Chapter – V

MONITORING OF PATHOGENIC INDICATOR ORGANISMS

5.1 INTRODUCTION

At any one time, 1.5 billion people worldwide are estimated to suffer from waterborne diseases and 3.4 million people die from directly or indirectly consuming water contaminated with bacterial, eukaryotic parasitic or viral pathogens (WHO/UNICEF 2000). Surface water bodies are presumed to be more susceptible to fecal contamination than groundwater reservoirs due to the absence of natural soil protection, filtration and possibly short distances between the occurrence of contamination and water extraction. It is argued that especially in the case of heavy rainfall the microbial loads of running water may suddenly increase substantially and reach reservoir bodies very quickly (Kistemann et al 2001). For this reason, monitoring microbiological raw water quality is an essential component of the protection strategy in catchment areas of surface drinking water reservoirs (Exner and Tuschewitzki 1993). The bacteriological quality of natural water is determined using indicators (faecal coliforms, *E. coli* and faecal *Streptococci*) their presence being associated with faecal contamination and which could imply the presence of human pathogenic agents. However, the use of these indicators are contested because the occurrence of allochthonous microorganisms and their survival in surface water is dependent on species, as well as environmental conditions and the presence of other organisms, which may be predators or antagonists. The permanency and metabolic stability of indicators and pathogens in water seems to be associated particularly with water parameters such as temperature, pH, solar radiation, electrical conductivity and salinity. Climate conditions, rainfall and catchment area runoff are also of great importance. *Salmonellae* are water and food borne pathogens frequently isolated from contaminated surface water and is amongst the main cause of high rates of intestinal infections in

developing countries. Recently, there has been much literature produced about listeria and listeriosis in developed countries where outbreaks and epidemics have been detected (Schaffter and Parriaux 2002). Pathogens are disease-causing microorganisms that are a serious threat to public health (Binderup et al 2002; Hunter 2003; Balbus et al 2004). Pathogens considered representative of those associated with waterborne disease include enteric viruses derived from human fecal contamination, bacterial pathogens, represented by *Escherichia coli* O157:H7 and the protozoan pathogens *Cryptosporidium* and *Giardia* (Ferguson et al 2003).

5.2 HISTORY OF MICROBIAL CONTAMINANTION

Microorganisms are present everywhere in our environment, soil, air, food and water. Also called microbes, microorganisms are living organisms, generally observable only through a microscope. Our exposure to them causes harmless microbial flora to establish in our bodies, although some microbes are pathogens and can cause diseases. These diseases are considered waterborne. If, the pathogens are transmitted by water, to infect humans or animals that ingest the contaminated water (Table 5.1). Diseases transmitted by water are primarily those found in the intestinal discharges of humans or animals coupled with presence of microbial contaminants in drinking water have plagued humans throughout history. Lin et al (1974) investigated bacterial assessment of Spoon river water quality. By using fecal coliform to fecal *streptococcus* ratios to sort out fecal pollution origins, it was evident that a concern must be expressed not only for municipal wastewater effluents to the receiving stream, but also for non-point sources of pollution in assessing the bacterial quality of a stream. Ambient water quality regulations make use of bacterial indicators because the density of an indicator in the water can be quantitatively linked potential human health risks (Cabelli 1983). The United States Public Health Services (USPHS) first conducted coliform indicator criteria on studies during 1940's and early 1950's. In the 1960's the USPHS adopted the fecal coliform standard by using a fixed ratio (18%) of fecal coliform to total

coliform bacteria (USEPA 1986). Indicator organisms are used as diffused pollution indicator as well. Maul and Cooper (2000) used enterococci and fecal coliform bacteria concentrations to assess the variability of water quality in an agricultural field during wet weather. Aitken (2003) investigated the potential risk of fecal contamination due to diffuse pollution on river catchments and coastal bathing water using indicator organisms. Indicator organisms are often used as a tool to identify the contaminant sources. Whitlock et al (2002) used fecal coliform to identify the contaminant sources in an urban watershed. The presence of *Escherichia coli*, a more common microbial constituent used for water quality examination indicates fecal contamination, since *E. coli* is the subset of fecal coliform. Fecal *streptococci* are also often used as an indicator. The U.S. EPA has published a protocol for developing pathogen TMDLs that provides guidance for this process (USEPA 2001).

Pathogen levels of surface water are sun light sensitive and are related to many other factors including temperature, salinity, moisture, soil condition, water body condition, and encystations. Bacteria show an affinity towards inorganic particle surfaces, and the role of chemical factors such as the pH and ionic strength of the solution is also important (Yee et al 2000). Cell factors such as hydrophobicity (Gannon et al 1991a) and the mineralogy and surface properties of the particles (Scholl et al 1990; Mills et al 1994) all influence the adsorption process. The settling and scouring process associated with suspended solids also affects the pathogen level of the water. Visits by people and livestock to surface water systems are common in developing countries, particularly in poor rural communities where most residents lack access to portable clean water. As a result, they usually obtain water for their daily needs from surface water systems that are often contaminated (Nevondo and Cloete 1999; Venter 2001; Obi et al 2002) and Table 5.2 describes detailed information about historical events in the provision of safe drinking water.

5.3 EMERGING CONTAMINANTS

Water borne microbial contaminants, however, have attracted renewed attention, both within the scientific community and among the public. Once thought to be under control, they are now referred to as the "emerging drinking water contaminants". What in fact is emerging is an expanded awareness of the presence of previously undetected microbial contaminants in drinking water and their effects on human health. The most frequently used microbial indicators of water quality are total coliforms (TC), fecal coliforms (FC) and fecal *streptococci* (FS), all considered indicators of recent fecal contamination (Godfree et al 1997).

Fecal contamination of water is considered a human health risk and there has always been a great deal of concern regarding the level of coliform bacterial counts in water. To establish a relationship between the concentrations of fecal indicators and the risk of illness upon using contaminated water, many epidemiological studies have been conducted (McBride et al 1998; Van Asperen et al 1998; Zamxaka et al 2004). Unsanitary means of disposing human waste and faecal droppings from livestock are routes through which faecal matter may enter aquatic systems. Faecal matter degrades water quality due to the possible introduction of pathogens, nutrients and organic matter (Vinneras et al 2003; Langergraber and Muellergger 2005; Vikaskumar et al 2007). To minimize health risk, it is often required to undertake regular monitoring of indicator parameters in aquatic systems (Kong et al 2002; McLellan and Salmore 2003; Noble et al 2003; Shah et al 2007). Such assessment studies are useful not only for evaluating health risk, but also for determining the course of action that may be required to solve the problem (Parveen et al 2001; Ahmed et al 2007; Graves et al 2007). In watershed management, the impacts of non-point pollution on the receiving water body are evaluated with consideration of its influence on the ecosystem and human health (Bryce et al 1999; Holas and Hrncir 2002). The origins of pathogens are various potential point pathogen sources like Waste Water Treatment Plants (WWTPs)

(Lipp et al 2001; Exall et al 2004), Combined Sewer Overflows (CSOs), Separated Sewer Overflows (SSOs) (Charles et al 2003), slaughterhouses, and animal feedlots (Gessel et al 2004). Potential non-point sources are illicit sewage connections, wildlife, septic systems, livestock, landfills, and pastures. Land application of manures or sludge is also one of the potential sources (McMurry et al 1998).

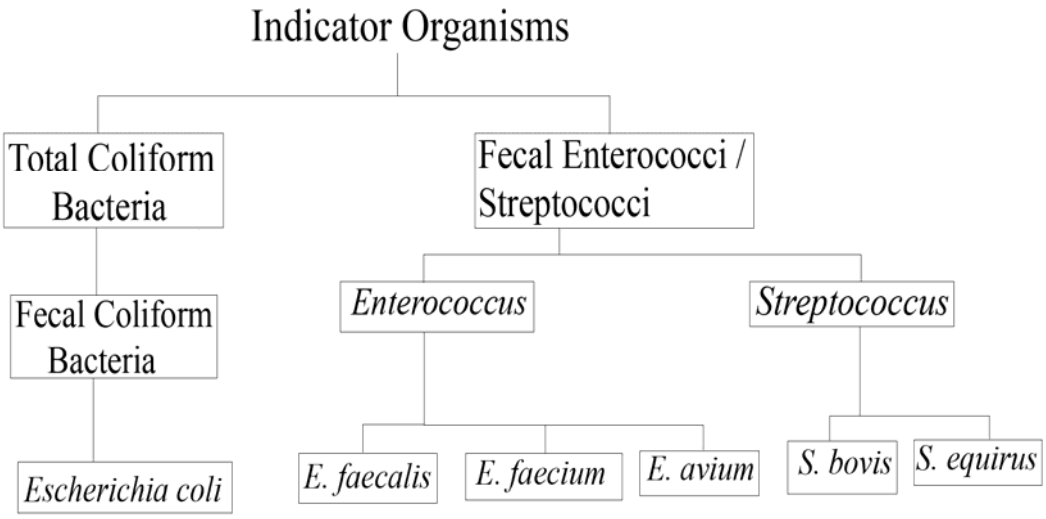

Figure 5.1 Species and relationship among indicator organisms

Fig 5.1 shown the relationships between indicator organisms (after EPA 2001), as faecal coliform is a subgroup of total coliform, it includes several species of coliform bacteria. The presence of *Escherichia coli*, common water quality examination constituent in microbial aspect, includes faecal contamination since *E. coli* is the subset of faecal coliform. Pathogen levels in the water can be estimated by measuring the pathogenic indicators concentration.

Pathogen levels in the water can be estimated by measuring the pathogen indicator concentration. Pathogen indicator organisms, often called indicator organisms, refer to pathogen associated microorganisms, typically chosen for easier isolation and identification of contamination. The indicator organism (i) should be easily detectable using simple laboratory tests; (ii) should not generally be present in

unpolluted water; (iii) should appear in concentrations that can be correlated with the extent of contamination; (iv) should have a die-off rate that is not faster than the die-off rate for the pathogens of concern (Thomann and Mueller 1987; USEPA 2001). Pathogen levels in surface water are regulated in many countries to guarantee water quality for recreational use, drinking water supply, and aquatic life protection. United Nation Environmental Programme (UNEP) and World Health Organization (WHO) have established criteria for coliform concentration for primary contact recreation purposes. Fecal coliform concentration of geometric mean of at least five samples should be less than 100/100 ml for 50% and less than 1000/100 ml for 90%. The U.S. EPA requires *E. coli* density to be less than 126/100 ml in fresh water to the logarithmic average for a period of 30 days of at least five samples (USEPA 1999). Tables 5.3 and 5.4 describe the international and national (BIS) drinking water standards or guidelines.

Accordingly, there is an emerging trend toward a risk-management, multiple barrier approach to providing safe drinking water. At present, *E. coli* appears to provide the best bacterial indication of faecal contamination in drinking water. This is based on:

1. The prevalence of thermotolerant (faecal) coliforms in temperate environments as compared to the rare incidence of *E. coli*
2. The prevalence of *E. coli* in human and animal faeces as compared to other thermo-tolerant coliforms
3. The availability of affordable, fast, sensitive, specific and easier to perform detection methods for *E. coli*.

Table 5.1. Bacterial species and related diseases

Name of bacteria	Major diseases
Escherichia coli	Urinary tract infection (UTI), enterotoxin, gastroenteritis, Traveler's diarrhoea, food born disease and vomiting
Pseudomonas aeruginosa	Opportunistic infection in man, giving rise to inflammations of middle ear and greenish pus,
Shigella spp	Acute bacillary dysentery, abdominal cramps and fever
Salmonella spp	Typhoid fever, diarrheal disease, gastroenteritis and Food borne disease
Vibrio cholerae	Epidemic cholera and diarrheal disease
Vibrio parahaemolyticus	Wound or ear infections, Food poisoning, gastroenteritis and diarrhoea
Streptococcus faecalis	Nonspecific diarrhoea, epididymal infections and nervous system infections

(Source: Rusin et al 1997; Stevens et al 2001; Kim et al 2005)

Table 5.2. Historical events in the provision of safe drinking water

1820's	The sand filter was put into use by James Simpson in the U.K. to clarify drinking water.
1852	Filtering water became law in London, England.
1854	John Snow linked the cholera outbreak in London to a specific water pump based on epidemiological evidence.
1881	Koch showed that pure cultures of bacteria were destroyed by hypochlorites.
1885	Frankland reported the first routine examination of water in London, using gelatin plate counts.
1887	Escherichia found *Bacillus coli* (now *E. coli*) ubiquitous in human faeces.
1890	Koch published the *Germ theory of disease*, linked the cholera outbreak to *Vibrio cholerae* in water.
1891	The Franklands introduced the concept of bacterial indicators. Recognizing that water can be a source of disease and that sick people shed pathogens along with their normal faecal flora. They concluded, "Organisms characteristic of sewage must be identified to provide evidence of potentially dangerous pollution."
1892	Schardinger in Australia suggested the use of *E. coli* as an indicator in water monitoring.
1893	Blachstein coins the term coliform.
1895	Smith independently introduced the use of *E. coli* and related organisms to indicate the possible presence of enteric pathogens in water in the US.
1904	Eijkman found a highly selective detection method for *E. coli*, using elevated temperatures, 44-46 ˚C.
1907	Winslow and Walker report that *E. coli* is largely faecal in origin while other coliforms are not.
1914	US Public Health Service Drinking Water Standard: one in five 10 ml samples can be *E. coli*$^{+}$.
1915	US Public Health Service standard tests for coliforms rather than *E. coli* based on assumption that coliforms are equally valid indicators of faecal contamination.

1919	Typhoid fever outbreak in Pforzheim (Germany) 400 deaths; drinking water proven as source. Led to establishing protected areas as sources of drinking water and decontamination of the water.
1948	Mackenzie finds that most coliforms are indole negative while *E. coli* is indole positive.
1948–1950	UK tests for *E. coli* with indole test; US uses faecal coliform (acid and gas at 44˚C).
1930–1950	Most developed countries introduce chlorination.
1974	Safe Drinking Water Act passed in US requiring US EPA to set enforceable standards for health-related drinking water contaminants.
1980	European Union (EU) establishes standards – Drinking Water Directive 80/778/EEC to apply to all water intended for human consumption.
1998	The EU Council Directive 98/83/EC on the quality of water for human consumption adopts *E. coli* and enterococci as microbiological parameters.
2003	The WHO recommends *E. coli* as the best indicator of faecal contamination.

(Source: Szewzyk et al 2000; Ashbolt et al 2001; Leclerc et al 2001; WHO 2003)

5.4 INDICATIVE ORGANISM OF FAECAL POLLUTION

5.4.1 Total coliforms

In a worldwide survey, 23 laboratories isolated over 1000 strains of coliforms from various types of water. It was found that 61% of the total numbers examined were non-faecal in origin (Gavini et al 1985). Total coliforms are classically defined as "All facultative anaerobic, gram negative, non spore forming, oxidase negative, rod shaped bacteria that ferment lactose to acid and gas within 48 hr at 35˚C or members of *Enterobacteriaceae* which are β-galactosidase positive" (APHA 1998). Blachstein coined the term "coliform" in 1893 to include bacteria resembling *E. coli* that are present in faeces and at the description above (Kornacki and Johnson 2001). It originally included a few well defined species, but the majority of coliforms were considered atypical (Leclerc et al 2001). With the development of more precise means to distinguish between species, the coliform group was redefined to be the

β-galactosidase-positive *Enterobacteriaceae* (Stevens et al 2001). Total coliforms are generally considered unreliable indicators of faecal contamination because many are capable of growth in both the environment and in drinking water distribution systems (LeChevallier 1990; Camper et al 1991; Szewzyk et al 1994). Within a system, coliforms are able to take advantage of the improved survival offered by indigenous biofilms (Power and Marshall 1988; LeChevallier 1990).

Total coliforms (TC) and fecal coliforms (FC) counts are the most widely used bacteriological procedures for assessment of drinking and surface water quality (Mcdaniels et al 1985). The TC bacteria test is a primary indicator of potability, suitability for consumption of drinking water. It measures the concentration of TC bacteria associated with the possible presence of disease causing organisms (Craun 1978). FC are selected members of the coliform group of bacteria which are able to ferment lactose at 44.5 ˚C are fairly specific for the feces of warm blooded animals and are commonly used as indicators of fecal pollution in water such as wastewater effluents, rivers, marine environments, recreational water and raw sources of drinking water supplies (Geldreich 1978). Instead, the EPA has designated TC bacteria as a standard to determine bacterial safety of water found in their wastes. The EPA maximum contaminant level (MCL) for coliform bacteria in drinking water is zero TC/100 ml (APHA 1998).

5.4.2 Vibrio like organisms

Vibrio spp are small, curved (comma shaped), Gram negative bacteria with a single polar flagellum. Species are typed according to their O-antigens. There are a number of pathogenic species, including *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*. *Vibrio cholerae* is the only pathogenic species of significance from freshwater environments. While a number of serotypes can cause diarrhoea, only O1 and O139 currently cause the classical cholera symptoms in which a proportion of

cases suffer fulminating and severe watery diarrhoea features such as the ability to produce a dialyzable heat labile haemolysin, active against sheep and goat red blood cells. The classical biotype is considered responsible for the first six cholera pandemics, while the El Tor biotype is responsible for the seventh pandemic that commenced in 1961. Strains of *V. cholerae* O1 and O139 that cause cholera produce an enterotoxin (cholera toxin) that alters the ionic fluxes across the intestinal mucosa, resulting in substantial loss of water and electrolytes in liquid stools. Other factors associated with infection are an adhesion factor and attachment pilus. Not all strains of serotypes O1 or O139 possess the virulence factors, and they are rarely possessed by non-O1/O139 strains.

5.4.3 *Escherichia coli*

E. coli was first introduced as an indicator of faecal contamination in 1893. In 1907, Winslow and Walker made the critical observation that *E. coli* was predominantly of faecal origin while other coliforms were not important (Ashbolt et al 2001). Because the known detection method at that time was inappropriate for routine testing for *E. coli*, thus coliforms were commonly used as an indicator instead. The recent identification of the β-glucuronidase enzyme being specific to 94–97% of *E. coli* and Huang et al (1997) allowed the development of detection methods that are simple, rapid, specific and sensitive. The possibility for direct detection of *E. coli* has led to a resurgence of interest in these bacteria as indicators of faecal pollution. Numerous studies have shown that methods available for *E. coli* are more accurate, specific and sensitive than those for thermotolerant coliforms (Feng et al 1982; Martins et al 1993; Huang et al 1997; Stevens et al 2001).

Table 5.3 International drinking water standards or guidelines

Parameter	Ontario	Canada	United States	Australia	New Zealand	United Kingdom	EU Directive	WHO
Guidelines or standards	S	G	S	G	S	S	S	G
Total coliforms	0/100 ml	0/100 ml	0/100 ml	0/100 in 95%		0/100 ml	0/100 ml	
Thermotolerant coliforms or *E. coli*	0/100 ml		0/100 ml	0/100 ml in 98%				0/100 ml
E. coli		0/100 ml	0/100 ml	0/100 ml	0/100 ml	0/100 ml	0/100 ml	0/100 ml
Enterococci					0/100 ml	0/100 ml		
Cryptosporidium parvum			99 % removal or inactivation			<1 oocyte / 10 L		
Clostridium perfringens including spores					0/100 ml			
Pseudomomas aeruginosa						0/250 ml		
Colony count 22˚ C					No abnormal Change	No abnormal change		
Colony count 37˚ C	<500 cfu/ ml	<500 cfu/ ml			No abnormal change	20/ ml		

(Source: Tallon et al 2005)

E. coli is a member of thermotolerant coliforms that produce indole from tryptophan, but is also defined now as coliforms with the enzyme β-glucuronidase. *E. coli* is an indigenous member of the intestinal flora of healthy humans and warm blooded animals, and comprises about 1% of the total bacterial biomass (Leclerc et al 2001). It is consistently found in larger numbers than the KEC coliforms, *Citrobacter, Klebsiella*, and Enterobacter in the faeces of humans, farm animals and pets. Only a small group of *E. coli* strains causes disease. The majority of strains are the natural inhabitants, commensal bacteria of the gastrointestinal tract of warm blooded animals (Salyers and Whitt 2002). There is often confusion around this, as many members of the public media discussing disease outbreaks do not make the distinction and simply refer to "*E. coli*" as the cause of the outbreak. The diarrheic disease causing strains in humans can be classified as belonging to one of six groups: enteropathogenic (EPEC); enterotoxigenic (ETEC); enteroinvasive (EIEC); enterohemorrhagic (EHEC); enteroaggregative (EAggEC); and diffusely adherent strains (DAEC).

Baudizˇsov´a (1997) also found that the other thermotolerant and total coliforms were capable of growth in non polluted river water while *E. coli* was not, and supports a recommendation for *E. coli* to be used as the sole indicator bacteria for recent faecal contamination. Although there is much support for *E. coli* as the definitive indictor of faecal pollution, some studies have shown that high concentration of *E. coli* can be found in tropical natural water systems (Carrillo et al 1985; Lopez -Torres et al 1987; Jimenez et al 1989). The effluents were coming from pulp and paper mills with no known sources of fecal contaminations (Archibald 2000; Gauthier et al 2001). Furthermore, it is recognized that *E. coli* may not be suitable as an indicator of some specific enteric pathogens.

5.4.4 *Salmonella spp*

Salmonella spp. belongs to the family *Enterobacteriaceae*. They are motile, Gram-negative bacilli that do not ferment lactose, but most produce hydrogen sulfide or gas from carbohydrate fermentation. Originally, they were grouped into more than 2000 species (serotypes) according to their somatic (O) and flagellar (H) antigens (Kauffmann White classification). It is now considered that this classification is below species level and that there are actually not more than 2–3 species (*Salmonella enterica* or *Salmonella choleraesuis*, *Salmonella bongori* and *Salmonella typhi*), with the species being subspecies. All of the enteric pathogens except *S. typhi* are members of the species *S. enterica*. Convention has dictated that subspecies are abbreviated, So that *S. enterica* serovar paratyphi A becomes *S. paratyphi* A.

5.4.4.1 Human health effects

Salmonella infections typically cause four clinical manifestations: gastroenteritis (ranging from mild to fulminant diarrhoea, nausea and vomiting), bacteraemia or septicaemia (high spiking fever with positive blood cultures), typhoid fever/enteric fever (sustained fever with or without diarrhoea) and a carrier state in persons with previous infections. About enteric illness, *Salmonella spp* can be divided into two distinct groups: the typhoidal species (*Salmonella typhi* and *S. paratyphi*) and the remaining non-typhoidal species. Symptoms of non-typhoidal gastroenteritis appear from 6 to 72 hr after ingestion of contaminated food or water. Diarrhoea lasts 3–5 days and is accompanied by fever and abdominal pain. Usually the disease is self limiting. The incubation period for typhoid fever can be 1–14 days but is usually 3–5 days. Typhoid fever is a more severe illness and can be fatal. Although typhoid is uncommon in areas with good sanitary systems, it is still prevalent elsewhere, and there are many millions of cases each year.

5.4.4.2 Source and occurrence

Salmonella spp. is widely distributed in the environment, but some species show host specificity. Notably, *S. typhi* and generally *S. paratyphi* are restricted to humans, although livestock can occasionally be a source of *S. paratyphi*. A large number of species, including *S. typhimurium* and *S. enteritidis*, infect humans and a wide range of animals, including poultry, cows, pigs, sheep, birds and even reptiles. The pathogens typically gain entry into water systems through faecal contamination from sewage discharges, livestock and wild animals. Contamination has been detected in a wide variety of foods and milk.

5.4.4.3 Significance in drinking water

Waterborne typhoid fever outbreaks have devastating public health implications. However, despite their widespread occurrence, non-typhoidal *Salmonella spp* rarely causes drinking water borne outbreaks. Transmission, most commonly involving *S. typhimurium*, has been associated with the consumption of contaminated groundwater and surface water supplies. In an outbreak of illness associated with a communal rainwater supply, bird faeces were implicated as a source of contamination. *Salmonella spp* is relatively sensitive to disinfection. Within a water safety plan (WSP), control measures that can be applied to manage risk include protection of raw water supplies from animal and human waste, adequate treatment and protection of water during distribution. *Escherichia coli* or (alternatively, thermotolerant coliforms) is a generally reliable index for *Salmonella spp* in drinking water supplies.

5.4.5 *Shigella spp*

Shigella spp is Gram negative, non-spore forming, non-motile, rod like members of the family Enterobacteriaceae, which grow in the presence or absence of oxygen. Members of the genus have a complex antigenic pattern, and classification is based on their somatic O antigens, many of which are shared with other enteric bacilli,

including *E. coli*. There are four species: *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei*, which cause human health effects.

Shigella spp can cause serious intestinal diseases, including bacillary dysentery. Over 2 million infections occur each year, resulting in about 6 lakhs deaths, predominantly in developing countries. Most cases of *Shigella* infection occur in children under 10 years of age. The incubation period for *shigellosis* is usually 24–72 hr. Ingestion of as few as 10–100 organisms may lead to infection, which is substantially less than the infective dose of most other enteric bacteria. Abdominal cramps, fever and watery diarrhoea occur early in the disease. All species can produce severe disease, but illness due to *S. sonnei* is usually relatively mild and self limiting. In the case of *S. dysenteriae*, clinical manifestations may proceed to an ulceration process, with bloody diarrhoea and high concentrations of neutrofils in the stool. The production of Shiga toxin by the pathogen plays an important role in this outcome. *Shigella spp* seems to be better adapted to cause human disease than most other enteric bacterial pathogens.

5.4.5.1 Source and occurrence

Humans and other higher primates appear to be the only natural hosts for the *shigellae*. The bacteria remain localized in the intestinal epithelial cells of their hosts. Epidemics of shigellosis occur in crowded communities and where hygiene is poor. Many cases of shigellosis are associated with day care centers, prisons and psychiatric institutions. Military field groups and travelers to areas with poor sanitation are prone to infection.

5.4.5.2 Significance in drinking water

A number of large waterborne outbreaks of shigellosis have been recorded. As the organisms are not particularly stable in water environments, their presence in

drinking water indicates recent human faecal pollution. Available data on prevalence in water supplies may be an underestimate, because detection techniques generally used can have a relatively low sensitivity and reliability. The control of *Shigella spp* in drinking water supplies is of special public health importance in view of the severity of the disease caused. *Shigella spp* is relatively sensitive to disinfection. Within a water safety plan (WSP), control measures that can be applied to manage potential risk include protection of raw water supplies from human waste, adequate treatment and protection of water during distribution. *Escherichia coli* (or, alternatively, thermotolerant coliforms) is a generally reliable index for *Shigella spp* in drinking water supplies.

5.4.6 Faecal *Streptococci* and *Enterococci*

Parallel to the work on coliforms, a group of Gram positive coccoid bacteria known as faecal *streptococci* (FS) were being investigated as important pollution indicator bacteria (Houston 1900; Winslow and Hunnewell 1902). Problems in differentiating faecal from non-faecal *streptococci*, however, initially impeded their use (Kenner 1978). Four key points in favour of the faecal streptococci were:

(1) Relatively high numbers in the excreta of humans and other warm-blooded animals.

(2) Presence in wastewater and known polluted water.

(3) Absence from pure water, virgin soils and environments having no contact with human and animal life.

(4) Persistence without multiplication in the environment.

It was not until 1957, however with the availability of the selective medium that enumeration of FS became popular (Slanetz and Bartley 1957). Since then, several media have been proposed for FS and/or enterococci to improve on the specificity.

Taxonomically FS are represented by various Enterococcus spp and *Streptococcus bovis* and *S. equinus* (WHO 1997). Of the faecal streptococci, the preferred indicators of faecal pollution are the enterococci. The predominant intestinal enterococci being *E. faecalis*, *E. faecium*, *E. durans* and *E. hirae*, in addition, other *Enterococcus* species and some species of *Streptococcus* (namely *S. bovis* and *S. equinus*) may occasionally be detected. These *streptococci* however, do not survive for long in water and are probably not enumerated quantitatively. Thus, for water examination purposes enterococci can be regarded as indicators of faecal pollution, although some could occasionally originate from other habitats.

5.4.6.1 Significance of the thermotolerant coliform: faecal streptococci ratio

Geldreich and Kenner (1969) proposed that a faecal coliform: faecal streptococci ratio of four or greater may indicate human pollution, whereas ratios of two or less may indicate animal pollution. There are many factors, however, that can jeopardise the usefulness of this ratio. Foremost is the quicker die off of coliforms in the environment and different counts from various media used for bacterial isolation (Geldreich 1976). Hence, the use of this ratio is no longer recommended unless very recent faecal pollution is being monitored (Howell et al 1995).

Enterococci, commensally organisms in gastrointestinal tract of human and animals have emerged as a leading cause of nosocomial infections (Murray and Weinstock 1999). *Enterococcus faecalis* (*E. faecalis*) and *E. faecium* are the two major pathogenic species in human, with sporadic infections caused by *E. durans*, *E. hirae* and other *enterococci* (Gilmore et al 2002). The presence of *enterococci* as an indicator of fecal contamination has been used in management of recreational water quality standards as it correlates best with the incidence of swimming related illnesses (USEPA 2003).

5.4.7 *Pseudomonas aeruginosa*

Pseudomonas is a gram negative rod that belongs to the family *Pseudomonadaceae*. More than half of all clinical isolates produce the blue green pigment pyocyanin. *Pseudomonas* often has a characteristic sweet odor. These pathogens are widespread in nature, inhabiting soil, water, plants, and animals (including humans). *Pseudomonas aeruginosa* has become an important cause of infection, especially in patients with compromised host defense mechanisms. It is the most common pathogen isolated from patients who have been hospitalized longer than 1 week. It is a frequent cause of nosocomial infections such as pneumonia, urinary tract infections (UTIs), and bacteremia. Pseudomonal infections are complicated and can be life threatening. *P. aeruginosa* is an opportunistic pathogen. It rarely causes disease in healthy persons. In most cases of infection, the integrity of a physical barrier to infection (eg, skin, mucous membrane) is lost or an underlying immune deficiency (eg, neutropenia, immuno suppression) is present. Adding to its pathogenicity, this bacterium has minimal nutritional requirements and can tolerate a wide variety of physical conditions.

The pathogenesis of pseudomonal infections is multifactorial and complex. *Pseudomonas* species are both invasive and toxigenic. According to Pollack (2000) that includes (i) bacterial attachment and colonization; (ii) local infection; (iii) bloodstream dissemination and systemic disease. The importance of colonization and adherence is most evident when studied in the context of respiratory tract infection in patients with cystic fibrosis and in those that complicate mechanical ventilation. Production of extracellular proteases adds to the organism's virulence by assisting in bacterial adherence and invasion. Numerous human diseases by having bath in rivers, lakes, ponds and coastal sea water are associated with the presence of opportunistic pathogens from *Pseudomonas, Aeromonas, Staphylococcus* and other microorganisms groups, being able to generate infections by contact with skin, mucous membrane,

nosopharyngeal cavity, respiratory ducts, eyes, ears and urogenital passages. Pyogenic infection of injuries, meningitis, urinary system, respiratory system, inflammation of the middle ear and eyes are typical diseases caused by contaminated water where *Pseudomonas aeruginosa* are found (Pellet et al 1983; Hernandez et al 1997; Rusin et al 1997). In the natural environment, *P. aeruginosa* has been labeled a surface water contaminant causing eye, ear, nose, and throat infections of swimmers (Hoadley 1968 &1977). In both situations, the bacterium is truly aquatic and does not long survive desiccations (Arseni and Koumentakou 1964; Skaliy and Eagon 1972; Lowbury 1975). However, the precise distribution of *P. aeruginosa* in a freshwater habitat has never been determined. Accordingly, it was our objective to ascertain the relative distribution of *P. aeruginosa* in a natural body of water.

Table 5.4 Bacteriological quality of drinking water

Organisms	Guidelines
All water intended for drinking	
E. coli or thermotolerant coliform bacteria[b,c]	Must not be detectable in any 100 ml sample.
Treated water entering the distribution system	
E. coli or thermotolerent coliform Bacteria [b]	Must not be detectable in any 100 ml sample.
Total coliform bacteria	Must not be detectable in any 100 ml sample.
Treated water in the distribution system	
E. coli or thermotolerent coliform Bacteria	Must not be detectable in any 100 ml sample.
Total coliform bacteria [d]	Must not be detectable in any 100 ml sample. In the case of large supplies, where sufficient samples are examined, must not be present in 95% of samples taken throughout any 12 month period.

(Source: BIS 2009)

a) Immediate investigative action must be taken if either *E. coli* or total coliform bacteria are detected. The minimum action in the case of total coliform bacteria is repeated, the cause must be determined by immediate further investigation.

b) Although, *E. coli* is the more precise indicator of faecal pollution, the count of thermotolerant coliform bacteria is an acceptable alternative. If necessary, proper confirmatory tests must be carried out. Total coliform bacteria are not acceptable indicators of the sanitary quality of rural water supplies, particularly in tropical areas where many bacteria of no sanitary significance occur in almost all untreated supplies.

c) It is recognized that, in the great majority of rural water supplies in developing countries, faecal contamination is widespread. Under these conditions, the national surveillance agency should set medium-term targets for progressive improvement of water supplies,

d) In the remaining five percent sample total coliform bacteria should not exceed ten per hundred ml.

5.5 SIGNIFICANT RESULTS

In India, more than 70% of the epidemic emergencies are either water borne or are water related (Khera et al 1996). Although a substantial amount of work has been carried out on common water borne pathogens in India, unfortunately only a little information is available on the emerging waterborne pathogens. A regular surveillance of resource and drinking water is one of the major mainstays of containing dreaded and often fatal water-borne diseases.

Rivers are the most important water resource. Unfortunately, river is being polluted by indiscriminate disposal of sewerage, industrial waste and plethora of human activities, which affects its physicochemical characteristics and microbiological quality (Koshy and Nayar 1999). Prevention of river pollution requires effective monitoring of physicochemical and microbiological parameters (Bonde 1977; Ramteke et al 1994).

Thus, detection and enumeration of indicator organisms are of primary importance for the monitoring of sanitary and microbiological quality of water (Kataria et al 1997; Gunnison 1999). The bacteriological examination of water has a special significance in pollution studies, as it is a direct measurement of deleterious effect of pollution on human health. For assessment of water, quality is not only the physicochemical characteristics of river water but also obtain information on whether the river conforms to prescribed standard of microbiological water quality (APHA 1998). The river Tamiraparani is one of the perennial rivers in south India. The salvation of majority of population in cities, towns, villages along the river basin and considered sacred. In the recent past, growing human population, industrialization, intensive agricultural practices and discharges of wastewater into the river have resulted in deterioration of water quality. The impact of these anthropogenic activities has been extensive that the water bodies have lost their self purification capacity. High levels of pollution indicator bacteria in river water is also a common problem in urban and rural areas that often leads to impairment of household and industrial uses. The river water was polluted by faecal contaminations to the extent that they were non-potable and unsuitable for recreational activity. Apart from this, there are routine releases of urban, rural sewage effluents; warm blood animal waste and agricultural waste also take part in water pollution.

This study was carried out to current levels of pollution indicator organisms as well as some groups of human pathogenic bacteria in different locations of the river basin and also focused on understanding the seasonal differences in the microbial groups. In this context that an assessment of microbial load of river water and the prevalence of emerging waterborne pathogens such as *E. coli*, *Salmonella Spp/Shigella Spp*, *Streptococcus faecalis, Vibrio cholerae* and *Pseudomonas aeruginosa* were undertaken in the Tamiraparani river, Southern peninsular, India. The sample was collected once in to two months interval along the river water and sediment samples. The water samples overall ranges (mean abundance; no.m l^{-1}) of the monitored groups

of bacteria were total viable counts 3.5 [$\times 10^3$] - 1.74 [$\times 10^5$] m l^{-1} (63165.9); total coliforms: 50 - 1.7 [$\times 10^4$] m l^{-1} (6473.9); total *streptococcus*: 0 - 1.6 [$\times 10^3$] m l^{-1} (552.5); Vibrio like organisms: 0 -1.6 [$\times 10^3$] m l^{-1} (158.5); *E. coli*: 0 - 4.9 [$\times 10^3$] m l^{-1} (1393); *Vibrio cholerae*: 0 - 1 [$\times 10^3$] m l^{-1} (111.8); *Salmonella spp/Shigella spp*: 0-280 m l^{-1} (68.5); *Streptococcus faecalis*: 0 - 420 m l^{-1} (94.1); *Pseudomonas aeruginosa*: 0 -260 m l^{-1} (57.4).

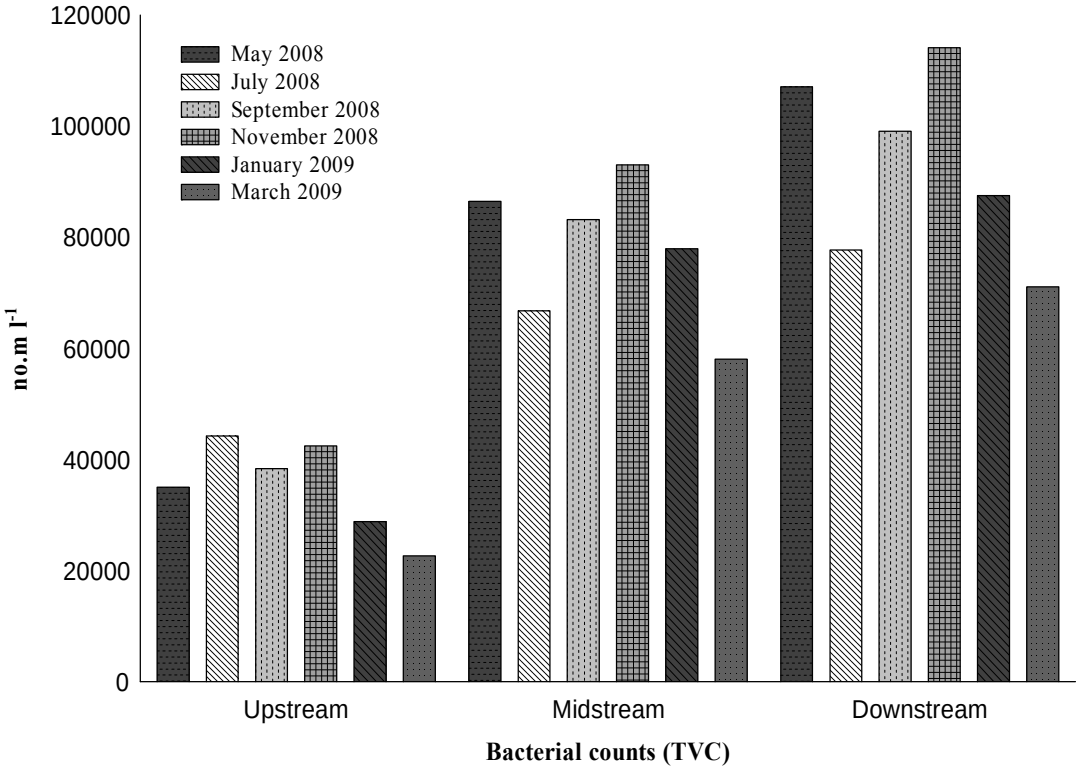

Figure 5.2. An average TVC counts in the river basin

From the sediment samples overall ranges (mean abundance; no.m l^{-1}) of the monitored groups of bacteria were total viable counts 2.5 [$\times 10^3$] -1.16 [$\times 10^6$] ml^{-1} (234103.2); Total coliforms: 160 - 1.24 [$\times 10^5$] m l^{-1} (20730.6); total *streptococcus*: 0 -1.01 [$\times 10^4$] m l^{-1} (1652.2); Vibrio like organisms: 0 - 6.2 [$\times 10^3$] m l^{-1} (657.3); *E. coli*: 0 - 4.0 [$\times 10^4$] m l^{-1} (3906.2); *Vibrio cholerae*: 0 - 5.3 [$\times 10^3$] m l^{-1} (562.4); *Salmonella spp/Shigella spp*: 0 - 3.0 [$\times 10^3$] m l^{-1} (249.4); *Streptococcus faecalis*: 0 - 1.3 [$\times 10^3$] m l^{-1} (213.8); *Pseudomonas aeruginosa*: 0 - 800 m l^{-1} (118.1). Tables 5.5 and 5.6 were shown mean, standard deviation, minimum, maximum, kurtosis, and

skewness of water and sediment samples for the present status of pathogenic indicator organisms into during sampling periods along the river basin. While the differences in their abundances between the sampling locations are statistically insignificant, strong signals of monthly variations could be discerned. Though our sampling locations were chosen to represent pollution gradience in the area, our observations during different months are helpful to understand the spatial variations and temporal differences of pollution indicators and many human pathogenic bacteria. These observations are useful to suggest that the non-suitability of Tamiraparani river for domestic purpose.

The total viable counts (TVC) were in order of magnitude of above 10^3 m l^{-1} for all sites in all the months respectively, which is substantially high (Fig 5.2). Most of the samples were found to have TVC higher than those prescribed in Bureau of Indian Standards of permissible limits BIS (2009). The TVC was higher in November (monsoon) month as compared to May (summer), January (winter) and other months in all province of river basin. Particularly, high TVC was found in S6 and S7 sites of upstream area during July (premonsoon). However, the TVC of mid and downstream regions were found higher in rainy season as compared to other months. The bacterial counts were increasing gradually from upper region to lower region, i.e. sites of the middle and downstream region were found to be more contaminated. The similar results were also obtained in sediment samples. Total viable counts in water samples were in highest loads during month of November and the least during March in both water and sediments. In water, the TVC mean ranged from 1.01 – 13.18 [$\times 10^4$] m l^{-1} during November and 3.5 – 86.0 [$\times 10^3$] m l^{-1} during March. In sediments, the TVC mean ranged from 2.4 – 94.0 [$\times 10^4$] m l^{-1} during November and 0.83 – 61.0 [$\times 10^4$] m l^{-1} during March. Variations in TVC counts were large in both months and region wise. In water, the overall average TVC concentrations were all the months 0.35 – 17.4 [$\times 10^4$] m l^{-1} and overall average sediments were in all the months 0.025 – 11.6 [$\times 10^5$] m l^{-1}. Similar was the case with TC and TS.

Commonly, the total coliform counts were relatively higher during November month than other months except Kuttralam (S6) and Tenkasi (S7). A lower count of total coliforms was obtained in March and July months. In lower region, high concentrations of TC were obtained in November month mainly in Eral (S17), Athoor (S18) and Punnakayal (S21) as in these locations people use the river water for bathing purpose cloth washing and site (S21) is a fishing harbor. Therefore, these contribute high level of pollution in lower province. Similarly, in middle region, higher counts were obtained in Tirunelveli (S13), Srivaikundam (S15), and Alwarthirunagari (S16) for the reason that, in these locations are holy and dense populated areas. Nevertheless, in upstream region, high counts were found in Kuttralam (S6) and Tenkasi (S7) in the month of July while more tourists visit in this month. Both places are prominent tourist and developing urban places, due to in this reasons we observed higher levels pathogenic indicator organisms beside the year.

The seasonal variability of the water sample bacterial groups such as TC, TS and VLO concentrations higher ranges from 70 - 1.7 [$\times 10^4$] m l^{-1}, 0 - 1.6 [$\times 10^3$] m l^{-1} during July and 0 - 1.6 [$\times 10^3$] m l^{-1} during May. The lower concentrations of TC, TS, and VLO ranged from 50 - 8.8 [$\times 10^3$] m l^{-1}, 0 - 7.6 [$\times 10^2$] m l^{-1} and 0 - 7.8 [$\times 10^2$] m l^{-1} during March. The sediments sample bacterial groups such as TC, TS and VLO concentrations higher ranged from 0.02 - 12.4 [$\times 10^4$] m l^{-1}, 0.01 - 10.1 [$\times 10^3$] m l^{-1} during July and 0 - 6.2 [$\times 10^3$] m l^{-1} during May. The lower concentrations of TC, TS, and VLO ranged from 160 - 3.8 [$\times 10^4$] m l^{-1}, 0 - 2.6 [$\times 10^3$] m l^{-1} and 0 - 3.4 [$\times 10^3$] m l^{-1} during March. However, in upper region TC, TS and VLO counts were highest in November month as compared to other months. Whereas, upstream region (S6 and S7) are contributes very high load of TC, TS and VLO during July followed by November, May, September, January and March. Particularly, July month as compared to other sites in upstream region does not get high amount of bacterial load (Figs 5.3, 5.4 and 5.5).

Table 5.5 Descriptive statistics of pathogenic indicators in water samples

Season	Bacterial types	Mean ± S.D	Minimum	Maximum	Kurtosis	Skewness
May 2008	TVC	69452.4±45636.8	7200	130000	-1.7	-0.3
	TC	7463.3±5418.9	230	13900	-1.8	-0.3
	TS	622.4±425.2	20	1100	-1.7	-0.5
	VLO	209.1±333.9	0	1600	16.9	3.9
	EC	1652.9±1297.2	30	3500	-1.7	0.0
	VC	137.6±207.7	0	1000	16.6	3.9
	SA/SH	86.7±65.3	0	260	1.0	0.7
	SF	119.5±92.9	0	300	-1.2	0.0
	PA	70.9±57.9	0	170	-1.3	0.2
July 2008	TVC	59823.8±49009.2	4000	174000	0.2	0.7
	TC	5689.5±4919	70	17000	-0.1	0.6
	TS	517.1±454.5	0	1600	0	0.6
	VLO	108.6±159.3	0	700	9.7	2.9
	EC	1237.1±1303.0	0	4800	2.2	1.4
	VC	79.1±121.4	0	540	10.8	3.1
	SA/SH	61.4±71.3	0	280	4.8	2.2
	SF	64.3±84.9	0	300	4.7	2.2
	PA	38.6±60.8	0	230	5.8	2.5
September2008	TVC	67871.4±46134.0	6500	134000	-1.7	-0.3
	TC	6926.7±5102.5	180	14000	-1.7	-0.3
	TS	580.5±421.7	20	1200	-1.7	-0.3
	VLO	161.9±231.7	0	1100	14.7	3.6
	EC	1495.7±1295.9	20	4300	-0.7	0.5
	VC	118.6±168.4	0	800	14.7	3.6
	SA/SH	70.5±52.9	0	170	-0.8	0.3
	SF	95.2±75.4	0	200	-1.6	-0.1
	PA	55.7±50.9	0	180	0	0.6

Table 5.5 Continue …

Season	Bacterial types	Mean ± S.D	Minimum	Maximum	Kurtosis	Skewness
November	TVC	76476.2±48760.5	10200	142000	-1.7	-0.3
2008	TC	8535.2±6218.0	300	16600	-1.8	-0.2
	TS	716.2±480.5	40	1400	-1.5	-0.3
	VLO	259.5±284.7	0	1300	8.8	2.5
	EC	2070.5±1616.7	40	4900	-1.5	0.0
	VC	180.0±196.9	0	900	8.8	2.5
	SA/SH	110.0±71.5	0	240	-1.0	-0.2
	SF	170.0±134.0	0	420	-0.9	0.3
	PA	90.0±76.0	0	260	-0.6	0.5
January 2009	TVC	59266.7±40998.0	4900	108000	-1.8	-0.3
	TC	5914.3±4379.6	100	10500	-1.8	-0.4
	TS	499.1±365.6	10	980	-1.8	0.4
	VLO	124.8±192.6	0	910	15.2	3.6
	EC	1123.8±917.4	10	2600	-1.6	0.0
	VC	91.4±142.2	0	670	15.1	3.6
	SA/SH	55.2±42.3	0	140	-1.0	0.2
	SF	61.4±48.9	0	120	-1.7	-0.4
	PA	40.0±37.4	0	130	-0.2	0.6
March 2009	TVC	46104.8±32921.0	3500	86000	-1.8	-0.2
	TC	4314.3±3374.6	50	8800	-1.8	-0.2
	TS	380.0±303.9	0	760	-1.8	-0.2
	VLO	87.1±164.5	0	780	17.8	4.1
	EC	778.1±659.2	0	1900	-1.6	0.1
	VC	63.8±111.7	0	530	17.1	4.0
	SA/SH	27.1±22.8	0	70	-0.7	0.5
	SF	33.3±27.5	0	70	-1.7	-0.2
	PA	17.6±17.3	0	60	0.0	0.7

TVC- Total viable count ; TC- Total coliforms ; TS- Total *Streptococcus*; VLO-Vibrio like organisms; EC-*Escherichia coli*; VC- *Vibrio cholerae*; SA/SF- *Salmonella spp/Shigella spp*; SF-*Streptococcus faecalis*; PA-*Pseudomonas aeruginosa*

Table 5.6 Descriptive statistics of pathogenic indicators in sediment samples

Season	Bacterial types	Mean ± S.D	Minimum	Maximum	Kurtosis	Skewness
May 2008	TVC	274976.2±246842.3	2500	870000	0.0	0.8
	TC	24591.4±22029.6	920	96000	-1.0	0.5
	TS	1944.8±160.3.3	50	4800	-1.2	0.3
	VLO	840.5±1394.5	0	6200	11.6	3.2
	EC	4212.4±4249.3	100	14000	0.3	1.1
	VC	719.5±1186.7	0	5300	11.8	3.2
	SA/SH	274±392.6	0	1500	6.1	2.6
	SF	245.2±216.5	0	700	-0.7	0.6
	PA	136.2±119.3	0	380	-0.8	0.5
July 2008	TVC	237904.8±313968.1	10100	1160000	3.6	2.0
	TC	22772.9±32814.7	200	124000	5.0	2.3
	TS	1734.8±2680.5	10	10100	6.1	2.5
	VLO	681.4±1229.7	0	4200	3.8	2.2
	EC	4213.3±7369.6	20	27000	6.2	2.6
	VC	600.0±1087.8	0	3800	4.2	2.3
	SA/SH	354.8±818.7	0	3000	7.7	3.0
	SF	212.9±345.6	0	1300	6.1	2.6
	PA	129.5±202.8	0	800	6.6	2.6
September 2008	TVC	221095.2±213437.6	16000	790000	0.8	1.1
	TC	20757.1±19015.9	800	62000	-0.8	0.6
	TS	1562.4±1326.6	40	4100	-0.9	0.5
	VLO	702.4±1225.8	0	5500	12.6	3.4
	EC	4947.6±8565.0	100	40000	15.5	3.7
	VC	608.1±1030.5	0	4600	12.0	3.3
	SA/SH	225.2±325.6	0	1300	6.8	2.6
	SF	193.8±162.2	0	500	-1.1	0.3
	PA	111.0±97.7	0	280	-1.3	0.3

Table 5.6 Continue …

Season	Bacterial types	Mean ± S.D	Minimum	Maximum	Kurtosis	Skewness
November 2008	TVC	336714.3±286552.8	24000	940000	-0.8	0.5
	TC	29333.3±26161.5	1100	86000	-0.8	0.5
	TS	2501.4±2118.8	60	7100	-0.7	0.5
	VLO	771.9±1257.2	0	5600	11.5	3.2
	EC	5690.5±5771.5	200	19000	0.3	1.1
	VC	628.6±948.3	0	4200	10.4	3.0
	SA/SH	341.4±485.9	0	1800	6.2	2.6
	SF	393.8±360.6	0	1100	-0.8	0.6
	PA	169.0±149.3	0	500	-0.1	0.8
January 2009	TVC	198390.5±180904.7	12700	640000	-0.1	0.8
	TC	15643.3±15626.2	500	51000	-0.3	0.8
	TS	1245.2±1061.0	20	3500	-0.7	0.5
	VLO	564.3±1014.0	0	4600	13.6	3.5
	EC	2762.9±2459.0	70	8000	-0.8	0.5
	VC	496.2±896.2	0	4100	14.3	3.6
	SA/SH	172.2±221.8	0	860	5.2	2.3
	SF	139.5±126.5	0	400	-1.0	0.5
	PA	92.9±83.3	0	250	-1.3	0.3
March 2009	TVC	135538.1±149910.5	8300	610000	4.0	1.8
	TC	11285.2±11086.5	160	38000	0.3	1.0
	TS	924.8±803.3	0	2600	-0.9	0.4
	VLO	383.8±760.3	0	3400	13.4	3.5
	EC	1610.5±1673.8	20	6000	1.3	1.2
	VC	321.9±608.7	0	2700	12.5	3.4
	SA/SH	127.6±190.8	0	750	6.7	2.7
	SF	97.6±95.2	0	300	0.0	0.9
	PA	70.0±70.9	0	200	-1.0	0.6

TVC- Total viable count ; TC- Total coliforms ; TS- Total *Streptococcus*; VLO-Vibrio like organisms; EC-*Escherichia coli*; VC- *Vibrio cholerae*; SA/SF- *Salmonella spp/Shigella spp*; SF-*Streptococcus faecalis*; PA-*Pseudomonas aeruginosa*

The values for TS were also found higher in the rainy season, which were similar to the findings of TC. On the other hand, in the upper region TS counts were low in most of the months. In the middle and downstream regions, higher counts were obtained especially S13, S15, S16, 17, S18 and S21. Interestingly, TS count was nil in some sites of upstream region such as Karaiyar (S1), Servalar (S2) and Manimuthar (S5) during March, July and January. The Vibrio like organisms (VLO) in downstream region was higher in most of the months particularly Punnakayal (S21), whereas, as compared to November the VLO concentrations was higher than May month. Similar to TS, the VLO ranges were nil in many sites of upstream region (S1, S2, S3, S4, S5, S8 and S9) during March, July and January. In middle province, locations are Tirunelveli (S13), Srivaikundam (S15) and Alwarthirunagari (S16) giving to increase the surveillance of VLO.

The abundance of five different types of pathogen indicator organisms such as *E. coli* (EC), *Streptococcus faecalis* (SF), *Salmonella spp/Shigella spp* (SA/SH), *Vibrio cholerae* (VC) and *Pseudomonas aeruginosa* (PA) in sediment samples during different months is shown in (Figs 5.6, 5.7 and 5.8). During November month, the bacterial counts of EC, SA, SF and PA were in the ranges of 200 - 19000 m l^{-1}, 0 - 1800 m l^{-1}, 0-1100 m l^{-1} and 0-500 m l^{-1} respectively. In the water, samples were observed lower concentration in the ranges of 0 - 1900 m l^{-1}, 0 - 70 m l^{-1}, 0-70 m l^{-1} and 0 - 60 m l^{-1} in the bacterial groups of EC, SA/SH, SF and PA respectively, during March. However, in sediments VC was high during May (0 - 5300 m l^{-1}). During November, the counts of EC, SA and SF were generally more in the entire region. Nagvenkar and Ramaiah (2009) made similar observations in Mandovi and Zuari estuary at Goa. However, the predominantly locations (S6 & S7) are dominant concentration in the *Vibrio parahaemolyticus* in estuary area of lower region such as Punnakayal (S21) in all the months. Counts of SF varied widely between seasons. Similar to most other pathogenic groups, the PA counts were low level in most of the sites in all seasons.

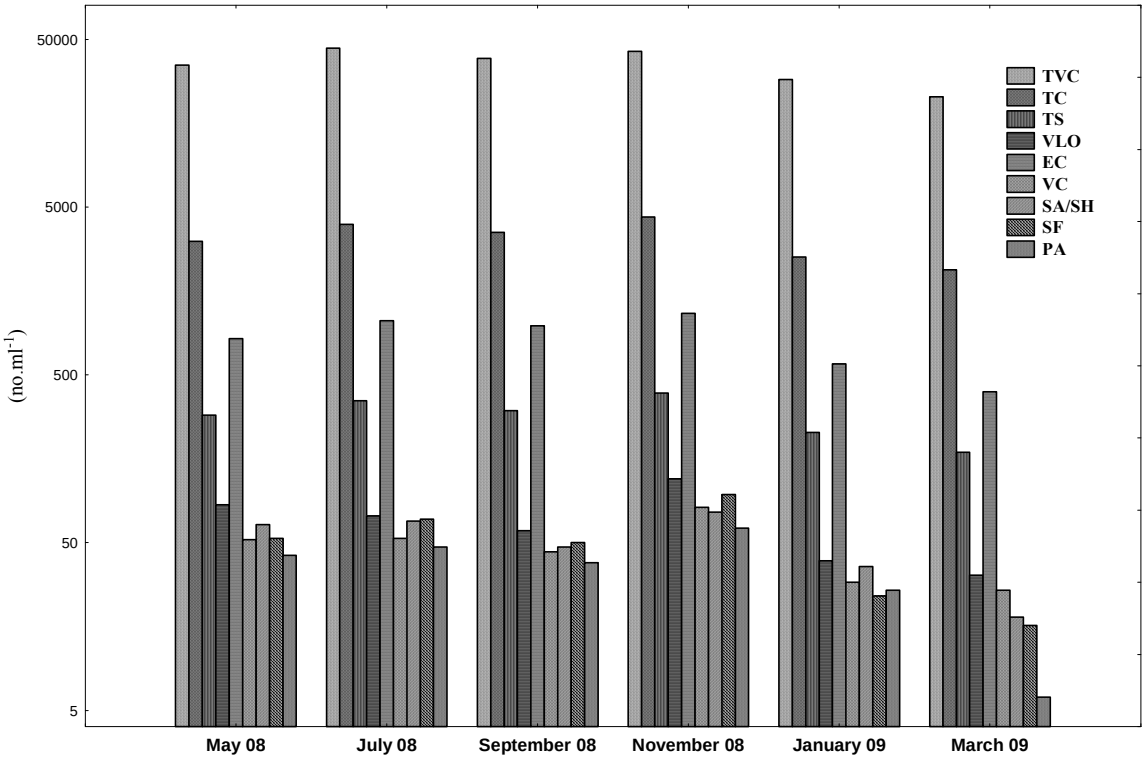

Figure 5.3 Temporal variations of indicator organisms in upstream region

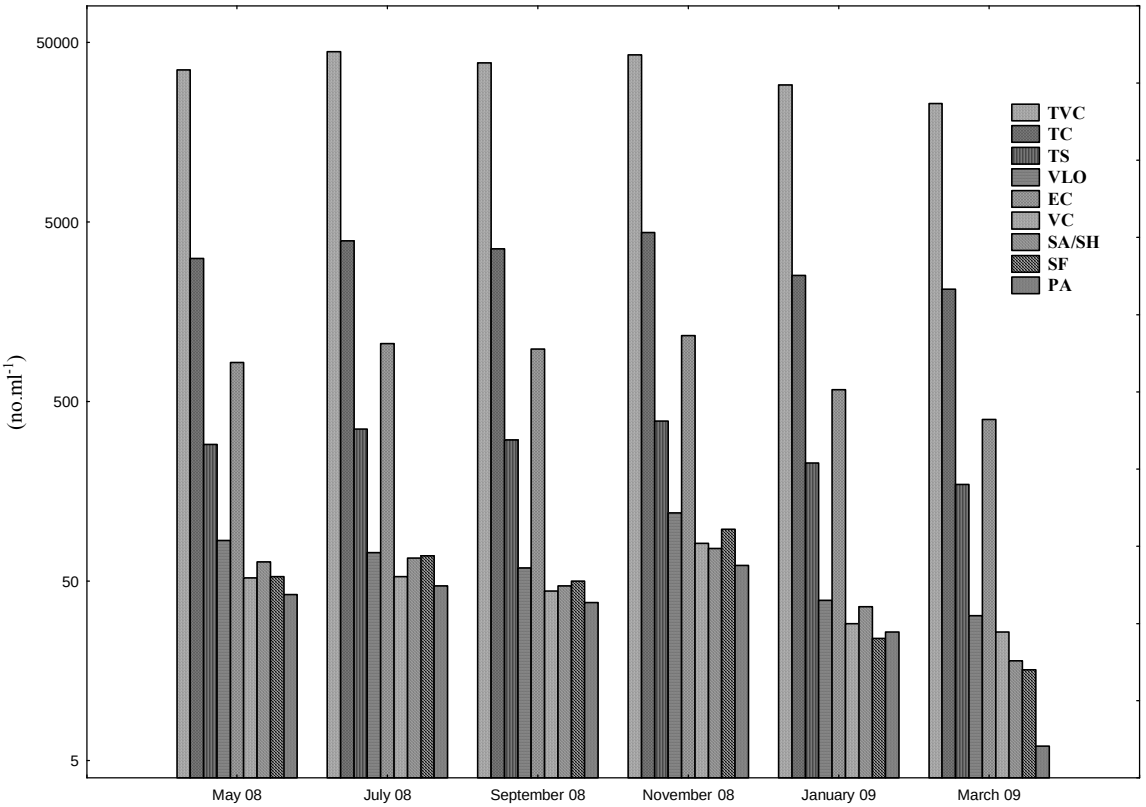

Figure 5.4 Temporal variations of indicator organisms in midstream region

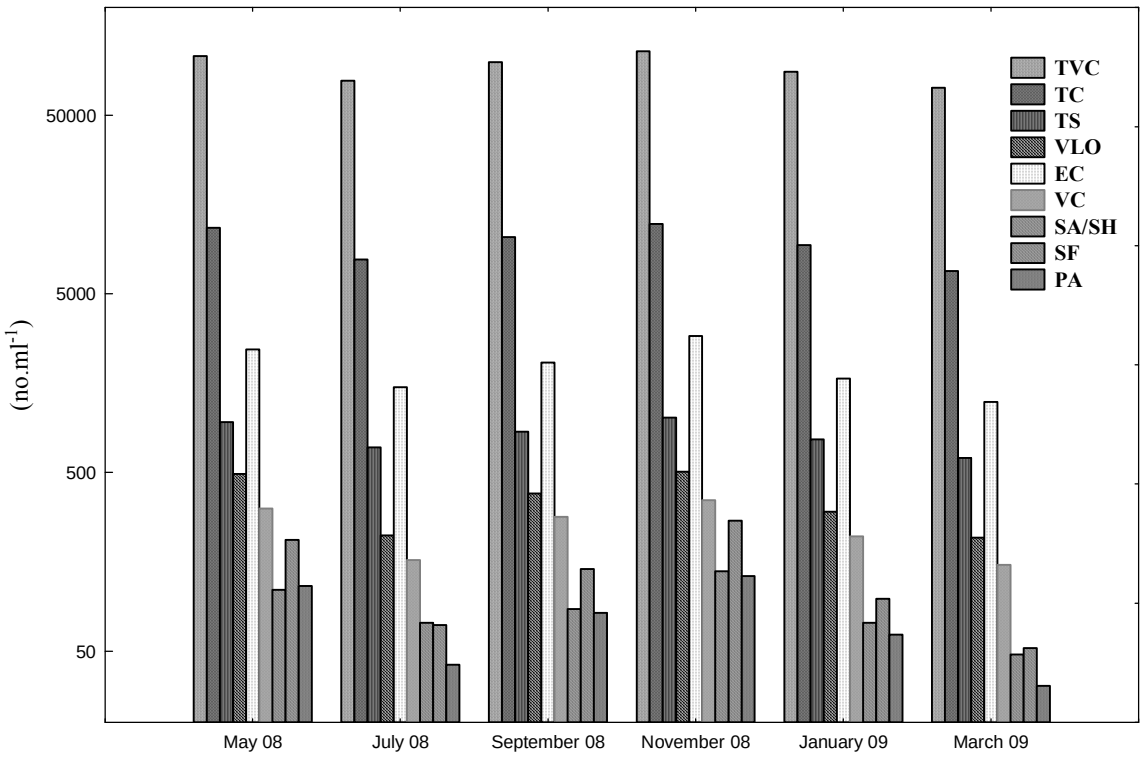

Figure 5.5 Temporal variations of indicator organisms in downstream region

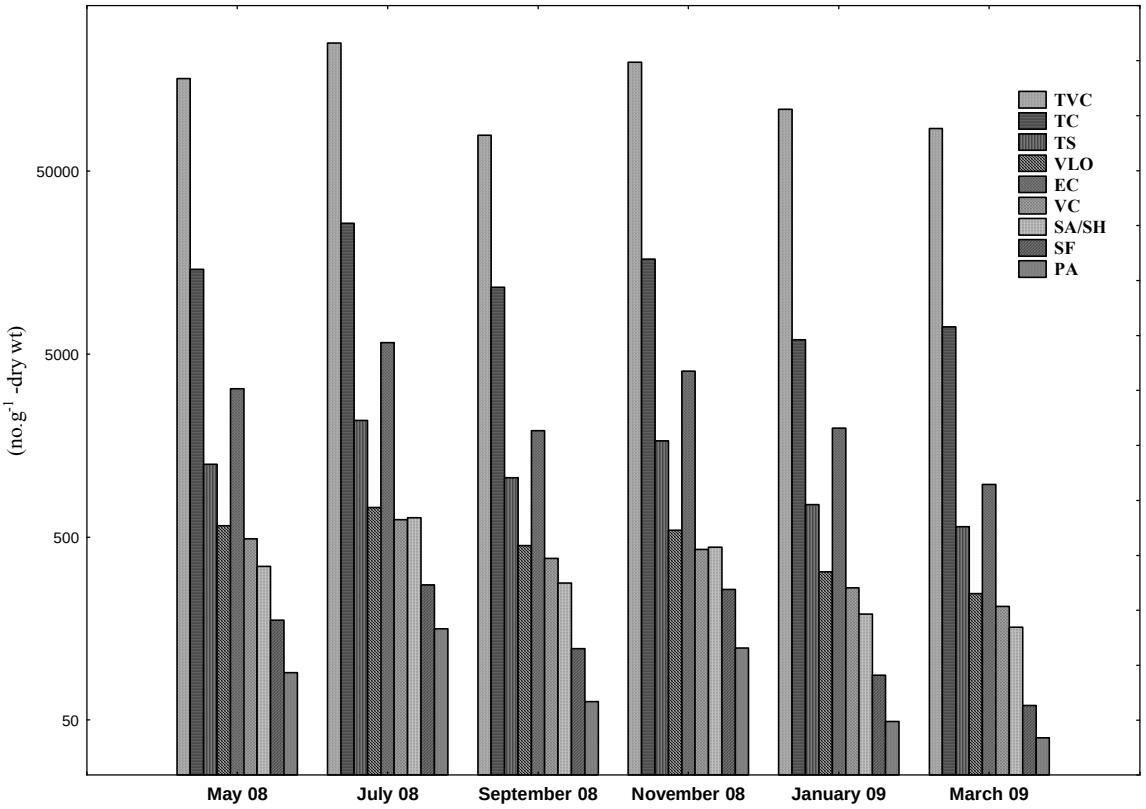

Figure 5.6 Upstream variations if indicator organisms in sediment samples

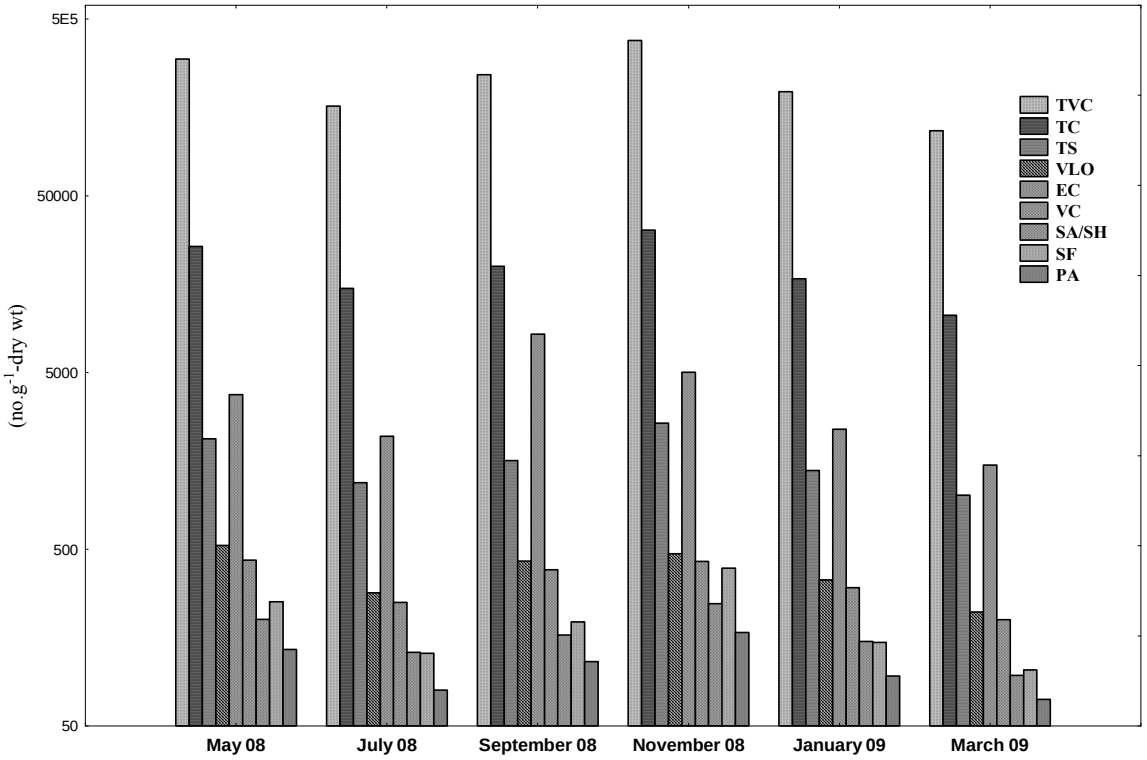

Figure 5.7 Midstream variations of indicator organisms in sediment samples

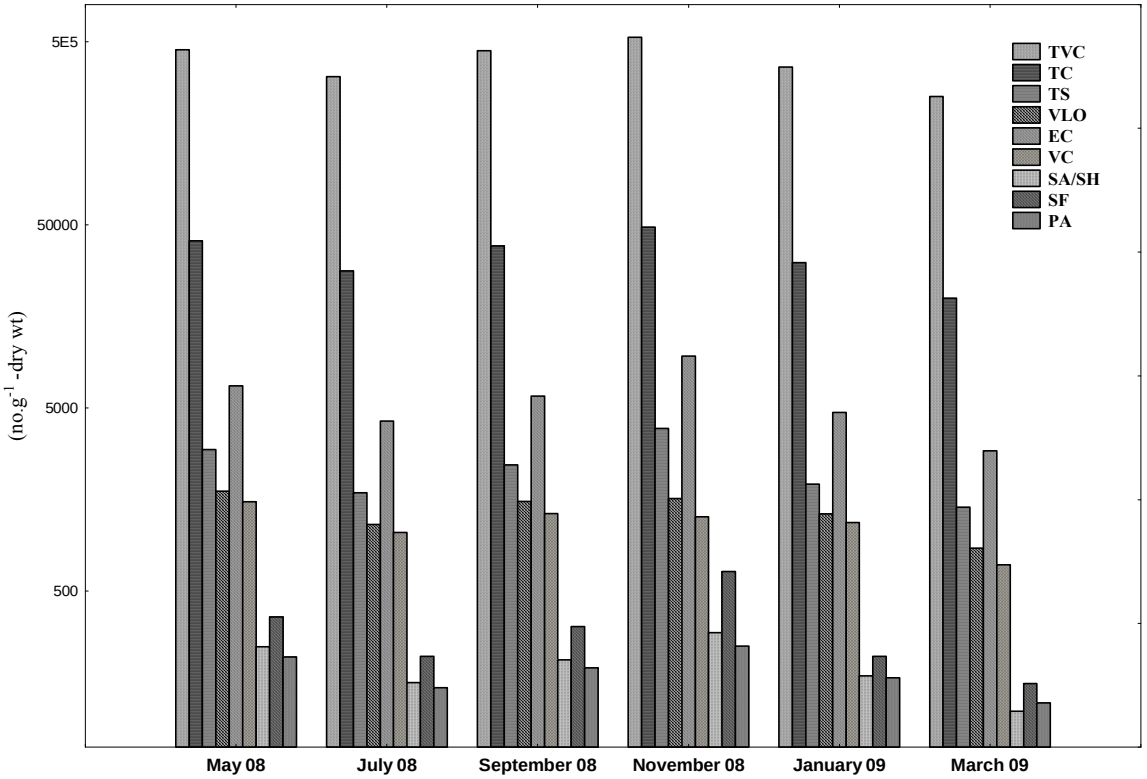

Figure 5.8 Downstream variations of indicator organisms in sediment samples

Briefly, during May and November, counts of human pathogenic like *E. coli* was generally high at several locations; their counts were lower during March. Counts of *Vibrio cholerae* and *Vibrio parahaemolyticus* (VP) showed a similar trend was also seen with other pathogens. Both VC and VP were obtained more counts during May (summer).The mean abundance of *Salmonella spp* and *Shigella spp*. were higher during the November followed by May, September, January, July and March. *Streptococcus faecalis* (SF) was also high in wet season.

5.6 DISCUSSION

Water from most sources is therefore unfit for immediate consumption without some sort of treatment (Raymond 1992). In present study, the TVC concentrations were observed in most of the places in higher levels of bacterial groups throughout the river basin. In fact, the Tamiraparani water is used for drinking, domestic purpose and Industrial activities. While the higher TVC values suggest that, direct use of river water for the domestic and Industrial purpose should be avoided. The TVC values were relatively higher in most of the places, which may be attributed to the presence of large population residing at the banks. The *E. coli* and coliform were prevalent in river water as well as effluent sample (Ramteke et al 1994; Ramteke 1995; Ramteke and Tewari 2002). Pollution indicator organisms such as TC, EC, and TS are routinely examined for an understanding of the preponderance of human pathogenic bacteria (Bordner and Winter 1978; APHA 1980). As is universally accepted, higher the counts of these organisms higher would be the incidence of human pathogenic bacterial species. It is adequate to realize the presence of pathogenic bacteria through indicator bacterial quantification. However, an understanding of the incidence of as many individual groups as possible would be necessary in river water management owing to the involvement of different sewage outfall, mass bathing, and other human and animal activities.

Further, as inestimable pathogenic bacteria will constitute the microflora of effluents discharged from domestic, urban, agricultural and certain manufacturing practices, quantifying different groups of pathogenic microbes ought to be part of such surveys. For instance, information on occurrence, abundance and distribution of potent human pathogens, *Vibrio cholerae* (causing cholera in humans), *Vibrio parahaemolyticus* (gastroenteritis), *Salmonella spp and Shigella spp* (typhoid fever; food poisoning), *Streptococcus spp* (meningitis and skin infections) and *Pseudomonas aeruginosa* (septicaemic conditions) are potent human pathogens.

Schillinger and Gannon (1985); Borst and Selvakumar (2003) indicated a significant amount of microorganisms associated with particles during rainfall runoff. If pathogens in rainfall runoff discharged from watersheds were presented in a form that could be absorbed in a suspended particle, sedimentation process should be implemented at site to reduce public health risk. Bacteria capable of causing human disease may contaminate the sand. Most microorganisms attached onto sediment. Stream sediments have been shown to contain faecal coliform at concentrations higher than those observed in the overlying water column. Sediments may contain 100 to 1,000 times the number of faecal indicator bacteria contained in the overlying water. Ashbolt et al (1993) observed that in the case of rainfall, the microbial loads of running water might suddenly increase and reach reservoir bodies very quickly. These observations explain the reason of increase of bacterial contamination from upper region to lower region. The total coliform counts were relatively higher in rainy season than summer and winter, which suggest role of precipitation on the sources and extent of microbial population. The values for TS were also found higher in the rainy season, which were similar to the findings of TC. While in rainy season, relatively higher values were obtained at all the sites. Similar finding were obtained in river Ganges (Sood et al 2008)**.**

TC and TS was obtained higher in the rainy season and negligible counts in winter season, which may be the relative frequency of TC from human sources increases in rainfall (Baghel et al 2005). The coliform bacterial population was lowest in the winter and highest in the monsoon, the pattern that was reported in earlier studies (Badge and Varma 1982; Badge and Rangari 1999). The coliforms population is increasing in monsoon months may be due to the rainwater that drained into the river, as it was the major source of bacterial population in the river water. Nagvengar and Ramaiah (2009) reported higher level of pollution indicator and pathogenic bacteria in mostly during November throughout the region. The most widely used indicators are the coliform bacteria, which may be the total coliform that got narrowed down to the faecal coliforms and the faecal streptococci (Pathak and Gopal 2001; Harwood et al 2001; Vaidya et al 2001). Concurrently, contaminations of water by enteric pathogens have increased worldwide (Craun 1986; Islam et al 2001). In rainy season, due to runoff of water having animal excreta, human waste, sewage waste from upper region to lower region leads to the high counts of pathogenic indicators in middle and lower regions which is densely populated and face heavy anthropological activity as compared to upper province.

From a comparative assessment of distribution and abundance of pollution indicator and human pathogenic bacteria in the typically tropical Tamiraparani river in the south India, it is inferred that the counts of all the groups are lower than Mandovi and Zuari river (Nagvenkar and Ramaiah 2009). In general, the highest abundance of all the examined groups was observed during November. However, most of the pollution indicator and human pathogenic bacteria counts are lower than those reported from the Seine river and its estuary (George et al 2001), Mumbai water (Ramaiah et al 2004), Czarna Hancza river (Niewolak 1998). In this study, most of the sites were not suitable for domestic purpose with respect to the maximum permissible limits of TC

and TS counts as per the standards laid by National River Conservation Directorate (NRCD), India.

Microbiologists rely on the principle that higher the incidence of sewage indicator bacteria in any environment, higher would be the chances for human pathogenic bacteria to be present (Brock et al 1994; Fujioka 2002). Nagvenkar and Ramaiah (2009) reported *Vibrio cholerae* is the dominant bacterium in the sewage discharges, it can compete and rapidly outgrow the native microflora leading to increased levels of indicator bacteria in natural water bodies. Pathogenic bacteria of human health concern have been studied mostly for their survival in the river environment (Niewolak 1998; Lipp et al 2001; Kim et al 2005; Baghel 2005; Sood 2008; Nagvenkar and Ramaiah 2009). It is evident that the abundance of pathogenic bacteria we studied fluctuates widely in the water and sediment samples in the study area.

Unsanitary means of disposing human waste and faecal droppings from livestock are routes through which faecal matter may enter aquatic systems. Faecal matter degrades water quality due to the possible introduction of pathogens, nutrients and organic matter (Vinneras et al 2003; Langergraber and Muellergger 2005; Vikaskumar et al 2007). Degraded water quality may result in increase in cost of drinking water treatment or loss of opportunities for recreation, aquaculture and fishing (Sinton et al 1998; Parveen et al 2001; Ebdon et al 2007; Edge and Hill 2007). Prominently, pollution with faecal matter may present significant health risk to the public (Sinton et al 1998; Byamukama et al 2005). The level of risk will depend considerably on the origin and level of contamination (Scott et al 2002). In particular, contamination from human excreta is of greater risk to public health as it is more likely to contain human-specific enteric pathogens although reliable epidemiological evidence is lacking (Sinton et al 1998). To minimize health risk, it is often required to undertake

regular monitoring of indicator parameters in aquatic systems (Kong et al 2002; Wheeler et al 2002; McLellan and Salmore 2003; Noble et al 2003 and Shah et al 2007). Such assessment studies are useful not only for evaluating health risk, but also for determining the course of action that may be required to solve the problem (Parveen et al 2001; Ahmed et al 2007 and Graves et al 2007).

` Pathogenic bacteria indicators are used to determine the presence of disease-causing organisms originating from fecal pollution. Indicators such as total coliform (TC), fecal coliform (FC), and fecal *streptococcus* (FS) are used because of the laborious technique and equally expensive equipment required to isolate pathogenic bacteria and viruses from water. The indicator organisms presently used for the monitoring of drinking water in developed countries are total coliforms and faecal coliforms and/or *Escherichia coli*, although the reliance on indicator organisms as the main source of information about the safety of drinking water is under review in many jurisdictions (Stevens et al 2001; Ashbolt et al 2001; WHO 2003). The water quality of major rivers varied widely with respect to total coliform (TC) and Faecal coliform (FC). In respect of total coliform and faecal coliform numbers, river Yamuna is leading with highest count of $307x10^6$ MPN/100 ml and $52x10^5$ MPN/100 ml respectively followed by Ganga ($45x10^5$ MPN/100 ml and $11x10^5$ MPN/100 ml), Brahmaputra ($24x10^4$ MPN/100 ml and $24x10^4$ MPN/100 ml), Mahanadi ($92x10^3$ MPN/100 ml and $54x10^3$ MPN/100 ml), Krishna ($84x10^3$ MPN/100 ml and $34x10^3$ MPN/100 ml), Godavari ($33x10^3$ MPN/100 ml and $1x10^4$ MPN/100 ml) (www.cpcb.nic.in). The river Tamiraparani as compared to Indian rivers Yamuna and Ganga has higher than other rivers in total coliforms count.

Unexpectedly, in present study, pathogenic microbes in most the water samples (especially middle and lower region) was found to be above the prescribed limits, irrespective to the fact that we studied the stretches of Tamiraparani where organic

matter is introduced mainly by sewage outfall and human activities. McLellan et al (2001) stated that faecal pollution indicator organisms could be used to a number of conditions related to the health of aquatic ecosystems and to the potential for health effects among individuals using aquatic environments. The presence of such indicator organisms may provide indication of water borne problems and is a direct threat to human and animal health. Dominance of these bacterial genera in Tamiraparani river system of Tamil Nadu suggests that they may be explored as indicators. A high count of pathogenic microbes in samples indicates the potential presence of pathogenic microbes of sewage and human origin that is source of microbial pollution. Other genera were also obtained which although were less abundant but still pose a threat if water is consumed untreated. Isolation of such potential pathogens from river water shows that the situation with respect to water quality is alarming in Tamiraparani rive.