.5	0.13	#	#	#
13/5	2.0	558.0	0.036	2.4	197.6	0.142	#	#	#
14/5	2.4	387.6	0.054	7.5	272.5	0.173	#	#	#
20/5	2.1	387.9	0.045	6.2	333.8	0.14	6.8	189.2	2.6
27/5	2.2	517.8	0.014	6.0	354.0	0.15	6.2	153.8	3.18
11/6	1.9	498.1	0.026	2.8	237.2	0.312	6.8	213.2	1.047
MAX	6.3	558.0	0.14	6.8	435.5	0.53	7.1	283.2	4.8
MIN	1.29	317.2	0.005	2.04	197.6	0.13	6.2	153.8	1.047
AM	2.88	455.0	0.037	4.89	311.5	0.225	6.68	222.9	2.63

BOD_5: Biochemical oxygen demand. DO: Dissolve Oxygen. *: rainfall day.
#: Not tested.

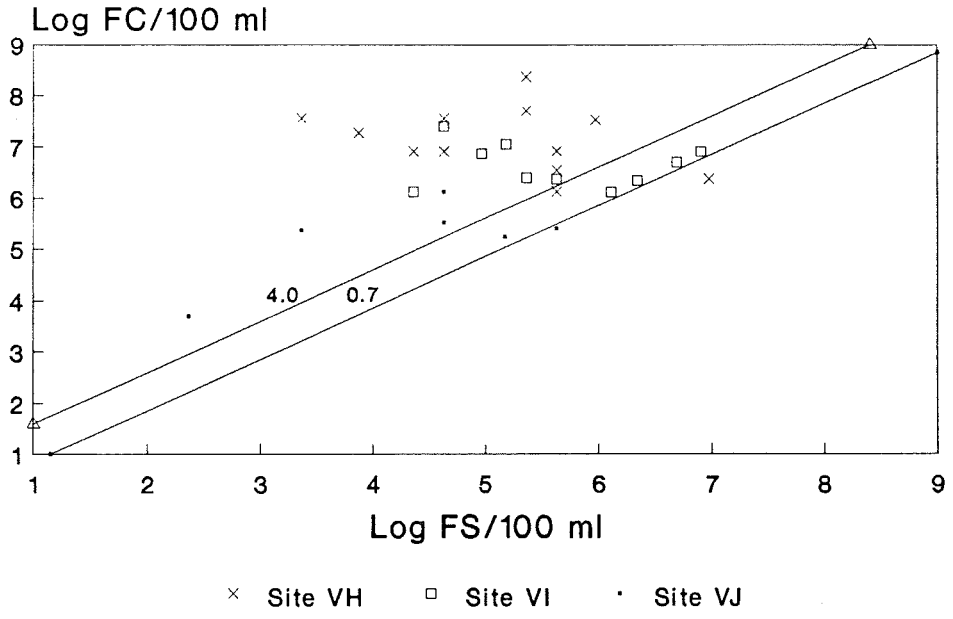


Figure 4.10: FC/FS ratios at sites VH, VI and VL.

The correlation coefficients between levels of indicators microorganisms and *P. aeruginosa* are summarized in Table 4.17. The correlation between total coliforms and faecal coliforms is significant at the levels of 0.001, 0.01 and 0.05 as considered for sites VH, VJ and VI respectively. No significant correlations were found between faecal streptococci and faecal coliforms at the levels of 0.05 for any site. The correlation between *P. aeruginosa* and indicators were 0.9452 ($p < 0.01$) for total coliforms and 0.8664 ($P < 0.05$) for faecal coliforms at site VJ. The correlation coefficient for *P. aeruginosa* and faecal streptococci at site VI was 0.6492; this is significant at the 0.05 level. No such correlations were found at site VH.

Table 4.17: Correlation coefficients between bacteria at sites VH, VI and VJ.

Site		TC	FC	FS
VH	FC	0.797[a]		
	FS	-0.412	-0.349	
	PA	0.256	0.033	0.162
VI	FC	0.643[c]		
	FS	-0.267	-0.336	
	PA	-0.125	-0.284	0.649[c]
VJ	FC	0.956[b]		
	FS	0.718	0.704	
	PA	0.945[b]	0.866[c]	0.736

TC: Total coliforms. FC: Faecal coliforms. FS: Faecal streptococci

PA: *P. aeruginosa* **a: $p < 0.001$. b: $p < 0.01$. c: $p < 0.05$.**

4.2.4. The Levels of Indicator Microorganisms and Pathogens in Combined Sewer and Beach Outfall Sediment in Valencia.

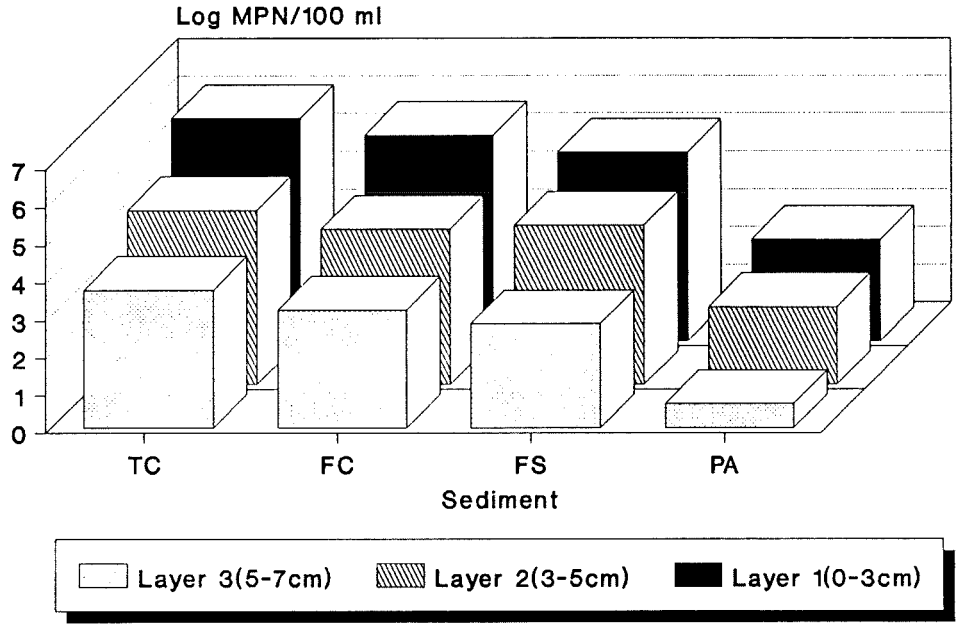
The data presented in Table 4.18 show the geometric mean of bacteria recovered from sediment samples at sites VH and VJ. The comparative ratios of the geometric mean of indicator microorganisms and pathogenic bacteria between water and sediment column (sediment / water) are also included. The levels of bacteria found in sediment at site VH were higher than those recovered from site VJ. The sediment / water ratios of total coliforms, faecal coliforms, faecal streptococci and *P. aeruginosa* were 0.0015, 0.0025, 0.35 and 0.012 respectively at site VH. However, the ratios at site VJ were higher than site VH, being 0.03, 0.024, 0.42 and 0.078 respectively.

Table 4.18: Levels of microorganisms recovered from sediments and sediment/water ratios at sites VH and VJ.

Site		Total coliforms	Faecal coliforms	Faecal streptococci	*Pseudomonas aeruginosa*
VH	22/3	$1.1x10^6$	$4.9x10^5$	$2.3x10^5$	$2.3x10^3$
	23/3	$7.9x10^5$	$3.3x10^5$	$2.3x10^4$	$9.5x10^2$
	10/4	$1.7x10^6$	$1.7x10^6$	$2.3x10^5$	$9.3x10^1$
	15/4	$8.0x10^4$	$3.5x10^4$	$2.3x10^3$	$2.3x10^2$
	30/4	$4.9x10^5$	$2.3x10^5$	$2.3x10^5$	$2.3x10^2$
	6/5	$4.9x10^5$	$1.3x10^5$	$1.5x10^5$	$2.3x10^3$
	13/5	$2.2x10^6$	$2.3x10^5$	$9.3x10^4$	$1.5x10^2$
	27/5	$2.3x10^5$	$7.0x10^4$	$2.3x10^4$	$4.3x10^2$
	11/6	$2.3x10^5$	$4.9x10^4$	$4.3x10^3$	$2.1x10^3$
	GM	$5.3x10^5$	$1.8x10^5$	$4.6x10^4$	$5.3x10^2$
	MAX	$2.2X10^6$	$1.7X10^6$	$2.3X10^4$	$2.3X10^3$
	MIN	$8.0X10^4$	$3.5X10^4$	$2.3x10^3$	$4.3X10^1$
	S/W	0.0015	0.0025	0.35	0.012
VJ	10/4	$1.7x10^4$	$5.0x10^3$	$9.5x10^5$	$3.9x10^3$
	15/4	$4.9x10^2$	$2.3x10^2$	$2.3x10^2$	$2.3x10^2$
	20/5	$5.0x10^3$	$1.7x10^3$	$9.3x10^3$	$4.3x10^1$
	27/5	$3.3x10^5$	$7.0x10^4$	$9.3x10^3$	$4.3x10^1$
	11/6	$1.7x10^4$	$7.9x10^3$	$2.3x10^3$	$9.3x10^1$
	GM	$1.2x10^4$	$4.1x10^3$	$8.5x10^3$	$1.7x10^2$
	MAX	$3.3x10^5$	$7.9x10^4$	$9.5x10^5$	$3.9x10^3$
	MIN	$4.9x10^2$	$2.3x10^2$	$9.3x10^1$	$4.3x10^1$
	S/W	0.03	0.024	0.42	0.078

GM: Geometric mean. MAX: Maximum. MIN: Minimum. S/W: Sediment / Water.

The trends by which the levels of bacteria decrease from the top layer to the bottom layer at site VH are showed in Fig. 4.11. Generally, the levels of bacteria in the bottom layer (3) were 100 fold lower than the top layer (1). *P. aeruginosa* presented a sharper decrease from top (4.9 x 10^2 MPN/g) to the bottom layer (4.9 MPN/g) than other bacterial indicators. The results demonstrated that indicator and pathogenic bacteria occur in the highest concentration within the upper layers of sediment; this may of course be resuspended during turbulence flow phases.



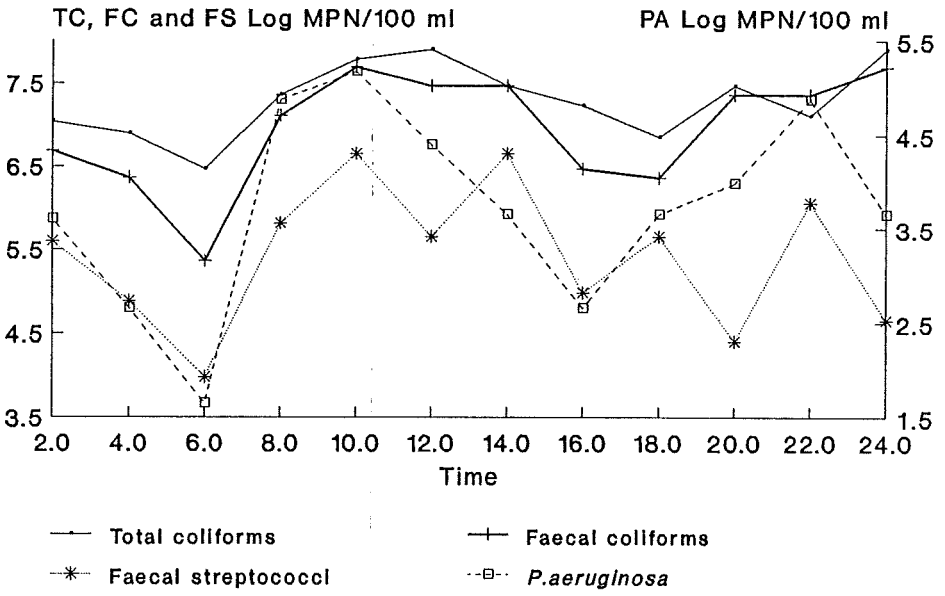
TC:Total coliforms FC:Faecal coliforms
FS:Faecal streptococci PA:P.*aeruginosa*

Figure 4.11: Geometric mean of bacteria from different sediment layer at site VH.

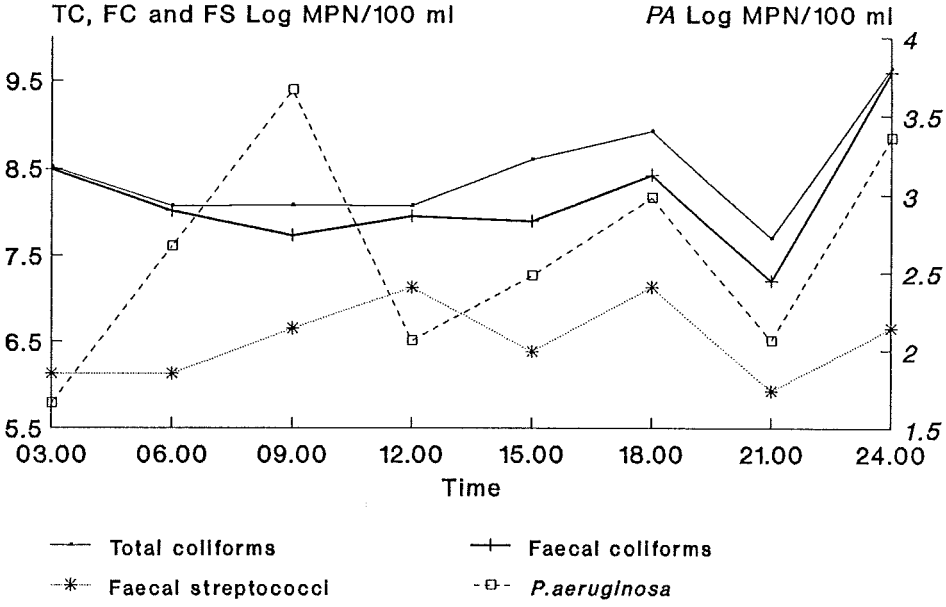
4.2.5. Diurnal Bacterial Patterns

Daily cycle tests for total coliforms, faecal coliforms, faecal streptococci and *P. aeruginosa* were conducted in sanitary wastewater in both North London and Valencia. Fig.4.12 a and b show the levels of indicator organisms recovered in samples taken continuously over 24 hours at sites LC (North London) and VH (Valencia).

At site LC in North London, the lowest levels of were recovered during the early



a: Site LC



b: Site VH

Figure 4.12: Diurnal bacterial pattern at sites LC and VH

morning (06.00). At this time levels of total coliforms were 3 x 10^6 MPN/100 ml, faecal coliforms were 2.3 x 10^5 MPN/100 ml, faecal streptococci were 9.5 x 10^3 MPN/100 ml and *P. aeruginosa* 4.5 x 10 MPN/100 ml. A sharp rise in the number of bacteria occurs around 06.00 - 08.00 hours with peak recovery of faecal coliforms (5.0 x 10^7 MPN/100 ml), faecal streptococci (4.5 x 10^6 MPN/100 ml) and *P. aeruginosa* (1.5 x 10^5 MPN/100 ml) at 10.00 hours. The levels of total coliform increased at the same time but peak values were obtained at about 12.00 hours (8.0 x 10^7 MPN/100 ml). Levels of total coliform and faecal coliform remain fairly stable for the 24h period, but levels of faecal streptococci and *P. aeruginosa* fall gradually after about 10.00 - 12.00 hours, than rise to a peak again at 22.00. The time of peak recovery in North London differed from that recorded in Valencia. At site VH, *P. aeruginosa* was at a maximum in the morning (09.00) at a level of 2.3 x 10^3 MPN/100 ml which was similar to the peak time in London. The highest levels of total coliform (3.3 x 10^9 MPN/100 ml) and faecal coliform (3.3 x 10^8 MPN/100 ml) recovered at site VH were at 24.00 hours. There was a smaller peak for total coliforms (7.9 x 10^8 MPN/100 ml), faecal coliforms (2.3 x 10^8 MPN/100 ml), faecal streptococci (9.3 x 10^6 MPN/100 ml) and *P. aeruginosa* (9.3 x 10^2 MPN/100 ml) at 18.00 hours.

4.3. Discussion

4.3.1. Occurrence and Levels of Indicator Microorganisms and Pathogenic Bacteria in Urban Sewage Discharges

The results of this study showed that the water quality of the urban stream, combined sewage, DWF sanitary wastewater and beach outfall was uniformly poor. The populations of indicators and pathogenic bacteria recovered were generally high. In North London, geometric means of indicator microorganisms and pathogenic bacteria found in DWF sanitary wastewater were generally about 100 - fold higher than in combined sewage. Total coliforms, faecal coliforms and faecal streptococci in combined sewage were about 10 - fold higher than in the receiving stream water with *P. aeruginosa* being some 100 - fold higher. The levels of indicator organisms and *P.*

aeruginosa, at the lower reaches of stream site LG were 4 - fold higher than found at the head of stream site LF, with *P. aeruginosa* being 29 - fold higher than site LF. The levels of *P. aeruginosa* were generally lower than levels of indicator microorganisms for all sites.

In Valencia, discharges from combined sewer (VI) contained significant qualities of total coliforms, faecal coliforms, faecal streptococci and *P. aeruginosa* with *Salmonella* being frequently isolated. The geometric mean densities of microorganisms in combined sewage (VI) approached the levels found in DWF sanitary wastewater (VH). The relatively low levels of organisms found at site VJ are likely to be due to natural die-off during self - purification in sewage. Generally, the water quality from a microbial point of view was still very poor at the beach outfall site VJ. High density of *P. aeruginosa* is hazardous to swimmers, having been associated with ear infections, primarily otitis externa (Favero *et al.*, 1964; Jones 1965; Cabelli *et al.*, 1979, 1982; Cabelli, 1983). The levels of *P. aeruginosa* found were very high during the study at sampling sites VH, VI and VJ, which demonstrates that sewage probably represents the major source of *P. aeruginosa* found in surface water. Sampling sites VH and VI were only a short distance from the centre of the Benimaclet catchment and thus there could be less time for die-off to deplete the bacterial population, and so the recovered levels of *P. aeruginosa* remain higher than at site VJ which is a further 2 miles away from the Benimaclet catchment. The results also confirm the suggestion made by Hoadley *et al.* (1968b) that populations of *P. aeruginosa* in excess of 1000/100 ml would imply very recent contamination.

4.3.2. Relationships between Indicators and Pathogenic Bacteria

The results of the correlation analysis demonstrate that there were good correlations between total and faecal coliforms for all sampling sites in both North London and Valencia. This is possibly due to the fact that the increase of concentration of total coliform and faecal coliform was nearly paralleled in combined and receiving water. In contrast, no significant correlations were found between faecal coliforms and faecal

streptococci in most of the water samples in the two cities except site LE, which could be affected by different faecal pollution sources. The relationships between *P. aeruginosa* and indicators were not consistent in urban sewage for either North London or Valencia. There were no significant correlations between *P. aeruginosa* and indicator bacteria in DWF sanitary wastewater at site VH. A high correlation coefficient ($p < 0.001$) between *P. aeruginosa* and indicator bacteria was obtained at site LE. No significant correlations were found between *P. aeruginosa* and total coliforms, faecal coliforms and faecal streptococci at sites LA, LD, LG and VI. Bacteria die-off or survival in the water environment has been broadly attributed to a variety of physical, chemical and biological factors and processes. Thus the different correlations between *P. aeruginosa* and indicators in urban sewage discharges probably reflect the characteristics of urban sewage from different sources of pollution and under different weather and water quality conditions. Bonde (1977) relates the presence of *P. aeruginosa* in faecal polluted waters to the incidence of faecal coliforms. The larger the numbers of coliforms the more frequently *P. aeruginosa* was isolated. Vicente *et al.* (1991) also suggests that total coliforms, faecal coliforms and faecal streptococci could be considered adequate indicators of the presence and density of *P. aeruginosa* in natural waters, as these parameters showed the best correlations with *P. aeruginosa.* According to the results in this study, there were not consistently such relationships observed at all sites in both cities.

One valuable application of the faecal streptococci indicator system in stream pollution investigations has been through correlations with the faecal coliform group. Faecal streptococci are present in the faeces of warm blooded animals and in wastewater polluted with such faeces. Faecal coliform bacteria, however, are more numerous than faecal streptococci in the faeces of man. During the study, most of the sampling sites in the two cities present FC/FS ratios greater than 0.7, suggesting more a human faeces origin in the urban sewage than animal according to Geldreich (1976)'s suggestion. Sites LA (37.5%) and LD (25%) possessed a higher percentage of FC/FS ratios of less than 0.7 than sites LB and LE. It is possible that more animal faeces have their origin at those two sites than at others included in the survey.

FC/FS ratios must however be applied with care. These correlations are most meaningful when developed from bacterial densities for samples taken from combined sewage and receiving stream. Once these organisms are diffused into the sewage and receiving stream, factors of water temperature, available organic nutrients, toxic metal ions such as copper, zinc, silver, etc., unfavourable water pH below 4.0 or above 9.0, and other ecological forces may alter the inter relationships between these indicator systems during flow - time. The use of a ratio relationship for stream samples would therefore only be valid during the initial 24h travel time down stream from point of pollution discharge into the receiving stream (Geldreich 1969). It is obvious that ratios must be interpreted with care and certainly they do not provide undisputed indicators of the pollution source.

4.3.3. Characteristics of Bacterial Loads

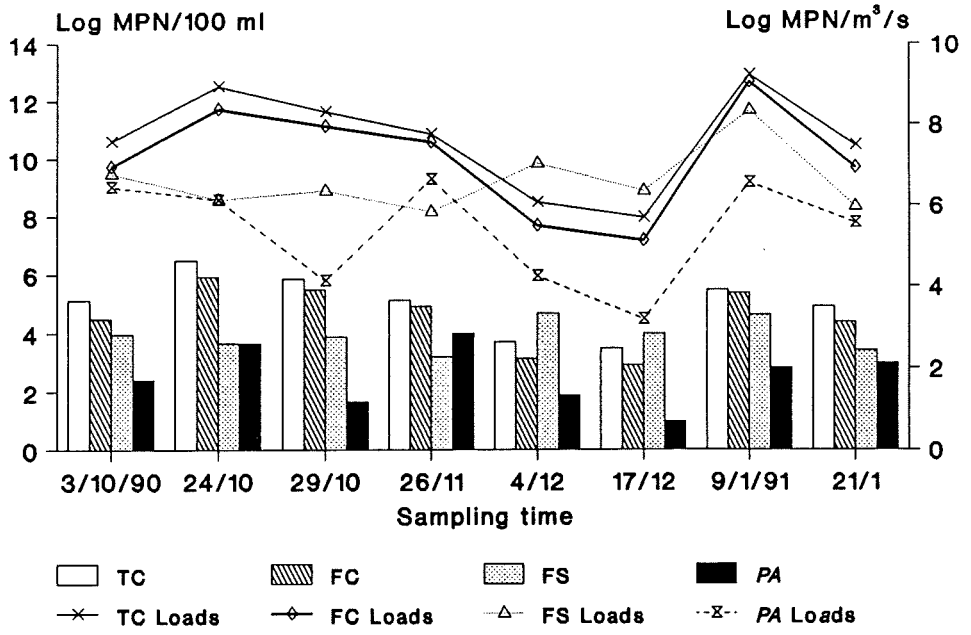
Figures 4.13 to 4.16 show the comparison between logarithm of bacterial densities (expressed as Log MPN/100 ml) and bacterial loads (Log MPN/$m^3.s^{-1}$) in order to compare the changes between bacterial levels and bacterial loads that occur between dry weather and storm events during the time of study. There were two typical winter rainfall events (26/11/90 with rainfall total of 8.6mm and an event on 9/1/91 with rainfall of 16.3mm) in North London and one rainfall event in the early summer (23/3/91 with rainfall of 9.5mm) in Valencia during the period of study.

Flow is a important factor in term of its effect on bacteria loads. At site LA, bacterial loads show sharp increases for both indicator bacteria and *P. aeruginosa* at a recorded flow of 0.54 m^3/s (Table 4.10) on the second rainfall day (9/1/91), even though bacterial levels recovered do not show any remarkable increase (Fig. 4.13a). This represents the result of a relatively high flow which was 20 - fold higher than the average DWF of 0.027 m^3/s. At sites LD, LE and LI, however, the changing trends of both bacterial loads and bacterial levels were very similar during the time of study. It is possibly due to the flow was not remarkably high during rainy days (Figs. 4.13b and 4.14). At site LC, the flow was 1.6-fold and 2-fold higher than average dry

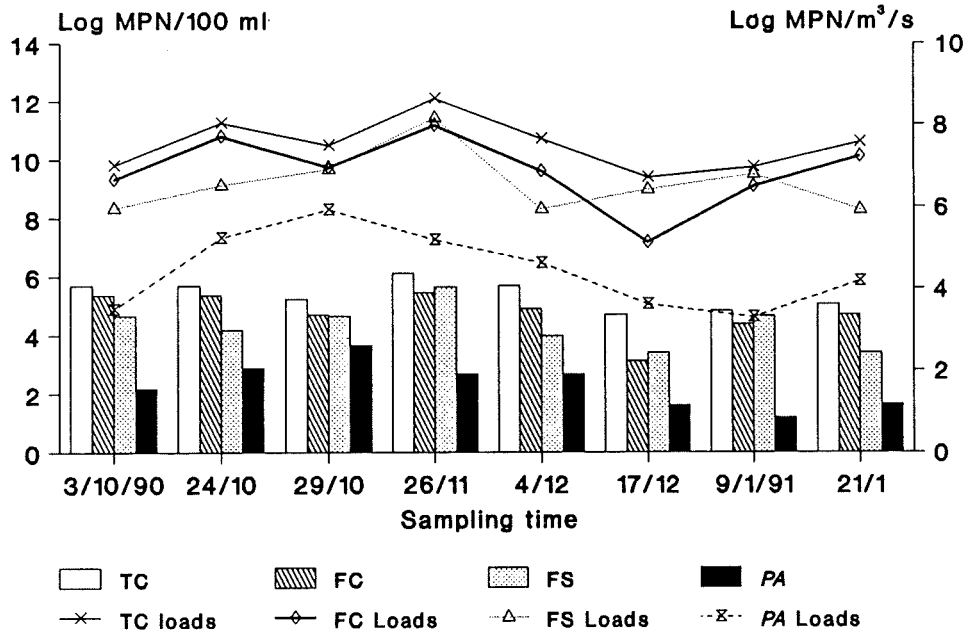
weather flow of 0.137 m^3/s on the first rainy day (26/11/90) and the second rainy day (9/1/91) respectively, but the levels of bacterial loads did not show notable changes during the rainy days. Similarly there were no significant changes of bacterial loads on the rainy day (23/3/91) at site VH, even though the rainy day flow was 5-fold higher than the average dry weather flow of 0.028 m^3/s (Fig. 4.15). These results demonstrate that bacterial loads only increase noticeably when flow is significantly higher than average dry weather flow.

Bacterial loads are also correlated with bacteria levels. At receiving stream site (LG) in London, although the highest flow of 0.853 m^3/s (Table 4.10) was recorded on the first rainfall day (26/11/90), which was 7.5-fold higher than the average DWF of 0.113 m^3/s, bacterial loads did not show any remarkable increase. On the second rainy day (9/1/91), the flow (0.345m^3/s, Table 4.10) was about 3-fold higher than the average dry weather flow of 0.113 m^3/s, but the levels of all indicator microorganisms and *P. aeruginosa* loads do show notable increases. Comparison of bacterial levels between the two rainfall days, show that bacterial levels of total coliforms and faecal coliforms were about 2 log scales higher on the second rainy day (9/1/91) than the first (26/11/90). Faecal streptococci and *P. aeruginosa* were also one log scale higher on the second rainy day than the first (Fig. 4.16a and b). It is possible the second rainy day sample taken was on the peak of bacterial flush, because higher levels of bacterial were also recorded on the second rainy day than the first. Bacterial level is correlated with both flow and flush peak, thus sampling time is a very important factor during the rainy day. Ellis (1985b) and Jefferies *et al.* (1989) have suggested that the peak microbial concentrations are often delayed behind the peak flow. Jacobs and Ellis (1991) have pointed out that only 36% of the total bacterial mass may be discharged during the first 65% of mass flow volume

Past research has demonstrated that the levels of indicator bacteria in stream water tend to increase during individual storm events (Matson *et al.*, 1978; McDonald and Kay, 1981; Hunter and McMonald, 1991). This has been explained either by the enhanced input of bacteria to stream water from a surrounding land store caused by the generation of storm runoff (Davis *et al.*, 1977) or by the wash-out of bacteria existing in stream

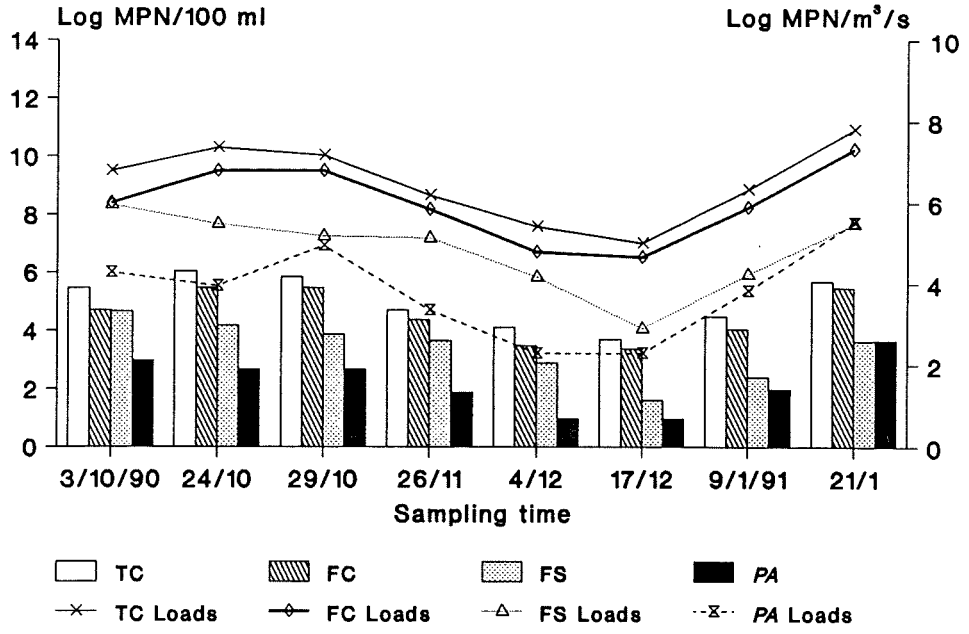


a: site LA

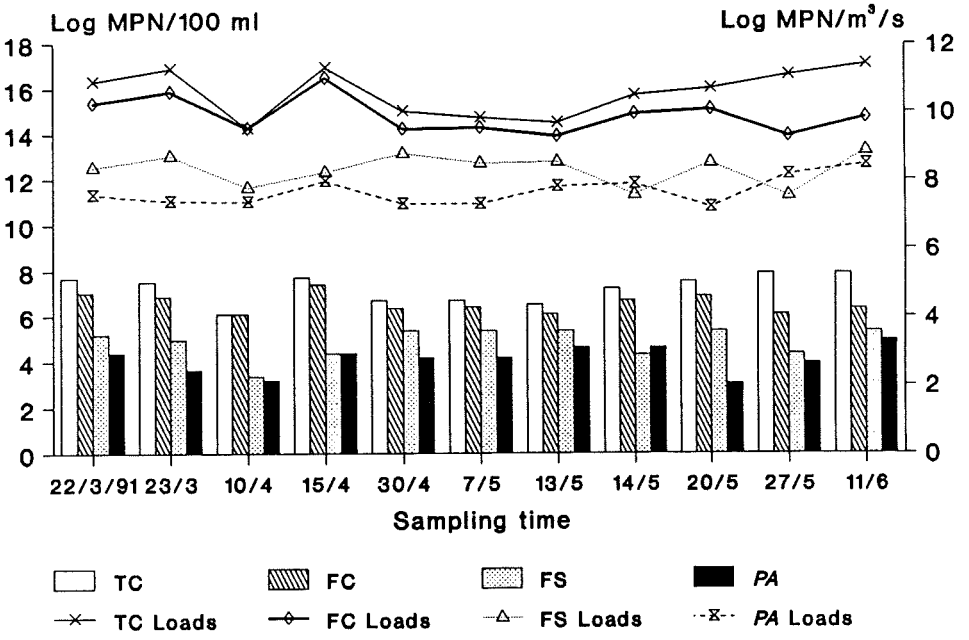


b: site LD

Figure 4.13 a and b: Relationships between the Levels of Bacteria and Loads at Sites LA and LD

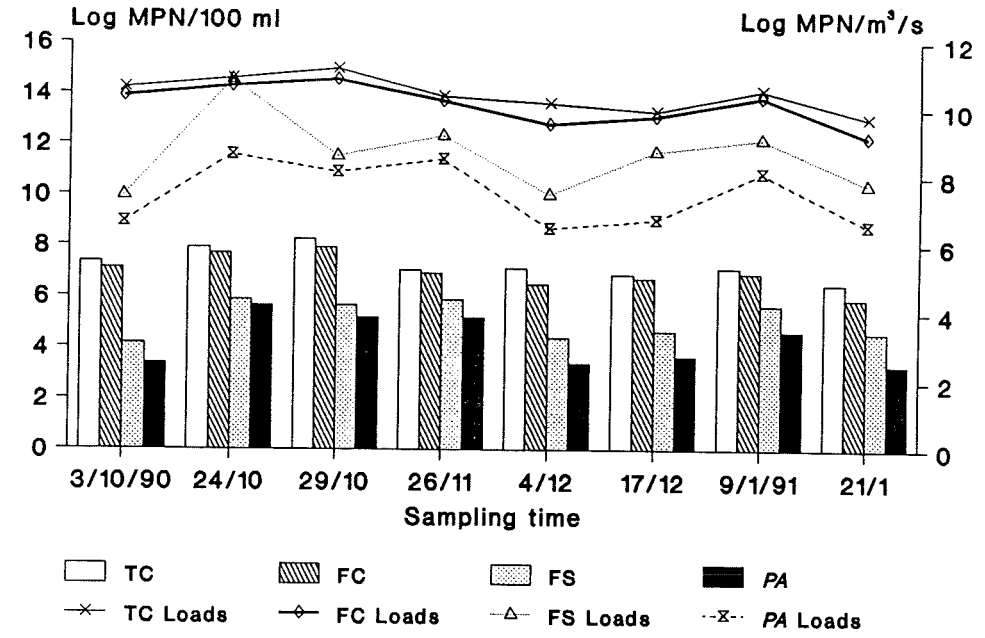


a: site LE

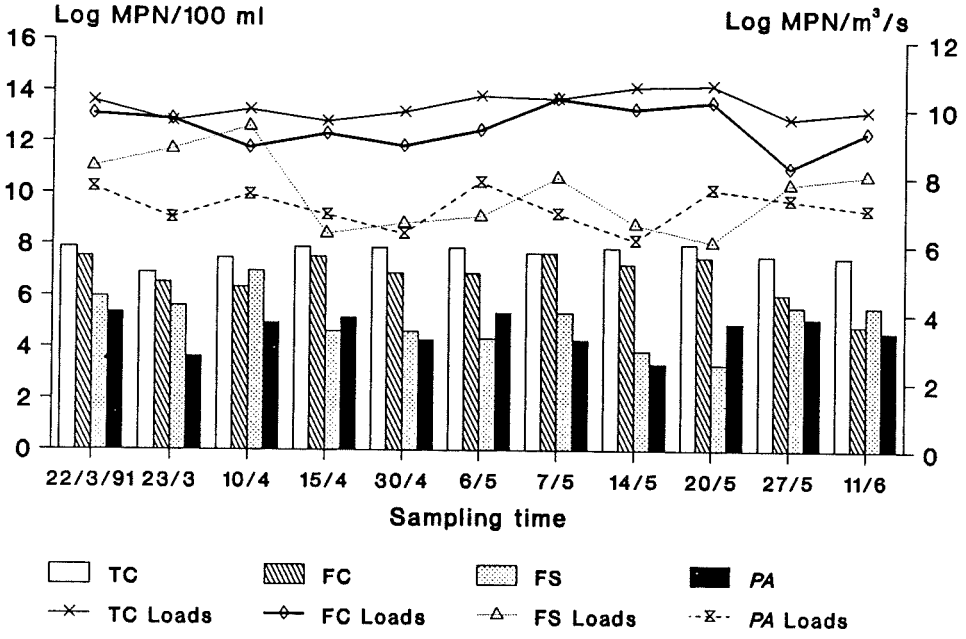


b: site VI

Figure 4.14 a and b: Relationships between the Levels of Bacteria and Loads at Sites LE and VI

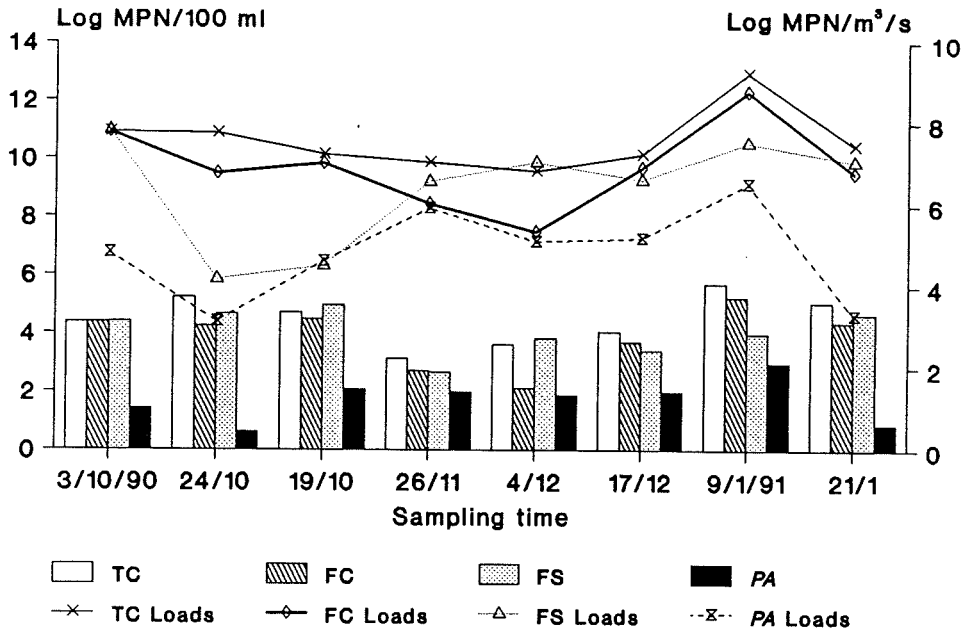


a: site LC

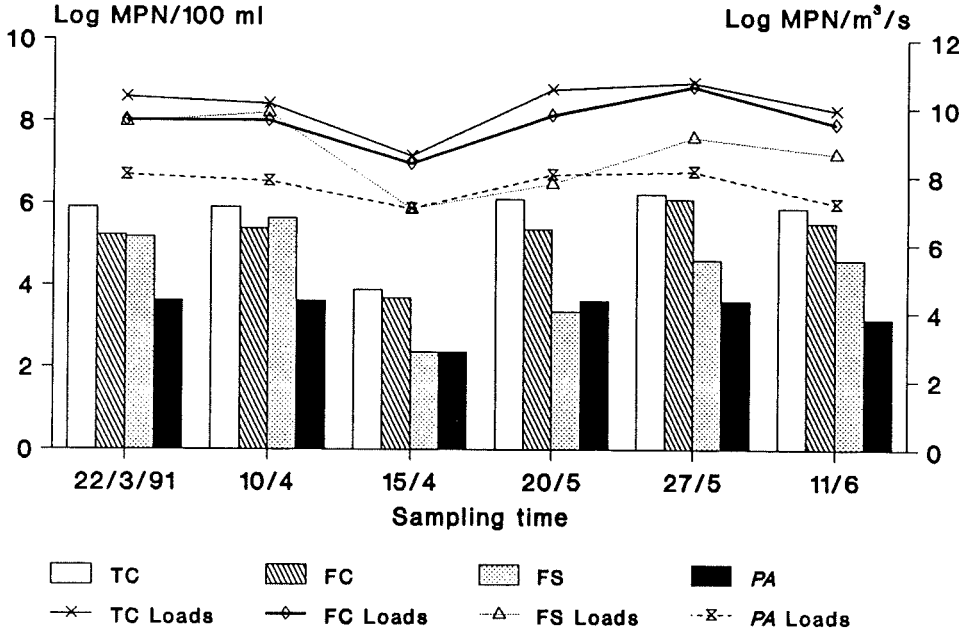


b: site VH

Figure 4.15 a and b: Relationships between the Levels of Bacteria and Loads at Sites LC and VH



a: site LG



b: site VJ

Figure 4.16 a and b: Relationships between the Levels of Bacteria and Loads at Sites LG and VJ.

bed sediments as stream discharges increase (Kay and Mcdonald, 1982). Kunkle (1970), Baxter - Potter and Gillil (1988) as well as Dan (1991) have also shown that there is a relationship between bacterial levels and flow. According to the results of the present study, the levels of bacteria recovered at combined sewage sites and receiving stream sites in both North London and Valencia showed similar relationships between flow and bacteria levels, but sometimes vary according to flow and sampling time.

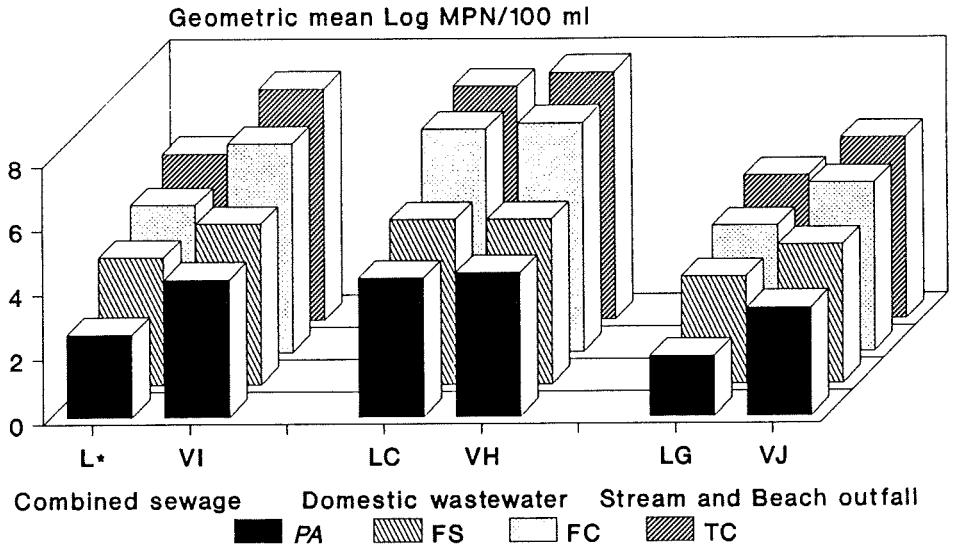
Flow is an extremely important environmental factor for bacterial pollution in urban sewage discharges according to the present study. According to the study, bacterial loads can be correlated with bacterial levels recovered during DWF and low flow rainy days. However, under high flow conditions, bacterial loads present a much more serious pollutant threat than bacterial levels.

4.3.4. Comparison of Indicator Microorganisms and Pathogenic Bacteria Levels between Sewage and Sediment for both North London and Valencia

The weather conditions differ markedly between North London and Valencia. Sampling in North London took place during a cold wet winter in comparison with the dry hot early summer period in Valencia. A comparison of the geometric means for urban sewage runoff between the two cities is shown in Figure 4.17.

Comparison of the bacterial levels recovered from sanitary wastewater at site LC (North London) and site VH (Valencia) are similar, although both sites are located in different countries and are exposed to different climate conditions. The mean sewage temperature was not found to be very different at either location. The sewer at site LC was at a depth of 2m under the ground in North London and even during the winter, the mean temperature is 15.9°C (Table 4.9), which is reasonably close to the mean of 19.5°C (Table 4.15) recorded at site VH during the early summer in Valencia. Furthermore, as they both carried DWF sanitary wastewater heavily polluted with human faeces, the levels of total coliforms, faecal coliforms, faecal streptococci and *P. aeruginosa* recovered were similar. *Salmonella* were found to be present in all samples at site VH

and in 85.7 % samples at site LC.



*: Mean value of sites LA,LB,LD and LE.
TC:Total coliforms FC:Faecal coliforms
FS:Faecal streptococci PA:P.*aeruginosa*

Figure 4.17: The Comparison of levels of bacteria recovered in combined sewage between North London and Valencia.

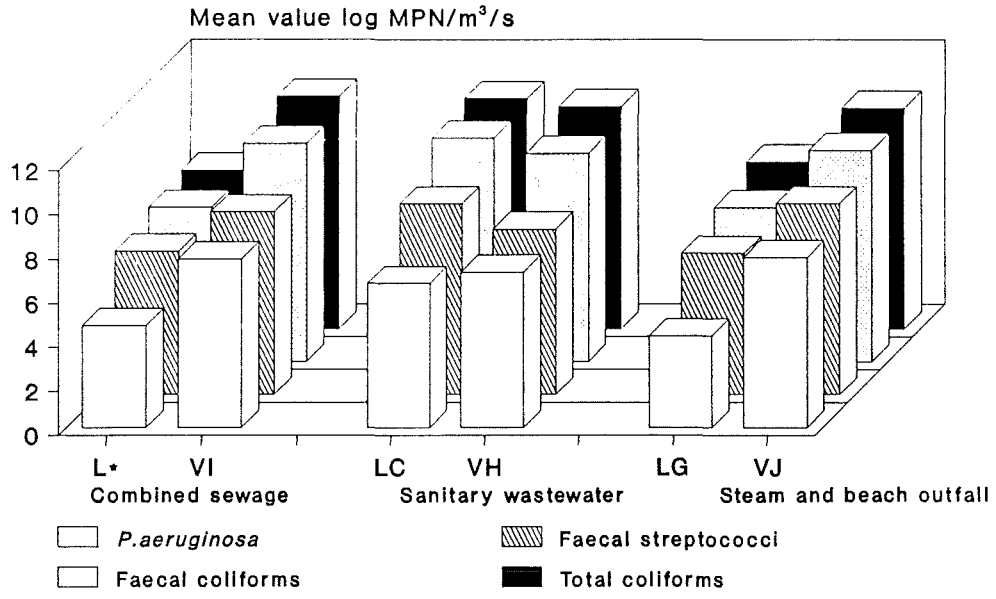
Comparison of the microbial quality in combined sewage between North London sites (LA, LB, LD and LE) and the Valencia site (VI) shows that the geometric means of bacteria in Valencia were obviously higher than the mean value for all combined sewage sites in North London. The levels of total coliforms, faecal coliforms and faecal streptococci recorded in Valencia were generally about 100 - fold higher than in North London with the levels of *P. aeruginosa* recorded in Valencia being 50 - fold higher than in North London and approached the levels of DWF sanitary wastewater in North London. Similarly, there were higher levels of bacteria recorded at Site VJ (beach outfall) in Valencia than site LG (lower reaches of receiving stream) in North London. For instance at site VJ, the geometric mean of total coliforms was 4.5 x 10^5 MPN/100

ml and of faecal coliforms was 3.0 x 10^4 MPN/100 ml. At site LG, the Geometric mean of total coliforms was 3.0 x 10^4 MPN/100 ml and faecal coliform was 7.9 x 10^3 MPN/100 ml.

Generally, the levels of combined sewage bacteria found under the hot dry climates conditions prevalent in Valencia were much higher than those observed under the wet and cold conditions of North London. The results also reflected the relative urban environmental health/sanitary conditions and degree of urbanization occurring in the two regions. There were more unpaved and open areas, building sites and undeveloped areas in the Benimeclet catchment of Valencia than in the well urbanized North London catchment.

Figure 4.18 shows the comparison of the bacterial loading between sanitary wastewater, combined sewage, receiving stream and beach outfall in North London and Valencia. In sanitary wastewater (sites LC and VH), the average bacterial loads at site LC (North London) were about 4.0 - fold higher than site VH (Valencia). This is due to a higher mean flow value (0.158 m^3/s) at site LC than at site VH (0.037 m^3/s). From the view point of public health, it is more polluted at site LC than at site VH. But *P. aeruginosa* loads were higher in Valencia than in North London, which result from higher levels of *P. aeruginosa* recovered at site VH than site LC. For the combined sewage, the levels of bacterial loads in Valencia (site VI) were higher than the mean values of sites LA, LD and LE in North London. Similarly, higher bacterial loads were identified at Site VJ (beach outfall in Valencia) than at Site LG receiving stream (in North London). These results are consistent with the bacterial levels recorded.

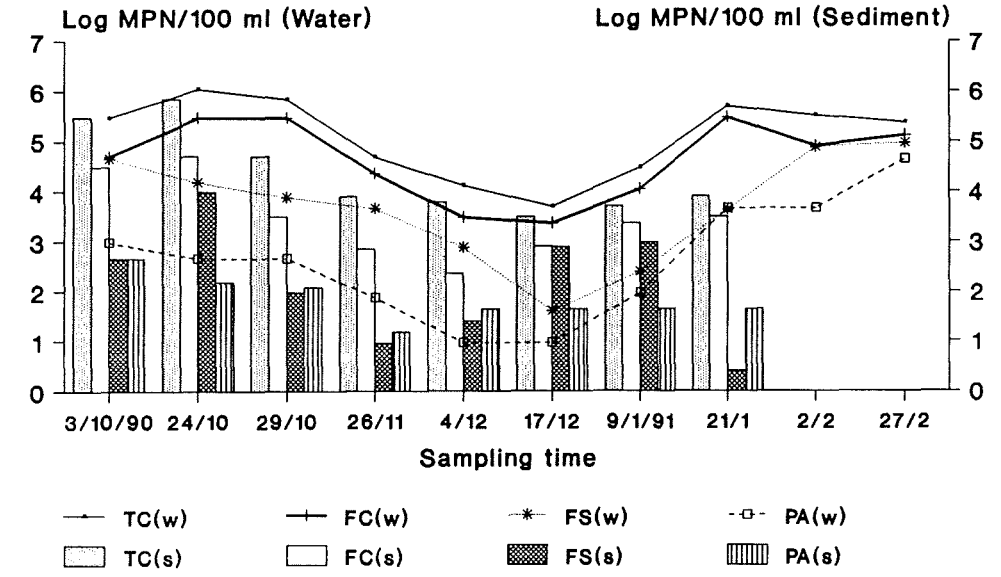
The combined sewage and beach outfall in Valencia were more heavy polluted than the combined sewage and receiving stream in North London. These results are also consistent with bacterial levels recorded.



*:Mean value of sites LA,LD and LE.

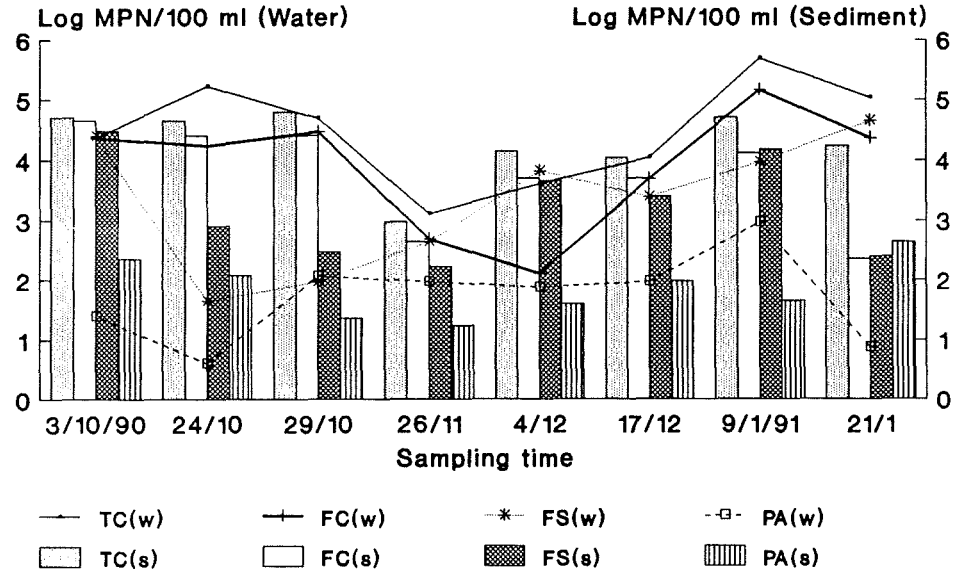
Figure 4.18: Comparisons of bacterial loads between North London and Valencia

The graphs of Figures 4.19 and 4.20 demonstrate different levels of bacteria in water and sediment existing during the time of study at sites LE, LG, VH and VJ. Generally, the variation in levels of bacteria found in sediment corresponded with levels of bacteria in water for combined sewage and receiving stream water. At site LE, faecal streptococci in sediment were recovered at higher levels than in sewage for the samples taken on 17/12/90 and 9/1/91. *P. aeruginosa* were also recovered at higher levels in sediment than sewage for samples on 4/12/90 and 17/12/90 (Fig. 4.19). Similar trends were also found at the receiving stream site LG and beach outfall site VJ on some sampling days (Fig. 4.20), but there was no such trends to be found at site VH. The variation of bacterial levels between water and the sediment column is possibly affected by variable urban combined sewage discharges. However, bacterial levels in sanitary wastewater were consistently higher in the water column than in sediment due to heavily polluted sewage effluent. In previous studies, many workers (Hendricks, 1971; Van Donsel and Geldreich, 1971; Grimes, 1975, 1980; Matson *et al.*, 1978) have found higher levels of microorganisms recovered from river sediments than water. In the



TC:Total coliforms FC:Fecal coliforms
FS:Faecal streptocci PA:*P.aeruginosa*
w:Water s:Sediment

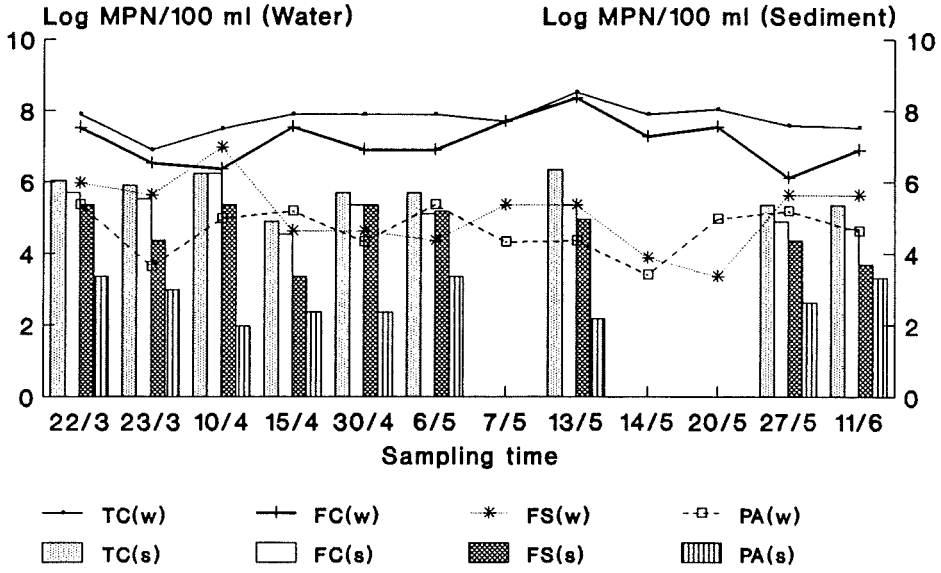
site LE



TC:Total coloforms FC:Faecal coliforms
FS:Faecal streptococci PA:*P.aeruginosa*
w:Water s:Sediment

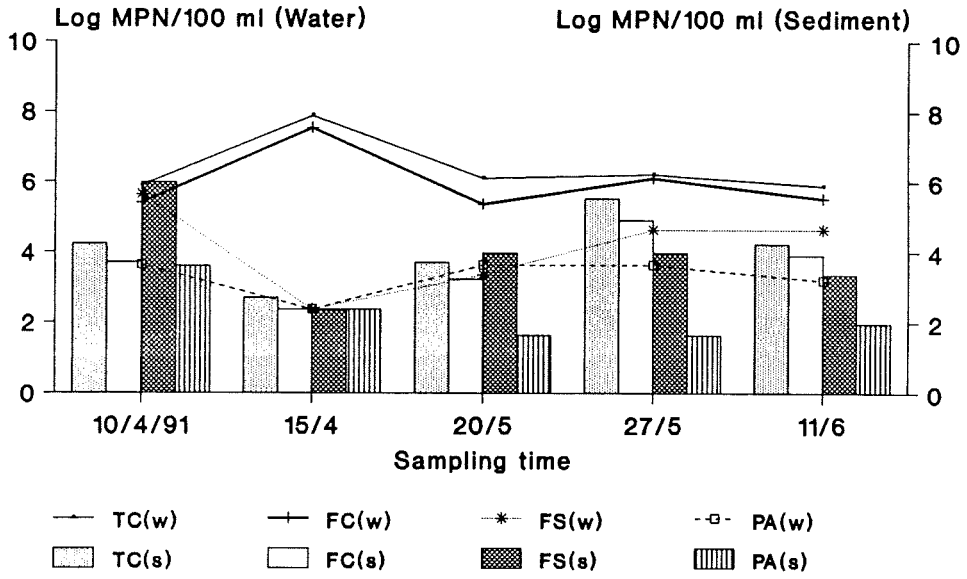
site LG

Figure 4.19: Comparisons of bacterial level in water and sediment at sites LE and LG



TC:Total coliforms FC:Faecal coliforms
FS:Faecal streptococci PA:P.*aeruginosa*
w:Water s:Sediment

site VH



TC:Total coliforms FC:Faecal coliforms
FS:Faecal streptococci PA:*aeruginosa*
w:Water s:Sediment

site VJ

Figure 4.20: Comparisons of bacterial Level in water and sediment at site VH and VJ

present study, however, the sediment/water ratios were variable in the urban receiving stream and combined sewage due to variable polluted sewage discharges.

The decreased bacterial levels from the top to the bottom layers of the sediment indicate particular characteristic of bacterial survival in sediments. However, at site LE, the levels of faecal streptococci were only slightly increased from layer 1 to layer 2, possibly due to faecal streptococci survival being longer in sediment than in the case for other indicators. Sayler *et al.* (1975) and Grimes (1980) have reported that faecal streptococci remain viable longer than other organisms in sediment. Kibbey *et al.* (1978) also showed that *S. faecalis* survived for long periods of time (at least 12 weeks) in coal and moist soil if sufficient nutrients were available.

The above analysis and discussion strongly supports the hypothesis that sedimentation is an important factor in distributing the bacteria vertically (Dan and Stone, 1991). Densities of bacteria in different layers of sediment greatly depend on the oxygen content and nutrient condition (Collins, 1977). The distinctive high survival rates of faecal streptococci found in sediment in this study indicates that each bacteria has its own specific survival rate and unique response characteristics that determine its distribution over depth and time.

4.3.5. Characteristics of Diurnal Bacterial Discharges in Sanitary Wastewater

In continuous effluent samples, the peaks of bacterial discharge were observed around 06.00 - 08.00 in the morning and 22.00 in the evening for site LC in North London. In Valencia, the peaks of bacterial discharge were observed at 18.00 and 24.00 in the evening for site VH. These peaks reflect the principal meal times and social domestic activities in North London which are just after breakfast and dinner time and in Valencia which are after lunch (taken from 14.00 - 16.00 in Spain) and late dinner. *P. aeruginosa* was higher at 09.00 from site VH following breakfast and during the early morning peak of domestic water use. The results therefore probably reflect normal social domestic activities patterns of North London and Valencia with the social

variations largely accounting for the quality of sewage.

This data show similar trends to previous works (Gameson, 1978; Jefferies *et al.*, 1989) with typical daily variations in bacterial counts occurring during cold mid-winter in North London and hot early summer in Valencia. FC/F ratios were greater than 4 throughout the 24 hours sampling period, indicating as would be expected, that the pollution was mainly of human rather than animal origin.

4.3.6. Principal Component Analysis of Selected Environmental Variables with Indicators Bacterial and *P. aeruginosa*

Principal Component Analysis (PCA) involves the extraction of the eigenvalues and eigenvectors from the matrix of correlation coefficients of the original variables. PCA finds successively the axes (components) of the hyperellipse defined by the data points in n-fold hyperspace. The axes have lengths (eigenvalues) and directions (defined by eigenvector coefficients). A distinctive characteristic of PCA is its data reduction capability. This multivariate technique enables one to see whether some underlying pattern of relationships exists, such that the data may be reduced to a smaller set of components (Nuttall, 1982).

The principal components were constructed for the data of DWF sanitary wastewater sites LC and VH, combined sewage sites LD and VI, receiving stream site LG and beach outfall site VJ, respectively. For site LC, the first two components accounted for 79.8 % of the total variance (Fig. 4.21a), There was almost the same contribution to the first axis (quantitative gradient) for *P. aeruginosa* (PA), total coliform (TC), faecal coliform (FC), faecal streptococcus (FS) and pH, but their position on axis 2 produced a difference between them with pH being above 0 point of axis 2 as well as TC and FC being below. PA and FS were above the zero point. The points representing TC and FC were also very close. The flow variable fell opposite to the bacteria along axis 1. However, for site VH with the first two components accounted for 57.3 % of total variation (Fig. 4.21b), most of the variations spread contributed on the left of the zero

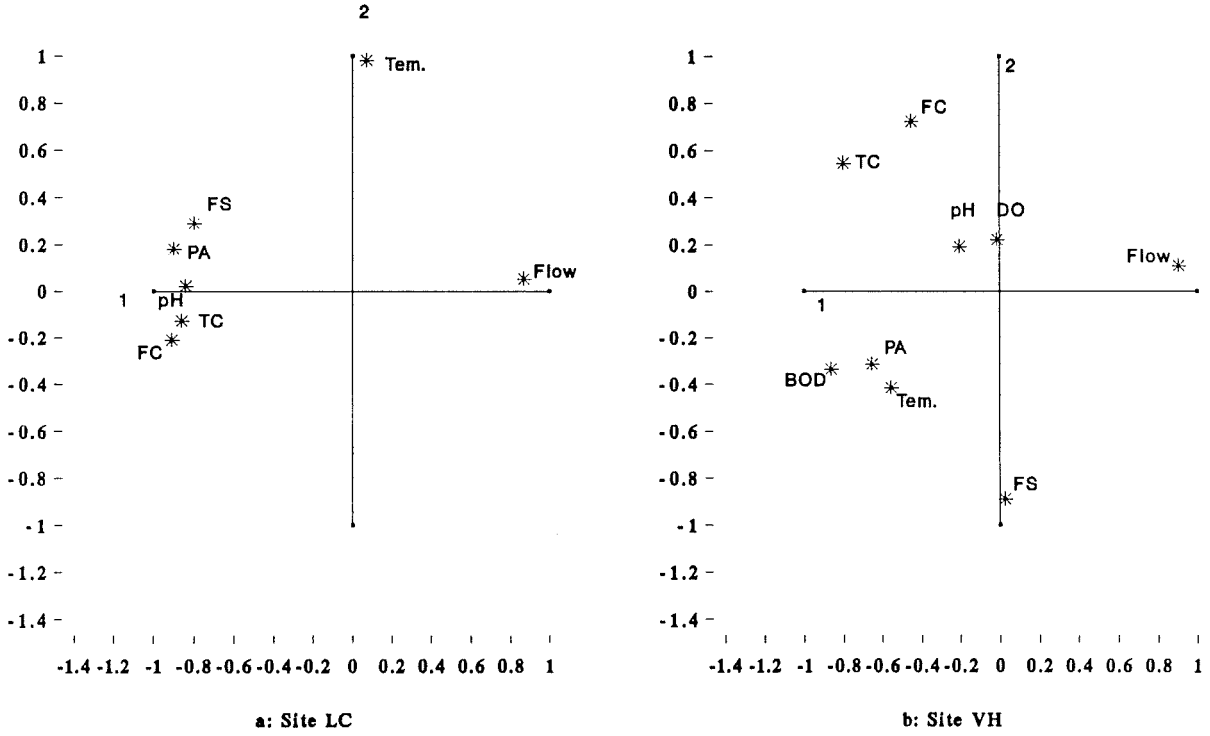
point to the first axis except flow. The major components for the two sites were dominated by flow. There were obvious negative correlations between bacteria and flow at site LC. Site VH also presented similar correlations between flow and TC, FC and PA respectively. BOD_5 was also included in this components for site VH.

A change in component structure was apparent for the combined sewage sites LD and VI with the first two components accounted of 73.1 % and 58.6 % respectively (Fig. 4.22a and b). For site LD, flow presented a relative high value to axis 2 and low value to axis 1. The value of temperature presented higher values to axis 1 at site LD than site LC. The points of bacteria location were packed. At site VI, FS and PA had almost the same contribution to the first axis as well as FC and FC. BOD_5 fell opposite to flow with FS and PA occurring along axis 1. TC and FC packed together with the position opposite flow to axis 1 as well as opposite to pH and DO on axis 2. Fig. 4.23a and b shows the first two components explained 63.4 % and 71.1% for site LG and site VJ, respectively. Generally, dispersive contributions of variations presented at site LG. TC and FC appeared relatively close as were PA and FS which were close to flow than TC and FC. TC, FC and PA were packed again at site VJ with FS being opposite to them on axis 1. Flow also presented the opposition relationship with bacteria at axis 1.

Generally, flow presented negative correlations with bacteria for sanitary wastewater samples in both North London and Valencia. The relationship of contributions were clearer for site LC than site VH, probably due to the underground sewer in North London being less effected by environmental factors than that part of the open sewer (VH) in Valencia. However, the closer relationship between flow, FS and PA were show in combined sewage samples at site VI than site LD. The results indicated that there are significant correlations between the levels of bacteria and flow in combined sewage, especially for FS and PA in Valencia. At sites LG and VJ, flow also presented better correlations with FS and PA than for TC and FC. From the view of the relationships between all variables, the snslysis showed there were more close relationships between BOD_5 and bacteria than flow and bacteria for all six sites. In the first component, BOD_5 was the more dominant variable at sites VH and VI, but at site VJ, BOD_5 was a subordinate variable. It is consistent with the low levels of bacteria

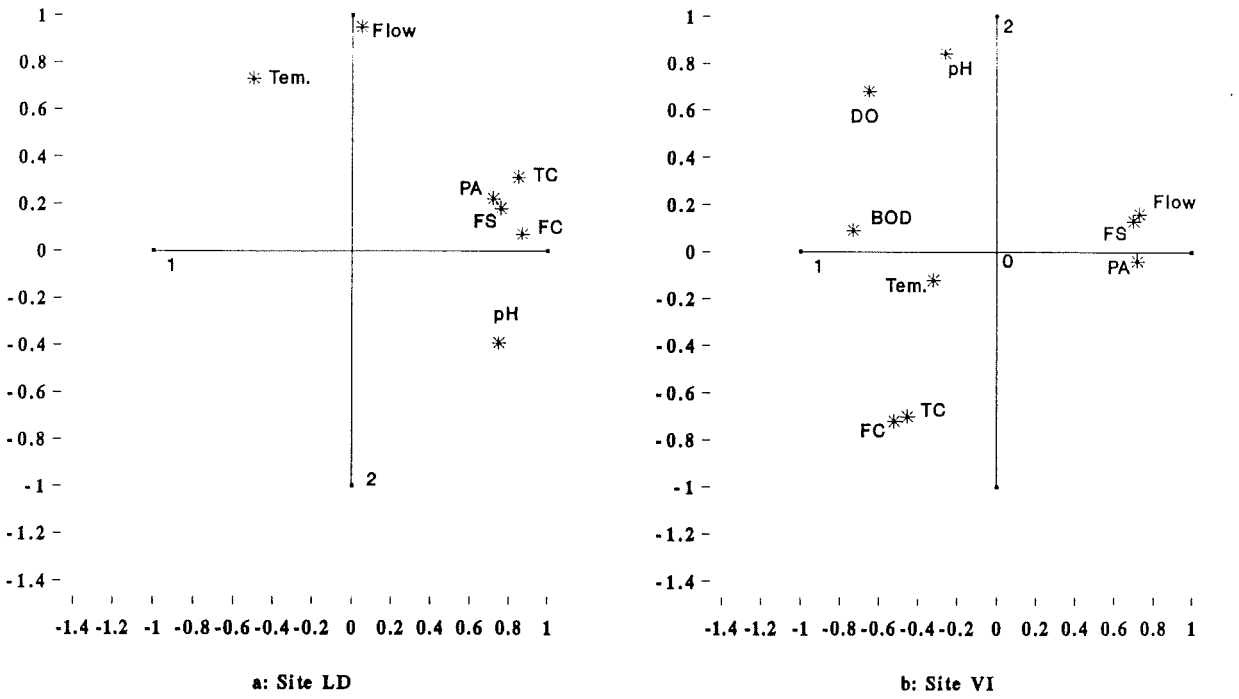
recovered at site VJ.

The results described here indicate the complex interrelationships of selected environmental factors and their influence on bacterial population dynamics. PCA allowed an identification of the degree of redundance between selected bacteria and environmental factors by the calculation of correlation coefficients. It seems to be difficult to establish an equation between selected microorganisms and environmental factors due to the problems brought about by the stabilization of the variance.



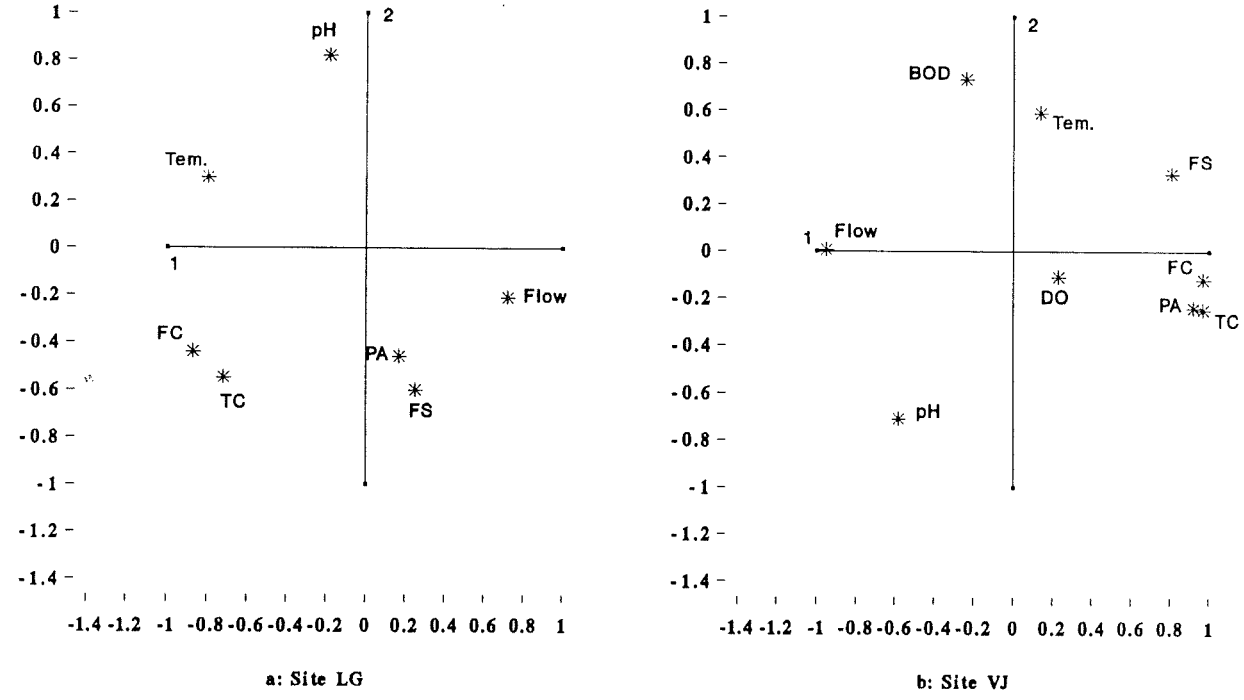
a b

Figure 4.21 a and b: Principal component analysis for sites LC and VH.



a b

Figure 4.22 a and b: Principal component analysis for sites LD and VI.



a b

Figure 4.23 a and b: Principal component analysis for sites LG and VJ.

4.4. Summary

1). In both North London and Valencia, the highest levels of indicators, *P. aeruginosa* and high percentage of *Salmonella* were recovered in DWF sanitary wastewater. High levels of indicators, *P. aeruginosa* and *Salmonella* were recovered in combined sewage, receiving stream water and beach outfall. The levels of bacteria in sanitary wastewater for both cities are very similar even given the different weather conditions. For combined sewage, the geometric mean of both bacteria levels and loads in Valencia were higher than in North London. Similarly, there were higher levels of bacteria recovered at the beach outfall in Valencia than from the receiving stream in North London. The quality of urban runoff waters was very poor from a microbiological point of view.

2). High levels of bacteria were also recorded in the sediments and the results demonstrated that most bacteria abounded in the top layer of sediments, this may of cause be resuspended by turbulence. For combined sewers, the levels of bacteria in sediment were lower than these in the water column. However, the levels of bacteria in sediment were higher than in the water in the receiving stream on rainy days. Faecal streptococci and *P. aeruginosa* showed distinctive high sediment / water ratios.

3). Bacterial loads are a useful parameter to be used in such a study as this. It has been suggested that the dynamic bacterial population can be determined by the calculation of an activity index expressed as Log $MPN/m^3.s^{-1}$. There are close relationships between bacterial level and flow during dry weather flow conditions and low flow rainy days. However under high flow conditions, bacterial loads present a more significant pollution threat than bacterial levels.

4). There were strong positive correlations between levels of total coliforms and faecal coliforms for all sampling sites for both North London and Valencia. There were no significant correlations between faecal coliforms and faecal streptococci. No consistent correlation between *P. aeruginosa* and indicators was found in combined sewage for

either North London or Valencia.

5. PCA analysis yields detailed information on the nature and strength of relationships between the variables, especially between the selected environmental factors and bacterial population in different kinds of water samples between the two cities. According to principal component analysis, flow presented a negative correlation with microorganisms for sanitary wastewater samples in both North London and Valencia. There were significant positive correlations between flow and microorganisms in combined sewage. There were closer relationships between BOD_5 and microorganisms than between flow and microorganisms.

6). One of the principal aims of the study was to determine whether variations in the bacterial content of sewage effluent could be correlated with time and people's daily activity. It can be concluded that such a conclusion can be made.

Chapter 5: Characteristics of Indicator Microorganisms and Pathogens in Road Surface Runoff

5.1. Introduction

Geldreich *et al.* (1968) have reported that storm waters from urban streets and suburban residential district have bacterial densities similar to storm water runoff from cultivated farm land. Other studies (Weibel *et al.*, 1964; Evans *et al.*, 1968; Sartor *et al.*, 1974; Qureshi, 1978; Gannon and Busse, 1989) have shown that runoff from street surfaces is highly contaminated with bacteria and is similar in many aspects to wastewater. In addition to faecal pollution indicator bacteria, the presence of pathogenic bacteria in stormwater has frequently been reported. Evans *et al.* (1968) demonstrated the existence of a potential health hazard by isolating *Salmonella thompson* from a separated sewer stormwater sample in an urban business district. Studies on sources of bacteria found in stormwater runoff from residential and light commercial area have indicated that bacteria are predominantly of non-human origin (Benzie and Courchaine, 1966; Geldreich *et al.*, 1968). However, in several instances pathogenic bacteria have also been isolated in runoff waters (Sartor *et al.*, 1974; Dutka, 1977; Qureshi and Dutka, 1979), demonstrating the existence of a potential health hazard.

This chapter provides some information on the non-human sources of microbial indicators found in storm runoff from different types of urban environments as a basis for comparison of the characteristics of stormwater runoff between North London and Valencia under different weather conditions.

5.2. Results

5.2.1. The Levels of Indicator Microorganisms and *P. aeruginosa* in Stormwater Runoff in North London

Four storm events were monitored during the study period. The levels of

microorganisms recovered in stormwater runoff samples (Table 5.1) reflected the surface runoff quality and the state of sanitation in the local neighbourhoods. The samples from site LL (Open Market) presented a higher geometric mean than other sites; total coliforms were 1.4 x 10^4 MPN/100 ml, faecal coliforms were 1.3 x 10^3 MPN/100 ml, faecal streptococci were 2.0 x 10^4 MPN/100 ml and *P. aeruginosa* were 1.2 x 10^2 MPN/100 ml (Fig. 5.1). The geometric mean level of faecal streptococci recovered at site LL was consistently higher than faecal coliforms at all five sites. The highest geometric mean for faecal streptococci (2.0 x 10^4 MPN/100 ml) occurred at site LL, followed by site LO (residential area, 8.9 x 10^3 MPN/100 ml), site LK (commercial area, 4.9 x 10^3 MPN/100 ml), site LM (car park, 1.1 x 10^3 MPN/100 ml) and site LN (urban main road, 1.0 x 10^3 MPN/100 ml). The levels of bacteria observed at site LN were relatively much lower than any other site for the first three samples (Table 5.1). However, levels of total coliform, faecal coliform and faecal streptococcus were unusually high for the fourth rainfall-melting snow samples on 12/2/91. *P. aeruginosa* levels were not particularly high. The levels of total coliforms, faecal coliform and faecal streptococcus for this winter event were 1.3 x 10^4 MPN/100 ml, 4.9 x 10^3 MPN/100 ml and 7.5 x 10^3 MPN/100 ml respectively.

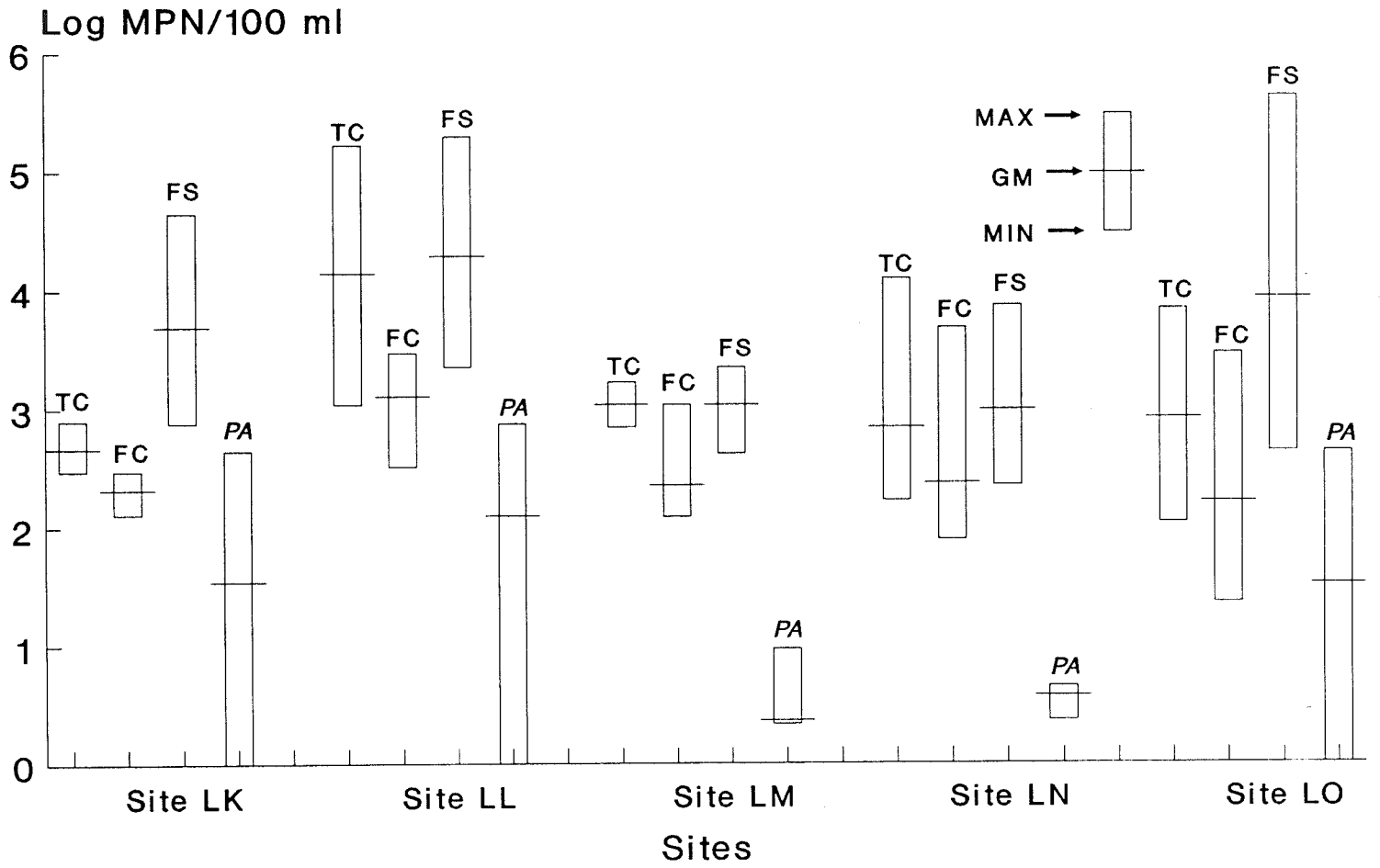
From both Table 5.1 and Fig. 5.1, it can be seen that microbial levels fluctuated over the time of study at each site except site LM where little variability is presented.
The levels of *P. aeruginosa* showed the biggest variation, fluctuating from a minimum of zero up to 7.5 x 10^2 MPN/ 100 ml at site LL. In contrast, the least variation in *P. aeruginosa* levels were found at site LN ranging from 2.3 MPN/100 ml to 9.3 MPN/100 ml (Fig 5.1).

Table 5.1: The levels of microorganisms in stormwater samples (MPN/100 ml)

Date	Site	TC	FC	FS	PA
25/11/90	LK	$8.0x10^2$	$3.0x10^2$	$4.5x10^4$	7.5x10
	LL	$1.7x10^4$	$3.0x10^3$	$1.5x10^5$	$4.5x10^2$
	LM	$1.7x10^3$	$1.1x10^3$	$9.5x10^2$	NF
	LN	$5.0x10^2$	8.0x10	$4.5x10^3$	4.5
	LO	$7.0x10^3$	$3.0x10^3$	$4.5x10^5$	$4.5x10^2$
8/12/90	LK	$3.0x10^2$	$1.3x10^2$	$7.5x10^3$	$4.5x10^2$
	LL	$1.7x10^5$	$3.0x10^3$	$2.0x10^5$	$7.5x10^2$
	LM	$8.0x10^2$	$2.3x10^2$	$7.5x10^2$	NF
	LN	$1.7x10^2$	8.0x10	$7.5x10^2$	4.5
	LO	$1.1x10^2$	3.0x10	$4.5x10^2$	2.5x10
15/12/90	LK	$4.3x10^2$	$2.3x10^2$	$7.5x10^2$	4.3x10
	LL	$1.1x10^4$	$9.8x10^2$	$2.3x10^3$	$7.5x10^2$
	LM	$7.5x10^2$	$5.0x10^2$	$4.3x10^2$	2.3
	LN	$2.3x10^2$	$1.1x10^2$	$4.3x10^2$	2.3
	LO	$5.0x10^2$	$3.0x10^2$	$7.5x10^3$	$1.1x10^2$
12/2/91*	LK	$4.9x10^2$	$2.3x10^2$	$2.3x10^3$	NF
	LL	$1.1x10^3$	$3.3x10^2$	$2.3x10^3$	NF
	LM	$1.3x10^3$	$4.9x10^2$	$4.3x10^3$	4.3
	LN	$1.3x10^4$	$4.9x10^3$	$7.5x10^3$	9.3
	LO	$7.9x10^2$	2.3	$4.3x10^3$	NF

TC: Total coliforms FC: Faecal coliforms FS: Faecal streptococci PA: *P.aeruginosa*

NF: Not found. *: Snow melt runoff.



MAX:Maximum MIN:Minimum GM:Geometric mean
TC:Total coliforms FC:Faecal coliforms
FS:Faecal streptococci PA:*P.aeruginosa*

Figure 5.1 Geometric Mean, maximum and minimum levels of bacteria from storm events in North London.

Figure 5.2 shows the relationships between faecal coliform and faecal streptococcus in storm water samples. All samples from the five sites had FC/FS ratios of consistently less than 0.7 except one sample at the car park site LM which had a ratio of between 4.0 and 0.7.

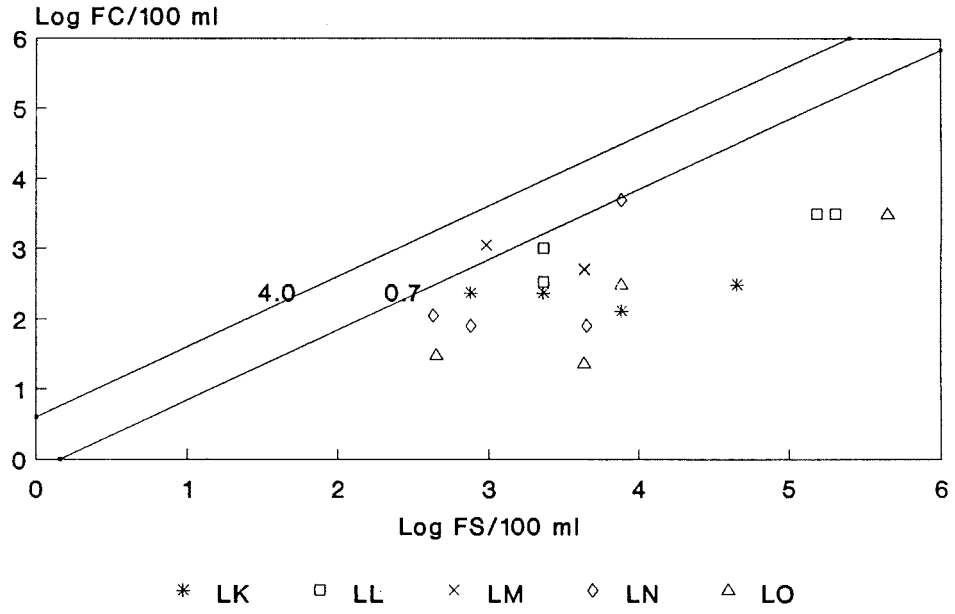


Figure 5.2 Relationships between faecal coliforms and faecal streptococci in stormwater samples in North London.

5.2.2. The Levels of Indicator Microorganisms and *P. aeruginosa* in Stormwater Runoffs in Valencia

Densities of microorganisms recovered from stormwater runoff at sites within Valencia are presented in Table 5.2. All results were from summer storms during 1990 and 1991. Geometric means and bacterial ranges for all sites are presented in Fig. 5.3. The highest geometric mean values for faecal streptococcus were found at sites VT (residential area, 1.2 x 10^6 MPN/100 ml) and VQ (open market, 3.6 x 10^5 MPN/100 ml). The highest geometric mean values for *P. aeruginosa* were also found at sites VT and VQ (1.2 x 10^2 MPN/100 ml and 6.4 x 10 MPN/100 ml respectively). At the

Table 5.2: Levels of microorganisms in storm water samples (MPN/100 ml)

Date	Site	TC	FC	FS	PA
16/5/90	VU	$7.0x10^3$	$2.2x10^3$	$2.3x10^5$	NF
25/5/90	VT	$1.1x10^5$	$4.9x10^6$	$9.5x10^6$	$2.3x10^2$
30/5/90	VP	$4.9x10^3$	$1.7x10^3$	$4.3x10^2$	4.0
	VU	$1.1x10^4$	$4.6x10^3$	$4.3x10^3$	4.3
7/6/90	VT	$1.1x10^5$	$2.8x10^4$	$2.1x10^6$	$1.5x10^2$
	VS	$2.2x10^4$	$1.3x10^4$	$4.3x10^3$	NF
9/6/90	VP	$3.3x10^4$	$2.3x10^4$	$4.3x10^4$	4.0
	VS	$4.6x10^4$	$1.7x10^4$	$4.3x10^4$	9.3x10
	VQ	$1.3x10^5$	$3.3x10^4$	$2.1x10^5$	$1.5x10^2$
11/6/90	VR	$1.1x10^6$	$3.5x10^5$	4.3x10	2.3x10
23/3/91	VP	$4.9x10^4$	$1.3x10^4$	$4.3x10^4$	4.3
	VQ	$1.7x10^5$	$4.9x10^4$	$2.3x10^6$	7.5x10
	VR	$1.7x10^5$	$3.3x10^4$	$4.3x10^4$	4.3
	VS	$3.3x10^4$	$7.9x10^3$	$4.3x10^4$	9.3
	VT	$1.3x10^5$	$4.9x10^4$	$4.3x10^5$	9.3
	VU	$4.9x10^4$	$4.9x10^3$	$2.3x10^2$	4.3
16/4/91	VP	$7.9x10^4$	$2.3x10^4$	$2.4x10^4$	7.5
	VQ	$1.7x10^4$	$1.5x10^4$	$9.3x10^4$	2.4x10
	VR	$1.3x10^4$	$3.3x10^3$	$1.2x10^2$	NF
	VS	$1.3x10^4$	$4.9x10^3$	$4.3x10^3$	NF
	VT	$3.3x10^4$	$7.9x10^3$	$2.3x10^5$	7.5x10
	VU	$1.1x10^4$	$4.6x10^3$	$2.3x10^2$	4.3

TC: Total coliform FC: Faecal coliform FS: Faecal streptococcus PA: *P.aeruginosa*
NF: Not found.

Valencia polytechnic campus site (VU), the lowest geometric mean values for *P. aeruginosa* (3.0 MPN/100 ml) were found.

The summary compiled in Fig. 5.4 shows FC/FS ratios in the stormwater runoff samples obtained in Valencia differs between the various sites. At Valencia polytechnic campus site VU, two samples showed FC/FS ratios greater than 4.0, one less than 0.7 and one between 4.0 and 0.7. The samples from residential site VT and the open market site VQ possessed FC/FS ratios of consistently less than 0.7. The samples from commercial site VP presented FC/FS ratios of between 4.0 and 0.7 for two samples with the remain two samples showing a ratio of less than 0.7. Valencia main road site VS had ratios of less than 0.7 for two samples; the other two samples fell between 4.0 and 0.7. The car park site VR was the only location where all the recorded FC/FS ratios were greater than 4.0.

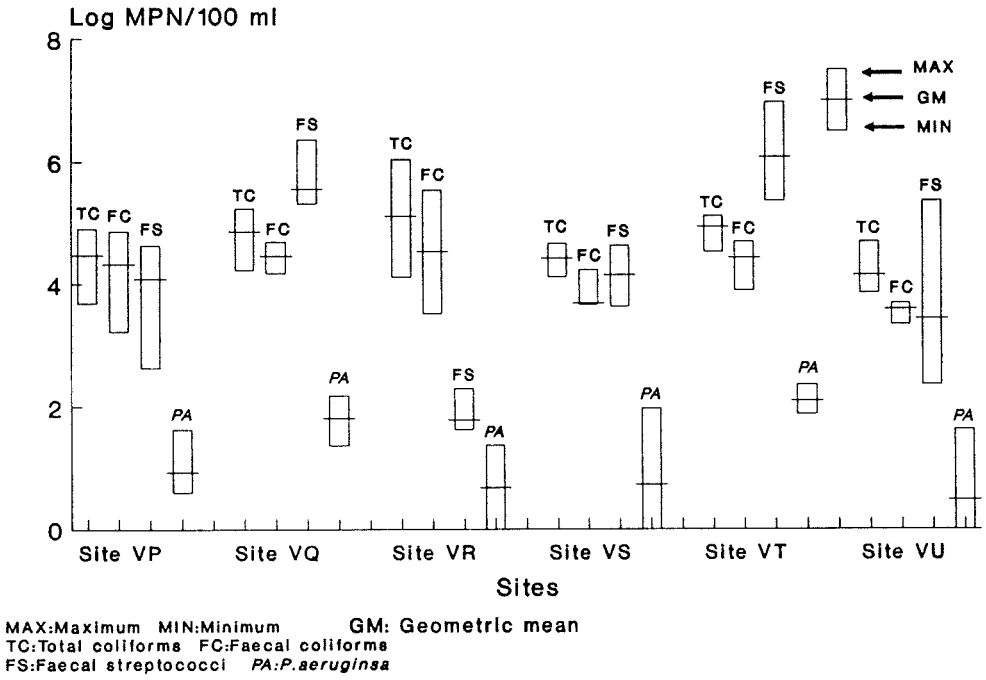


Figure 5.3: Geometric means, maximum and minimum levels of bacteria from storm events in Valencia.

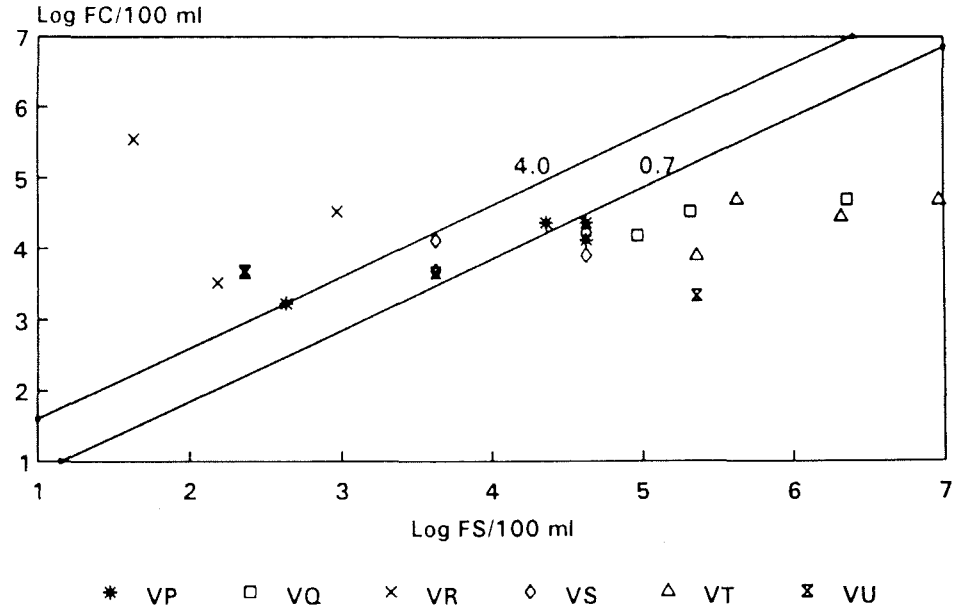


Figure 5.4: Relationships between faecal coliforms and faecal streptococci in stormwater samples in Valencia.

5.3. Discussion

5.3.1. Sources and Characteristics of Indicator Microorganisms and *P. aeruginosa*

All stormwater samples collected during the study period, from both North London and Valencia, contained significant levels of indicator bacteria. The levels of total coliforms, faecal coliforms and faecal streptococci approached dilute sewage concentrations during each event at some sites, thus re-emphasizing the potential health hazards due to high levels of indicator microorganisms which are contained within stormwater runoff.

In the rainfall-snow melt event within the North London catchment (12/2/91), levels of total coliform, faecal coliform and faecal streptococcus found at site LN were higher than in any other samples. During this event, the ground was frozen and relatively impervious to the rain, therefore, it is possible that rainfall-snow melt mixtures were responsible for the high levels of microorganisms. Snowfall/slush in urban areas may

harbour and preserve microorganisms, especially of animal faecal origin, which eventually reach storm sewers and receiving waters. Another factor which may have impact on the high levels of microorganisms, was roads which were paved by sand after snowfall for traffic safety. The sand probably contributed some external microorganisms into the road surface runoff. These results are consistent with Qureshi and Dutka (1979)'s findings in Ontario during winter period.

The extent of "dirtiness" of the source associated with the particular land use activity will inevitably have an effect on the microbial quality of storm water runoff. The major background sources of urban surface pollution are of faeces from animals and birds which are washed by rainfall from the roof and street surface. At site VU (Valencia Polytechnic campus), which is located in the eastern suburban area of Valencia, the high levels of coliform could be derived from birds. The microbial densities in urban stormwater runoff also reflect the local population densities, the local state of sanitation as well as the nature of social activities. For instance, the relatively high levels of faecal streptococci consistently found from open market (sites LL and VQ) and residential areas (sites LO and VT) in both North London and Valencia were probably due to the concentrated social and commercial activities which take place in these relatively enclosed areas. The results would also tend to confirm that animal faeces are the main sources of bacterial pollution in urban stormwaters.

The varying range of bacterial levels found in storm water runoff from the different sampling sites reflect the inherent local characteristics of microbial pollution associated with nonpoint sources. Relatively large scale variation in *P. aeruginosa* and faecal streptococcus occurred at commercial site LK, open market site LL and residential site LO in North London, where more anthropogenic activity leads to contamination than at the car park site (LM) and the main road site (LN). This reflects the characteristics of urban non-point polluted sources which are inevitable affected by man's social activity in central urban areas. In Valencia, few such trends were to be found. Inversely, there were relatively small scale variations of bacterial levels in storm water samples between the different sites. This probably due to the levels of bacteria in stormwater samples at each Valencia site being constantly higher than those recovered in North London, so the fluctuation of bacterial levels were also affected by nonpoint

pollution sources.

5.3.2. Comparison of Microorganisms Levels in Storm Water Runoff between North London and Valencia

The levels of bacteria recovered in stormwater runoff between North London and Valencia clearly showed different microbial qualities under the different prevailing weather and sanitary conditions. The bacterial geometric means at each site within North London and Valencia are shown in Fig. 5.5.

The bacterial geometric mean densities recovered in storm water runoff within Valencia, particularly during the hot dry summer period, were higher than those found in North London during the cold wet winter at the commercial sites (VP), open market sites (VQ), urban main road site (VS) and residential site (VT). At the car park site (VR), total coliforms and faecal coliforms within Valencia were higher than London, but faecal streptococci and *P. aeruginosa* were lower than London. The geometric means of total coliform and faecal coliform at all sites were several hundred fold higher in Valencia than North London. Commercial activity does have an impact on the sanitary environment of road surfaces and the microbial quality of storm water runoff even in the cold winter conditions of the UK, which has unfavourable temperatures for survival of the high levels of microorganisms.

The bacterial hierarchy observed in storm water runoff from the various sampling sites in North London and Valencia vary according to the type of bacteria recorded. For instance in North London, in terms of the degree of faecal streptococci pollution at each site the following order of incidence was noted: open market > residential area > commercial area > urban main road > public car parking. In Valencia, the order was residential area > open market > urban main road > commercial area > Polytechnic campus > public car parking. The order reflects the characteristics of urban storm water runoff pollution which are influenced by the principal land use and social activity. The open market and residential areas are main major nonpoint pollution sources being derived from animal and rodent faeces as well as from dust, litter and normal biogenic

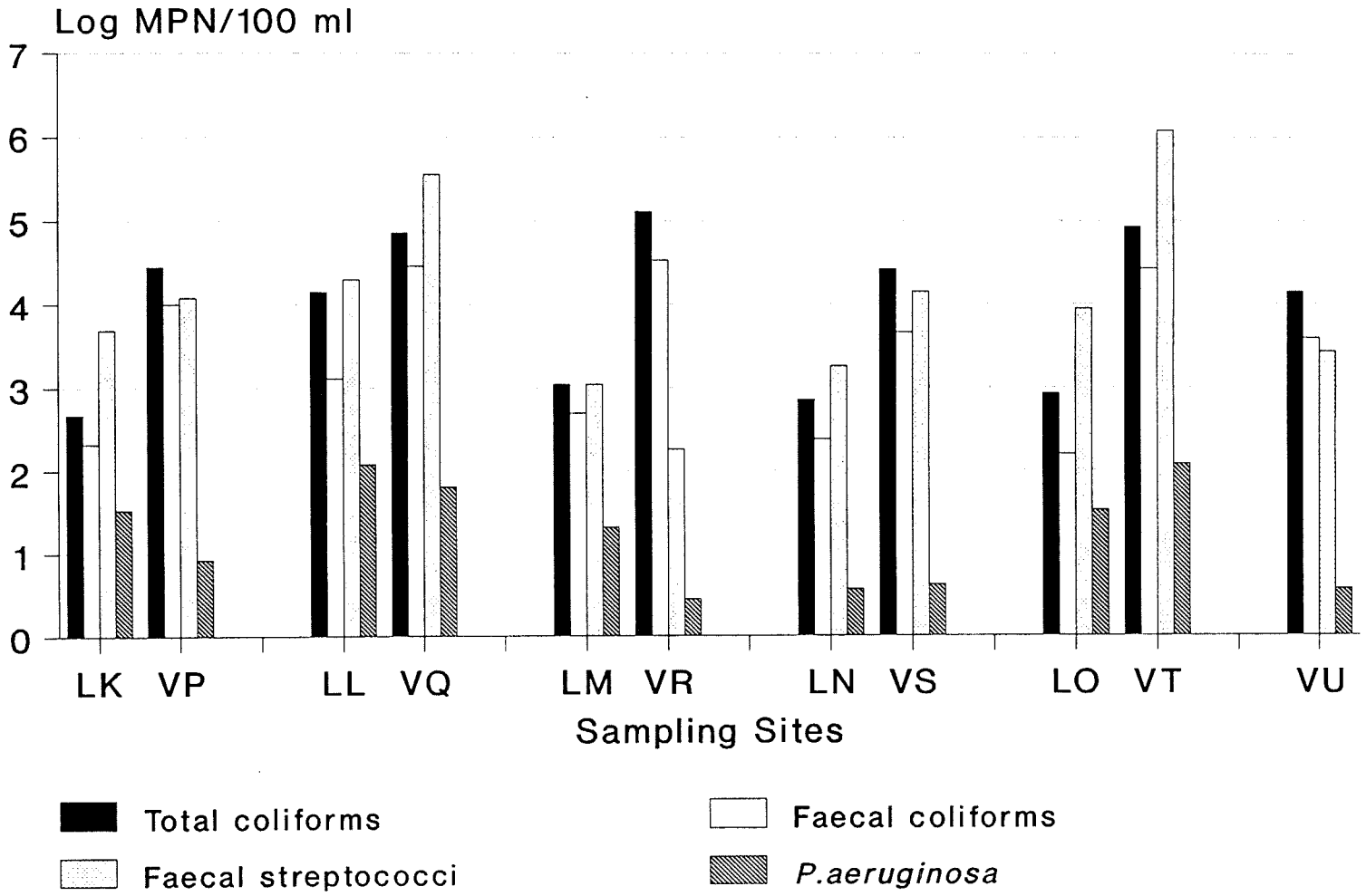


Figure 5.5 Comparison of Bacterial level in Storm Water Runoffs between both North London and Valencia

synthesis of organic materials accumulating on the surface.

Several other investigators (Burm and Vaughan, 1966; Benzie and Courchaine, 1966; Geldreich *et al.*, 1968; Gannon and Busse, 1989) have reported high levels of total coliform, faecal coliform and faecal streptococcus found in stormwater. Geldreich *et al.*(1968) reported seasonal variations (with autumn densities higher than winter) in the levels of indicator bacteria in storm water. Benzie and Courchaine (1966) as well as Qureshi (1978) found that the levels of coliforms were lower in storm water runoff during the winter and early spring than for the warmer weather periods. Similar results were obtained in the present study as the total coliform, faecal coliform and faecal streptococcus densities were considerably higher (in Valencia) in samples obtained during summer than those collected in winter periods (North London).

Comparison of FC/FS ratios in stormwater runoff from different sampling sites showed a distinct difference between North London and Valencia. In North London sites, most samples had FC/FS ratios less than 0.7; thus the pollution was highly likely to be of animal origin according to Geldreich and Kenner, 1969. In Valencia, three samples had FC/FS ratios greater than 4.0 at the public car park site (VR) with two samples greater than 4.0 at the polytechnic campus site (VQ) as well as other samples being FC/FS ratios between 4.0 and 0.7. According to Geldreich and Kenner (1969) 's suggestion, there would be slightly more of human pollution origin in Valencia than London.

Some published literature has reported conflicting information in respect of FC/FS ratios. Hussong *et al.* (1979) for example, found the FC/FS ratios in waterfowls faeces to be similar to those of human faeces. Wheater *et al.* (1979) found FC/FS greater than 4.0 in faecal samples of cattle, sheep, pigs, ducks and turkeys. Olivieri *et al.* (1989) also concluded that the applicability of the conventional FC/FS ratio to the determination of sources of bacterial population in urban runoff is questionable. Therefore the FC/FS ratio should not be accepted unconditionally as a suitable criteria for bacterial pollution, especially in stormwater runoff containing multiple contaminant sources. Based on the results of this study, the comparison of FC/FS ratios between North London and Valencia has nevertheless provided some useful information in respect of the differences in pollutant origins. Most samples had FC/FS ratios of less

than 0.7 in both cities, thus the pollutant was highly likely to be of animal origin.

5.4. Summary

1). High levels of faecal pollution indicator bacteria and notable elevated levels of *P. aeruginosa* were found in stormwater samples from both North London and Valencia. These results indicate that impermeable surface urban storm water discharges can be major sources of intermittent microbial pollution to urban receiving waters. Water bodies receiving untreated storm water are therefore unlikely to be suitable for bathing, swimming and general contact recreation because such discharges can be potential causes of human and animal infections.

2). Higher levels of microorganisms and pathogenic bacteria were found in stormwater runoff within Valencia under warm summer conditions than North London under the cold winter, but for the view of public health hazards, the levels of microorganisms in stormwater runoff in North London are still not acceptable.

3). Distinctive levels of bacteria associated with varying land use activity have been identified. Storm water from high population density urban neighbourhoods having generally poor sanitary conditions contained higher densities of microorganism indicators and of *P. aeruginosa*. The levels of bacteria in storm water from these areas approached levels found in raw sewage. In contrast, stormwater from cleaner low population density neighbourhoods was associated with lower levels of bacteria.

4). The results of the study showed the importance of faecal streptococci rather than total coliforms to be a more realistic microbial indicator of stormwater pollution. The FC/FS ratio can provide some first order, screening information on sources of pollution in urban stormwater.

Chapter 6: Ratios of Faecal coliforms and Faecal streptococci in Stored Water Samples and Bacterial Release from Combined Sewer Sediment

6.1. Introduction

The survival of a microorganism in an environment to which it is not indigenous is dependent upon its ability to withstand physical, chemical and biological conditions. Knowledge of the survival characteristics of indicator microorganisms and pathogenic bacteria in a variety of aquatic environments is of prime importance to public health. Although there have been many studies on the survival of microorganisms in aquatic environments both in situ and in the laboratory (Vasconcelas and Swartz, 1976; Rhodes and Kator, 1988; Lim and Flint, 1989), the characteristics of FC/FS ratios in different urban runoff waters are less well defined. This may, however, proved a useful approach to ascertain whether bacterial pollution of waters is of human or animal origin. The aim of this part of study is therefore to determine the relative change of FC/FS ratios in DWF sanitary wastewater, combined sewage and receiving streams under different controlling temperatures. The methods were carried out as described in section 3.5 of Chapter 3.

Several studies of bacterial survival have been carried out in sediment systems, which have indicated that sediments may play an important role in the survival and distribution of indicator and pathogenic bacteria within the water environment. (Van Donsel and Geldreich, 1971; Matson *et al.*, 1978; Labelle *et al.*, 1980; Laliberte and Grimes, 1982; Burton *et al.*, 1987 and Marino and Gannon, 1991). Sediment results as described in Chapter 4 have also revealed very high levels of indicator and pathogenic bacteria in combined sewers and receiving stream. Apparently, high concentrations of indicator and pathogenic bacteria in the sediments can be due to extended survival (Gerba and McLead, 1976; Goyal and Adams, 1984 and Dan and Stone, 1991). Matson *et al.* (1978) and Grimes (1975) have observed a phenomena of bacterial resuspension / release from the sediment during storms and dredging operations in rivers. These studies have demonstrated that sediments can harbour many bacteria and thus could pose a potential

health hazard as a result of bacterial release from sediment, especially in the case of urban streams which have variable discharges. The present study was undertaken to investigate the pattern of bacterial release from combined sewer sediment under continuous flow condition. The methods were carried out as described in section 3.6 of Chapter 3.

6.2. Results

6.2.1. Ratios of Faecal coliforms and Faecal streptococci in Stored DWF Sanitary Wastewater and Combined Sewage Samples

The significance of die-away rates of FC/FS ratios was investigated in samples from sites LA (combined sewage) and LC (DWF sanitary wastewater, Figs 6.1 and 6.2). FC/FS ratios shown at zero time represent levels recovered at beginning of experiment. Figs. 6.1 and 6.2 illustrate the change of FC/FS ratios over 120 hours at 20°C and 4°C in the dark.

At 4°C, the FC/FS ratio were relatively stable during the 120 hours of the experimental period for combined sewage and DWF sanitary wastewater samples. FC/FS ratios ranged between 1.22 and 1.36 in sanitary wastewater and 1.08 to 1.18 in combined sewage.

At a temperature of 20°C, FC/FS ratios in DWF sanitary wastewater and combined sewage samples showed almost parallel fluctuation trends. In DWF sanitary wastewater, the FC/FS ratios ranged between 1.22 and 1.69 with the ratio on the final day being 1.63. For combined sewage, FC/FS ratios ranged between 1.16 and 1.6 with the ratio on the final day being 1.30.

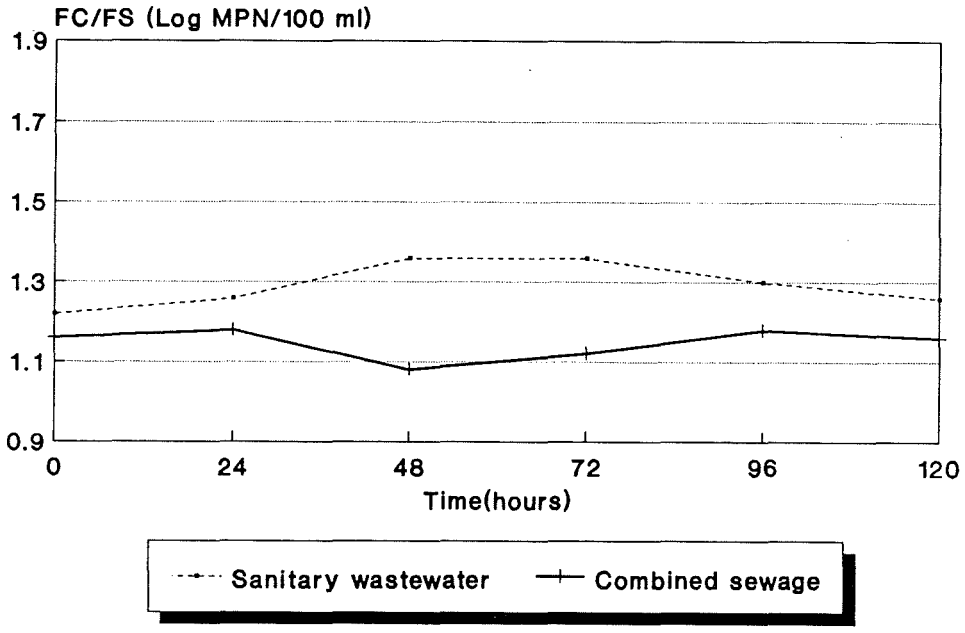


Figure 6.1: Changing Ratios of FC/FS in stored samples at 4°C.

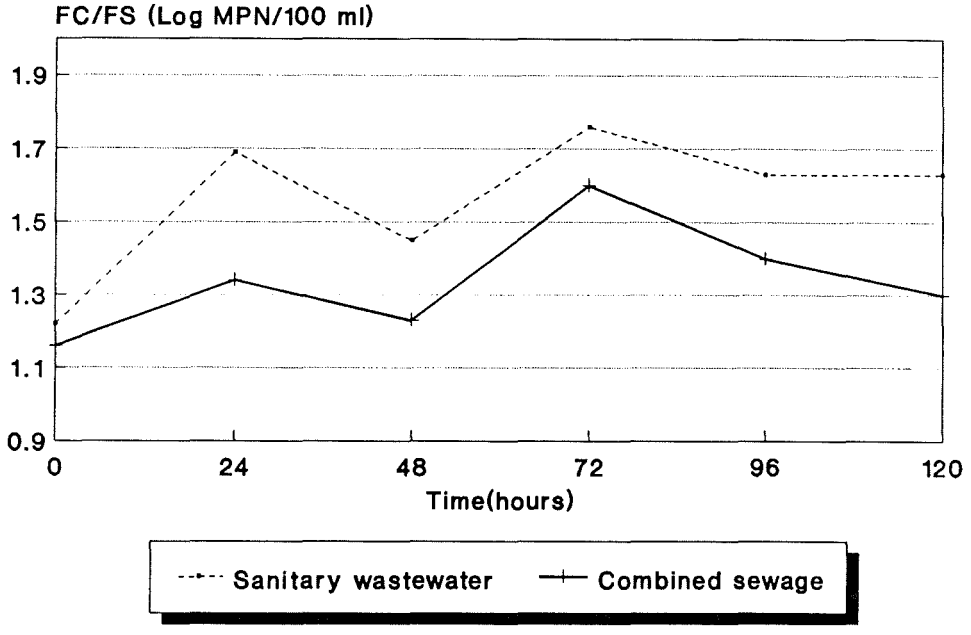


Figure 6.2: Changing Ratios of FC/FS in stored samples at 20°C.

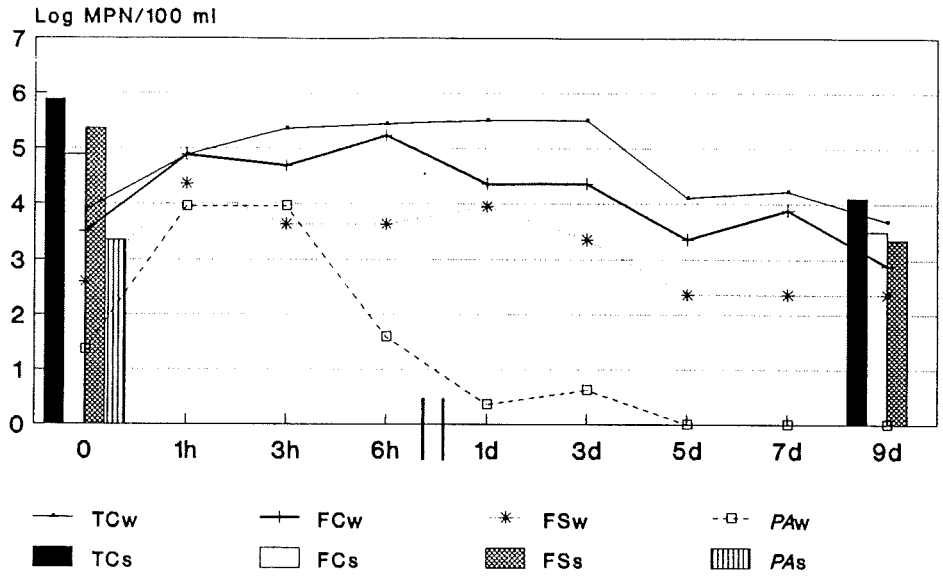
6.2.2. Experiments of Microorganisms Release from Combined Sewer Sediment

The main aim of this part of the study was to investigate the release of bacteria from urban combined sewer sediment. The trends of bacterial release varied among the tested bacteria and the time of the experiments. Fig. 6.3 illustrates the typical release curves observed.

All test bacteria increased sharply in the water column during the first hour (Fig 6.3). The release of total coliform rose continuously until the third day to 3.3 x 10^5 MPN/100 ml, than decreased to 4.9 x 10^3 MPN/100 ml by the ninth day. Faecal coliforms increased up to 1.7 x 10^5 MPN/100 ml within 6 hour, then decreased to 7.9 x 10^3 MPN/100 ml by the ninth day. For faecal streptococci, high levels of bacteria were released from sediment into the water in the first hour, but then gradually declined to 2.3 x 10^2 MPN/100 ml at the end of experiment. The highest release of microorganisms was observed between 1 and 3 hours for *P. aeruginosa*, but the numbers then decreased rapidly and the organisms were not detected after the 3rd day.

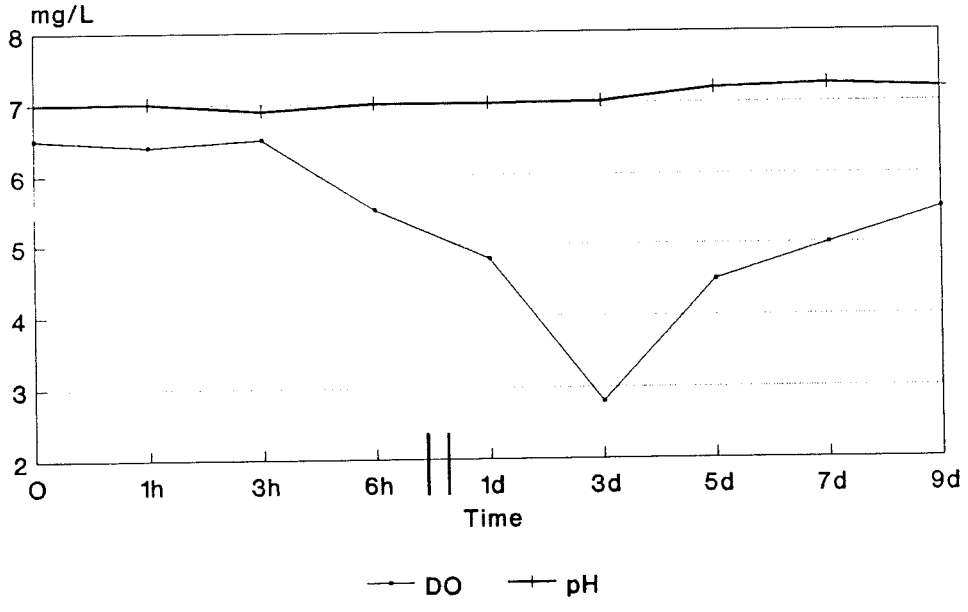
The levels of sediment bacteria also show noticeable changes during the experiment (Fig 6.3). Before the experiment, the levels of total coliforms in sediment were 7.9 x 10^5 MPN/100 ml, faecal coliforms were 7.9 x 10^4 MPN/100 ml, faecal streptococci were 2.3 x 10^5 MPN/100 ml and *P. aeruginosa* were 2.3 x 10^3 MPN/100 ml. At the end of experiment (the ninth day), the levels of total coliform decreased to 1.3 x 10^4 MPN/100 ml, faecal coliform decreased to 3.3 x 10^3 MPN/100 ml, faecal streptococci decreased to 2.3 x 10^3 MPN/100 ml and *P. aeruginosa* was not found. Thus, the levels of indicators had decreased by about 1.5 - 2.0 log MPN/100 ml during the experiment.

Fig. 6.4 shows the level of dissolved oxygen and pH recorded during the experiment. Dissolved oxygen decreased gradually from the third hour (6.5 mg/l) to the third day (2.8 mg/l), then increased by the ninth day to 5.5 mg/l. pH remained stable level during the experiment at about 7.0 ± 0.2.



w:water s:sediment
TC: Total coliforms FC: Faecal coliforms
FS: Faecal streptococci *PA:P.aeruginos*

Figure 6.3: Bacteria Release from sediment of Combined Sewer.



DO: Dissolved Oxygen

Figure 6.4: Dissolved Oxygen and pH Changing Pattern in Water during the experiment

6.3. Discussion

6.3.1. Ratios of FC/FS in Stored Domestic Wastewater and Combined Sewage Samples

Generally, FC/FS ratios show a relatively smaller fluctuation range at 4°C than 20°C in samples of DWF sanitary wastewater and combined sewage during the 120 hours experiments. This is probably the result of low temperature inhibiting bacterial metabolism, thus both faecal coliforms and faecal streptococci present slow die-way rates and FC/FS ratios remain relatively stable. The use of FC/FS ratio as a means of determining whether pollution is of human or animal origin has been questioned by many previous workers (Hussong 1979; Olivieri, 1989). McFeters *et al* (1973) point out that the FC/FS ratios depend upon differential die-away rates of faecal coliform and faecal streptococcus. Faechem (1974) suggested that different die - away rates may increase the value of FC/FS ratio in determining the source of pollution. According to the present study, the temperature dose have an important impact on the FC/FS ratio. FC/FS ratios at a temperature of 4° C remain relatively stable when compared to 20°C for DWF sanitary wastewater and combined sewage.

6.3.2. Bacterial Release from Combined Sewer Sediment

This study showed the patterns of indicator bacteria released from combined sewer sediment and also confirmed that bacteria can be resuspended / released into the water column by activities which disturb the sediment. This confirms the findings noted in previous literature which state that aquatic sediment can serve as a reservoir for indicator bacteria (Sayler, 1975; Gimes, 1980).

Matson *et al* (1978) have found that gradual increases in indicator microorganism levels in water or decreases in sediment can be significantly correlated with river flow. They suggest that the physical characteristics such as river flow at each sampling site regulate the sediment-water equilibrium of indicators. Thus, wherever contaminated sediment is present, the accumulation in and release of bacteria from the sediment is possible. Once

sedimentation occurs, the fate of the bacteria is regulated by their ability to metabolize benthic nutrients, withstand predatory pressure as well as metabolically compete with other microorganisms (Labiberte and Grimes, 1982). This probably explains the phenomenon of variable release rates for different bacteria observed during the experiment. Again, indicator and pathogenic bacteria occur at the highest concentration in the upper layers of sediment, which is more easily resuspended by turbulence. In the present study, most *P. aeruginosa* were released in the first 24 hours, which reflected their mobile characteristics. The changing trends in DO concentrations in the ambient water column reflects the changes recorded in bacterial levels. As the bacterial numbers increase in the water column, dissolved oxygen decreases due to bacterial metabolism, but increases again as bacterial levels decrease in the water column (Fig. 6.4).

High levels of indicator bacteria were still found on the ninth day in the sediment during the experiment. Stable bacterial survival in sediment indicated that nutrients should not be bacterial limiting for at least nine days and confirm extended bacterial survival in sediments.

The data from this experiment also indicate that bacteria can be resuspended easily even under low flow conditions and different amounts of each bacteria can be released at different times. Most of the bacteria reach release peaks within 6 hours. The characteristics of bacterial release do have significance, especially for combined sewage with variable discharges. Bacteria will be released easily when the population of bacteria is unsaturated within the water column. This study suggests that the sediment reservoir allows extended bacterial survival. Furthermore, the sediment of urban receiving streams and combined sewers should be considered of equal importance to bacterial density determinations in the water column; the two are closely related.

6.4. Summary

1). The changing FC/FS ratios depend upon different die-away rates of faecal coliform and faecal streptococcus at different times after the water is contaminated by faeces.

2). There were different changing FC/FS ratios for different kinds of water. The temperature is an important factor which impacts on the ratio. There were relatively small changes in FC/FS ratio at the lower temperatures compared to the higher.

3). Bacteria can be easily resuspended / released from sediment. *P. aeruginosa* were more easily released than other indicator bacteria during the experiment. The peak of bacterial release was found in the first 6 hours. The sediments function as major reservoirs of total coliforms, faecal coliforms and faecal streptococci for at least 9 days and for *P. aeruginosa* for up to 5 days.

Chapter 7: Conclusions.

7.1. Summary of Major Findings.

1). Discharges from urban combined sewage systems contain large quantities of total coliforms, faecal coliforms, faecal streptococci and *P. aeruginosa* as well as a high percentage of *Salmonella*. The geometric mean of samples collected during the various periods of discharge of combined sewage in both North London and Valencia for total coliforms ranged from 1.0 x 10^5 MPN/ 100 ml to 1.7 x 10^7 MPN/100 ml, faecal coliforms ranged from 1.8 x 10^4 MPN/100 ml to 3.3 x 10^6 MPN/100 ml, faecal streptococci ranged from 4.7 x 10^3 MPN/100 ml to 9.8 x 10^4 MPN/100 ml and *P. aeruginosa* ranged from 2.7 x 10^2 MPN/100 ml to 1.8 x 10^4 MPN/100 ml. *Salmonella* was found in 100 % of samples at the combined sewage sites VH and VI in Valencia and in 42.9%, 14.2% and 12.5% and 11% of samples at sites LA, LB, LD and LE respectively in North London. A comparison of geometric mean and percentage indicates that considerably larger quantities of indicator microorganisms, *P. aeruginosa* and *Salmonella* were found in Valencia during the warm summer than North London during the cold winter.

High levels of indicator microorganisms, *P. aeruginosa* and *Salmonella* were recovered at the beach outfall in Valencia. Geometric mean of total coliforms were 4.5 x 10^5 MPN/100 ml, faecal coliforms were 1.7 x 10^5 MPN/100 ml, faecal streptococci were 2.0 x 10^4 MPN/100 ml and *P. aeruginosa* 2.2 x 10^3 MPN/100 ml as well as 83.3 % of samples were found to contain *Salmonella*. Significant levels of indicator microorganisms and *P. aeruginosa* were recovered at receiving stream (LG), total coliforms, faecal coliforms, faecal streptococci and *P. aeruginosa* being 3.0 x 10^4 MPN/100 ml, 7.9 x 10^3 MPN/100 ml, 2.1 x 10^3 MPN/100 ml and 7.0 x 10 MPN/100 ml respectively. No Salmonella was recovered over the time of study at this site.

Comparison of levels of indicator microorganisms and pathogenic bacteria in DWF sanitary wastewater are very similar between North London and Valencia. Total and faecal coliforms were about 10^7 MPN/100 ml, faecal streptococci were about 10^5

MPN/100 ml and *P. aeruginosa* 10^4 MPN/100 ml. *Salmonella* was recovered in sanitary wastewater from 100 % of samples in Valencia and from 87.5 % of samples in North London.

Generally, the quality of urban runoff waters was very poor from a microbiological point of view for both cities. Recently, urban reservoirs and coastal waters are increasingly being utilised for recreational purposes. Such water bodies are subject to intermittent impermeable surface water discharges which have been shown to carry significant levels of microorganisms and pathogens. Primary and secondary contact activities with such waters could therefore carry a risk of ingestion and infection.

2). High levels of indicator microorganisms and *P. aeruginosa* were recovered in sediments from the DWF sanitary wastewater sewer, combined sewer, receiving stream and beach outfall. The highest levels of total coliforms, faecal coliforms, faecal streptococci and *P. aeruginosa* were 5.3 x 10^5 MPN/100 ml, 1.8 x 10^5 MPN/100 ml, 4.6 x 10^4 MPN/100 ml and 5.3 x 10^2 MPN/100 ml respectively in sediment of DWF sanitary wastewater (VH). Total coliforms, faecal coliforms and faecal streptococci were about 10^4 MPN/100 ml, 10^3 MPN/100 ml and 10^3 MPN/100 ml in sediment of combined sewer (LE) and receiving stream (LG) respectively. High levels of *P. aeruginosa* (10^2 MPN/100 ml) in sediment of beach outfall than combined sewer and receiving stream with 10^1 MPN/100 ml. The highest ratios of sediment to water appeared in the receiving stream. The ratios of sediment to water for total coliform was 0.63, faecal coliform was 0.94, faecal streptococcus was 1.38 and *P. aeruginosa* 5.42. The lowest ratios of sediment to water appeared in DWF sanitary wastewater. The ratios of sediment to water were 0.0015, 0.0025, 0.35 and 0.012 for total coliforms, faecal coliforms, faecal streptococci and *P. aeruginosa* respectively. The ratio of sediment to water of faecal streptococci showed the highest value for sites LE, VH and VJ. At site LG, *P. aeruginosa* presented the highest ratios of FC/FS. High levels of *P. aeruginosa* were found (3.8 x 10^2 MPN/100ml) in sediment at receiving stream site (LG) than the combined sewage site (LE, 6.9 x 10 MPN/100ml).

Indicator microorganisms and *P. aeruginosa* occurred at the highest level in the superficial upper layer of sediments. There are distinctive differences in bacterial

distribution found at different depths of sediment. Each bacterial species has its own specific survival rate and unique response characteristics that determine its distribution over depth and time. The sediment reservoir allow indicator microorganisms and *P. aeruginosa* to have extended survival and can be resuspended by flow.

3). Bacterial pollutant loading was considered in this study. This index provided the better evidence of the dynamics of sanitary bacteria in water. From the view point of bacterial loads, the results were not very different between North London and Valencia, as far as combined sewage flows are concerned because flow rates recorded were higher in Valencia than in North London. For DWF sanitary wastewater, the results of bacterial loads were higher in Valencia than North London. The highest bacterial loads were found at a beach outfall in Valencia, which had higher bacterial loads than the equivalent receiving stream in North London. Bacterial loadings are of considerable importance in terms of the management of sewage prior to entry into recreational waters. Of equal importance is the effect of environmental factors on the survival and mortality of pathogens in the receiving water as well as dilution factors and dispersal rates.

4). There were strong positive correlations between levels of total coliforms and faecal coliforms at all sites. There were significant correlations between *P. aeruginosa* and total coliforms, faecal coliforms and faecal streptococci at most of the combined sewage sites.

According to principal component analysis undertaken for six sites, flow presented a negative correlation with microorganisms for sanitary wastewater samples in both North London and Valencia. There were significant positive correlations between flow and microorganisms in combined sewage. There were closer relationships between BOD_5 and microorganisms than flow.

5). There are significant relationships between bacterial content of sewage effluent with time and man's daily social activity for DWF sanitary wastewater sewers in both North London and Valencia.

6). Stormwater runoff from high population density urban neighbourhoods with generally poor sanitary conditions contained high levels of indicator microorganisms and *P.*

aeruginosa. The highest levels of indicator microorganisms and *P. aeruginosa* were found at an open market site in North London and in both residential areas and open market sites in Valencia. The levels of indicator microorganisms and *P. aeruginosa* in the stormwater from these areas approached levels found in raw sewage. Higher levels of indicator microorganisms and *P. aeruginosa* were found in Valencia stormwater during the warm summer periods than in North London over winter.

Storm water from clean low population density neighbourhoods had lower levels of indicator microorganisms and *P. aeruginosa*. Distinctive different levels of bacteria were recorded from different urban areas due to varying nature of urban activity and land use.

7). FC/FS ratios proved to be useful indicators of faecal pollution in urban surface water runoff during the time of study. But based on the experiment of changing FC/FS ratios, the ratio appears to depend upon different die off ratios of faecal coliforms and faecal streptococci in the time following faeces deposition and their incorporation in run off. There was a relatively small changing range of FC/FS ratios under 4°C in sanitary wastewater and combined sewage than at 20°C conditions.

FC/FS ratios for road surface runoff are low which suggests more animal sources than human in both cities. In contrary, FC/FS ratios in combined sewage are suggesting of more human than animal sources. The receiving stream showed mixed characteristics of FC/FS ratios. It is clear that the ratios of FC/FS must be used with care and certainly do not provide undisputed indicators of pollution source.

8). Indicator microorganisms and *P. aeruginosa* can be resuspended and released from sediment. The highest release peak of total coliforms were 3.3×10^5 MPN/100 ml, faecal coliforms were 1.7×10^5 MPN/100 ml over 6 hours, faecal streptococci were 2.3×10^4 MPN/100 ml on the third day (72 hours) and 9.3×10^3 MPN/100 ml for *P. aeruginosa* in the first hour. High levels of indicator microorganisms were found on the ninth day indicating that aquatic sediment can serve as a reservoir and can lead to extended survival. These observations indicate that the quality of sediment microbiology should be investigated as part of the evaluations for public health safety associated with sewage discharges.

7.2. Suggestions for Further Work.

1). The studies described in Chapter 4 have indicated the bacterial loading rates associated with urban stormwater runoff. It would be of considerable interest to undertaken further investigations on the loading characteristics of bacteria found in urban surface water runoff, associated recreational reservoirs and at beach outfalls. Such discharges and receiving waters have significance for public health. Further research should be taken to study a catchment and its hydrology in conjunction with the microorganisms which the living populations of the catchment introduce into the various water flows. If an understanding can be gained as to mechanisms controlling the input of faecal contaminants into the water flow, and the manner in which the concentrations of these contaminants fluctuate, it will be possible to taken rational steps to control the water environmental capacity.

2). Chapter 6 has outlined the results of bacterial release experiments carried out with slow flow in a water tank. Further studies should be taken to examine bacterial release under variable flow conditions associated physico - chemical factors for both water and sediment column to determine the relationships between bacterial level and controlling environmental factors. The different kinds of sewer sediment also need to be examined, especially for storm drain sediment which serve as reservoirs of faecal bacteria, thus increasing the health hazard to receiving waters. This could enable a modelling of bacterial release in urban aquatic ecosystems and help an understanding of their mechanisms under more realistic and mobile conditions. Studies of bacterial contribution in different layers of sediment from different kinds of sewer also need to be carried out to determine the characteristics of bacteria accumulation and transportation for systems of different ages, transporting capacities and network design.

3). The results described in this thesis indicate limited information on how physical and chemical changes affect microbiological populations in urban surface water runoff. In order to further understanding the survival and die - off of microorganisms in aquatic environment, it is very important to know more about the complex interrelationships of environmental factors and their influence on microbiological population dynamics. The

mortality rate of bacteria in natural waters is governed by many factors, including predation, toxic compounds, salinity and temperature. None of these exert the same degree of lethality on all organisms. In any particular situation mortality rates are difficult to establish for even one group of organisms since the ability to resist hostile environments depends on physiological function. Further work should be undertaken to relate microbiological populations in water to environmental factors and develop a mathematical model in order to understand the mortality rate of bacteria in water. The importance of organic particulate matter as a predictor of microorganism and pathogenic bacterial changes also suggests a need for a detailed analysis of the sources that contribute to the particular load.

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