REVIEW OF LITERATURE
2.1 GENERAL

India covers a coastline of about 7500 km and 25% of the total population live in coastal areas. An unique feature of the subcontinent and Indian ocean is its monsoon. India is one of the wettest countries in the world with an annual rainfall of 1000 km$^3$. There are 14 major, 44 medium and 162 small rivers in India with an average annual runoff of 1645 km$^3$ and this results in a sediment discharge of 500 million tons each year (Glasby and Roonwal 1995)

With a population growth rate of about 2% p.a. and an economic growth rate of 4% p.a., India is likely to face serious environmental problems and chemical pollution in the future. The aquatic body has become the ultimate receptacle for land based pollution and it is interesting to assess the stress on coasts caused by this phenomenon.

The 1st international conference on Molluscan shellfish safety (ICMSS 1994) conducted at the University of NSW, Sydney, discussed the practices and standards concerning the contamination, purification and

Human and animal excreta as well as domestic sewage inevitably carry a variety of human pathogens into surface and ground waters or directly into coastal waters. These microbiological agents include pathogenic bacteria, virus, protozoa and several more complex multicellular organisms which can result in a variety of gastro-intestinal diseases. Other organisms are more opportunistic in nature, infecting susceptible individuals through body contact or by inhalation of contaminated aerosoles (Geldreich 1989).

Fig. 3 - The chief pathways of human exposure in the aquatic environment
In terms of ubiquity and severity of health consequences, there are four important pathways of microbial pathogens from their faecal origin back to man. (Helmer et al. 1991)

- Drinking water supplies from contaminated surface waters or ground waters.
- Recreational activities in coastal bathing waters, particularly water sports having body contact.
- Consumption of contaminated shellfish, especially when eaten uncooked or undercooked.
- Irrigation of agricultural crop production areas with reused domestic and urban sewage.

Riverine and lake pollution has been a serious health risk as it acts as the major source of water directly or indirectly for human and animal consumption in different ways (Pathak et al. 1993a).

Studies on seasonal fluctuation in bacterial density in the water column and benthic epilithon at Koremasa, River Tamagawa in Tokyo (Morikawa and Tonozuka 1994) revealed that water quality was highly influenced by the pollutant discharge at the site. Anuradha et al. (1993) reported that the presence of pathogenic bacteria in river water is dangerous to health especially in a country like India, where large number of people use river water for various purposes. *Aeromonas, Escherichia coli, Vibrio, Salmonella, Shigella* etc in addition to cause gastroenteritis, dysentery,
cholera, typhoid and paratyphoid fevers, can result in eye, skin and wound infections also (Back *et al.* 1980; Galbraith *et al.* 1980; Wilson 1980; Black *et al.* 1981; Ramachandran and Varghese 1984; Adkins *et al.* 1987; Levine 1987; Manjula *et al.* 1993; Shobha *et al.* 1993).

In an epidemiological study of New York City ocean beaches, Cabelli *et al.* (1979) reported a higher incidence of gastroenteritis among bathers at beaches affected by sewage pollution from Hudson River. Animal manure and human recreational activities in the vicinity of oyster growing areas were important sources of pollution (Bouchriti *et al.* 1992). Dillon and Sellers (1984) studied the bacterial pollution in the estuaries of Mersey in the United Kingdom. A number of similar reports are available from Israel (Yoshpe and Shuval 1972; Yoshpe 1981; 1982) and French coasts (Moreau *et al.* 1971). Glasby and Roonwal (1995) also suggested that estuaries and backwaters are more affected by pollution than the open sea.

Quality can be considered as fitness for use (Snell and Perry 1992). The shellfish consumption which is higher in coastal areas constitutes one of the principal kinds of human exposure to microbial pollution. Oysters harvested from polluted waters have a well-documented history of causing food-borne diseases (Yoovidhya and Fleet 1981). As it comes several thousand metric tons per year, the harvest and consumption of shellfish is an economically important activity in the coastal waters of all most all countries. But it involves remarkably higher risks. The shellfish
growing water, if it is located in the vicinity of inhabited urban areas, it will be definitely subjected to varying degrees of sewage pollution. WHO/UNEP (1977; 78) discussed the microbiological limits adapted by several countries and recommended interim criteria for shellfish and its growing waters. The shellfish samples could be considered acceptable, if the FC concentration in 100 ml of shellfish flesh plus intravascular fluid did not exceed 300.

Hespanhol (1991) mentioned that conventional secondary and tertiary treatment systems such as activated sludge, chemical aided sedimentation, rapid sand filtration and disinfection originally designed for protection of receiving waters were supposed to attain specific standards. Traditional approaches based on potential risks, (qualitative presence of pathogens in waste water, soil or crops and non-detection of diseases caused by them), has lead to the development of very restrictive standards in many countries, which have been difficult to implement and enforce (WHO 1994).

Due to its increased significance, Dodgson (1928) and Colwell and Liston (1960; 61) have done remarkable studies on the public health aspects of shellfish bacteriology. It has been reported (Reichenback-Klinke 1973) that bacteria do not constitute the normal flora of fish and shellfish, and their presence indicate its exposure to water contaminated with microbes by various means. This is of primary significance as a source of
occupational diseases to fishermen and fish handlers. Shewan (1977) reported the presence of very high bacterial load in fish from tropical waters, as compared to that in temperate zone. In marine prawn, the total viable count reported was $10^3$-$10^7$.g$^{-1}$ (Surendran et al. 1983). It was mentioned (ICAR,1983), that total heterotrophic bacterial population varied from $10^6$-$10^7$.cm$^2$ on the body surface and $10^6$-$10^8$.g$^{-1}$ in the intestinal content of *Penaeus indicus*.

Pathogenic bacteria and virus in domestic waste presented a potential health hazard to consumers of organisms grown in waste water ponds (Ogbondeminu and Okoye 1992). Parvatheesam and Gupta (1994) assessed the aerobic heterotrophic bacteria of a lake polluted by the effluents from textile mills in a semiarid climatic zone in Rajasthan. Mathews (1994) identified sewage outfalls, industrial effluents and the Golf course runoff as the major reasons behind the estuarine fin fish contamination in South Carolina estuaries. Like water, sediments are able to hold large bacterial populations. Under certain specific conditions, sediments trap considerable quantities of bacterial pollutants. (Rittenberg et al. 1958; Hendricks 1971; Babinback et al. 1977). But the capacity was generally dependent upon the nature and granularity of the sediments.

Prieur (1981 a;b) and Durairaj et al. (1983) reported the instances of shell fish accumulate autochthonous bacteria into the gills and intestine from the surrounding water and sediment. They also mentioned
that, the total count of surrounding water was significantly lower than that in the oyster.

2.2 PHYSICO- CHEMICAL FACTORS

2.2.1 Influence of physico-chemical factors on bacterial flora in aquatic environment

The aquatic habitat is always put under the influence of several hydro-biological factors. Painchaud et al. (1995) reported that bacterial abundance in low-salinity waters of estuaries is not caused by salinity-related mortality of fresh water bacteria, as the mixing time between fresh and marine (>20 °/oo) waters is relatively long (days). He pointed out that mortality of fresh water bacteria can be an important process in estuaries with shorter mixing time (hours).

Enteric bacteria are known to be more resistant to environmental factors than enteric viruses (Shuval 1967). The influence of environmental and hydrographical stress on indicator bacteria in natural watermasses were studied in detail. (Bissonnette et al. 1975). In an investigation in the canal communities along the Texas coast, Goyal et al. (1977) found that bottom sediment in shallow and canal systems could act as reservoirs of enteric bacteria which might be resuspended in response to various environmental factors and recreational activities. Hydrobiology of polluted water bodies have been the subject of several investigations. Detailed studies were carried out in Cochin backwaters, (Sankaranarayanan and Qasim 1969;

Fujioka et al. (1981) reported that sunlight is the primary influencing factor controlling the survival of indicator bacteria suspended in water under field conditions and that 90% of sewage-borne FC and FS suspended in sea water at 15-20°C can be inactivated within 30-60 minutes respectively on exposure to sunlight. Numerous evidences (Gameson and Saxon 1967; Fujioka et al. 1981; Kapuscinski and Mitchell 1981; Mc Cambridge and McMeekin 1981) are available, suggesting that sunlight is bactericidal and play a key role in controlling the survival of indicator bacteria in natural waters. It was also revealed that highest bacterial counts were obtained in fishes at ambient temperatures (28 ± 2°C), and 1-2 log unit decrease occurred in counts by incubation at 37°C and 80°C.

Chamberlin and Mitchell (1978) suggested that under field conditions, sunlight is probably the most effective bactericidal agent in natural waters. Further evidences for the bactericidal effect of sunlight has been provided by Bellair et al. (1977) and Mc Cambridge and McMeekin (1981). Even though this is the situation, the possible bactericidal effect of sunlight is not proved beyond doubt and specific recommendations to prevent exposure to bacteriological water samples to sunlight are not given
in water quality manuals, including standard methods (APHA 1980; APHA, AWWA and WEF 1992).

Hydrodynamic factors such as prevailing winds, currents etc play a very important role in dilution and dispersion of contaminants (Volterra et al. 1984). Prieur et al. (1987) showed that halophobic bacteria made a significant proportion (up to 50%) of fresh water bacterial communities in the Gironde River in France. It has also been suggested that sediment-flocculation (Rittenberg et al. 1958), presence of microbial toxins (Aubert et al. 1964), nutrient deficiency (Carlucci and Pramer 1960 a), lysin by bacteriophage (Carlucci and Pramer 1960 b) and predation by other microorganisms (Enzinger and Cooper 1976) are involved in inactivation and destruction of bacteria in natural water bodies.

In the St. Lawrence estuary, Painchaud and Therriault (1985;1989) and Painchaud et al. (1987) have shown that heterotrophic activity and bacterial abundance decreased sharply between salinities of zero and 10 °/00. Neerja et al. (1991) observed an inverse relationship between the FC positive strains of Klebsiella and growth at 10°C. Studies in the watersprings of Kunashir Island, Savvichev and Nikitin (1992 a;b) proved that oligotrophic bacteria are less tolerant to higher temperature than the eutrophic ones and they differ in their halotolerance.
A survey of *Vibrio cholerae* non-01 from surface waters and sediment samples was carried out in the aquatic environment of Hiroshima city (Ciira *et al.* 1991) and found that salinity and pH did not have any obvious influence on growth and distribution of *Vibrio* sp in water. But Lovejoy *et al.* (1993) reported that dilution of fresh water bacterial community by estuarine mixing contributes to the decline of bacteria in low salinity waters along with the decline of planktonic protozoan grazers. Influencing effect of higher salinity on coliform bacteria was reported by Carlucci (1960 b) also.

A pH range of 6-8 was found optimum for the growth of *Escherichia coli* in sewage (Gaddad and Reddy 1992). In a study of survival, the ability of certain bacteria under in situ conditions of temperature and pressure, Takizawa *et al.* (1993) found that culturable portions of autochthonous and allochthonous bacterial communities at waste disposal sites are inhibited by high pressure and low temperature. Barillier and Josette (1993) observed that overaging 33%, the growth yield in river water did not show any trend with temperature in the range of 8-25ºC, but increased with the concentrations of dissolved organic carbon in the range of 2-12 mg of C./litre⁻¹. McMeekin *et al.* (1993) proposed that low temperature and water salinity are the major constraints on microbial growth in lakes. Chai *et al.* (1994) reported the variations of THB, TC and FC in relation to temperature, salinity, dissolved oxygen, depth, seasons and geographic location in shell fish growing waters of Chesapeake Bay.
The effect of solar radiation on the survival of *Escherichia coli* *Salmonella zanzibar* and a faecal *Streptococcus* strain in sea water was tested in laboratory experiments and survival of *Escherichia coli* was tested under natural light conditions at Davis Station, Antarctica. Exposure to artificial light of wave lengths 290-800 nm caused a rapid decline in viability of each strain examined. Faecal bacteria were rapidly inactivated when exposed to sunlight in Antarctic waters (Statham and McMeekin 1994).

2.3 QUANTITATIVE DISTRIBUTION OF HETEROTROPHIC BACTERIA

2.3.1. Faecal and sewage indicator bacteria

Since time immemorial, contamination of water and food with human and other animal faeces were considered to be harmful. The assumption that, indiscriminate discharge of animal and human faecal wastes into water bodies of recreational purposes as a source of potential public health hazard has lead historically into the search for a typical microbial indicator capable of monitoring the water quality. Thus, the coliform group of bacteria were found as the most suitable ones to serve the purpose.

Regular monitoring of coliform bacteria directly indicates the rate of microbiological pollution of drinking water (WHO 1980). Better understanding of the source and significance of the different components of the coliform group, faecal coliforms were selected as the typical indicators of sewage and faecal pollution in inland waters and estuaries. The
epidemiological studies carried out in the United States (USEPA 1980) and the Mediterranean Institutes in Malaga and Tarrogonia proposed that faecal *Streptococci* and faecal coliforms are the two quality indicators that best correlate with the morbidity rate of gastro intestinal symptoms and now it has been finalised that FC estimation is sufficient to indicate faecal pollution (WHO 1991).

The use of bacteria as an indicator of water quality commenced in 1880 when von Fritsch described *Klebsiella pneumonia* and *Klebsiella rhinoscleromatis* as microorganisms of human contamination. Following it in 1885, Escherich identified *Bacillus coli*, a bacterium of human faecal origin and speculated that such microbes could be used for identification and estimation of faecal contamination in water. He put forth *Bacillus coli* as an indicator of faecal pollution and this was used in the sanitary evaluation of water bodies. Subsequently, these coliform bacteria were isolated from the gut contents of fresh water fishes (Amyot 1901; Johnson 1904).

Later studies confirmed beyond doubt that coliforms are not usually associated with the normal intestinal flora of fin fish and shellfish and their presence indicate a recent faecal contamination (Gibbons 1934 a,b). Microbial analysis of 60 fishes caught in three areas of Flat head lake, Montana, substantiated the theory that fish carry a significant population of bacteria and fungi on their body surface and in their digestive tracts (Potter and Baker 1961). Also it was proposed that fishes, if they
somehow acquire pathogenic microbes, could act as potential carriers and contaminate distant unpolluted places (Geldreich and Clarke 1966).

Ramteke et al. (1990;91) suggested that more than half of the available natural drinking water sources in rural areas of India are contaminated with coliforms exhibiting increased antibiotic resistance. Antibiotic resistant and metal tolerant bacteria make their appearance as a result of exposure to aquatic environments contaminated with metals and faecal wastes from homoeotherms by co-incidental co-selection of resistance factors (Trimoney et al.1978; Sterritt and Lester 1980; Calormis et al. 1984). In a study of indicator bacteria in Cochin backwaters, Chandrika (1986) proposed that faecal coliforms are the most suitable indicator bacteria for faecal contamination in aquatic system. Pathak and Gopal (1994) also supported this view.

2.3.1.1 Indicator bacteria in fin fish and shell fish

Studies on gut bacterial flora of some fresh water fishes in tropical water (Fasanya et al. 1988) revealed that, most predominant microbes isolated from the skin and gills of the fish, belonged to faecal coliform in naturally contaminated oysters and it could be as high as 39,000 MPN/100 g and still reduced to values below 50 in 48 h (Hunt et al. 1976; Haven et al. 1978). More or less similar data was obtained by other workers also (Heffernan and Cabelli 1970; Piel et al. 1974).
Detailed studies of Fuhrmann et al. (1984) proved that some members of the family enterobacteriaceae are associated with organs and skin lesions of fresh water fish. Buras et al. (1987) noted down that fish grown in pond containing waste water, accumulate faecal bacteria in their body and at a critical concentration of about $10^4$ / ml of water, a measurable level of bacteria could be observed in the fish flesh. More or less similar findings were obtained by Ogbondeminu and Okoye (1992). In a study of microflora in waste water aquaculture ponds, MPN of TC/FC varied between $0.5 \times 10^4 - 12.0 \times 10^4$ in water (Bhowmik et al. 1995).

Microorganisms associated with fish has been subjected to thorough investigation at the turn of this century. Primary stimulus for these studies was the recognition of the involvement of shell fish as potential carriers of different human enteric diseases. It was identified that fish and marine invertebrates inhabiting unpolluted waters did not carry the type of bacteria found in the intestine of human and other animals. It was also noted down that the bivalve molluscs could cleanse themselves of enteric bacteria if held in clean flowing waters (Karunasagar and Karunasagar 1991). The faecal coliform count was reported to be higher in oysters than in the surrounding water and sediment in Yaquina estuary, USA (Arnold et al. 1992).
2.3.1.2 Indicator bacteria in water and sediment

1950's witnessed the onset of study of bacterial indicators in coastal and interior waters (Stevenson 1953; Moore 1954 and 59; Nusbaum and Garvar 1955; Orlob 1956). Then only the relevance and significance of coastal and inland water pollution by bacteria caught attention of the scientific world. A plenty of reports on the presence of bacterial pollution indicators were critically discussed and the importance have been established.

Faecal indicator organisms and the total heterotrophs in Cochin back water was studied by Santhakumari (1966); Chandrika and Pillai (1975) and Raveendran et al. (1978). Sastry et al. (1969) reported the presence of coliforms and Enterococci in natural waters of Bhopal city. Investigations of Gallagher and Spino (1968) suggested that all total coliform occurrences could be associated with faecal pollution. Naturally occurring microbes, enteric bacteria and the stocked strains of pathogenic species were investigated in North Oconee River, a typical stream of the northern Georgia piedmont in Clarke Country near Athens by Hendricks (1972). His data indicate that the enteric organisms are able to survive and proliferate in very stringent nutrient conditions.

Seasonal occurrence and distribution of bacterial pathogens and indicators in Italian coastal waters (Lombardo 1973; Parvis et al. 1975; Boeddu et al. 1977; Izzo et al. 1983; Volterra et al. 1984), Long Island
Sound (Birley and Buck 1975) and the bacterial pollution of Chesapeake Bay (Sayler et al. 1975; Carney et al. 1975) were worked out in detail. Grabow et al. (1974) and Cooke (1975) suggested that coliform bacteria can no longer be regarded as harmless indicators of pollution.

*Escherichia coli* is widely used as an indicator of faecal contamination (Kapuscinski and Mitchell 1981). The quantitative and combined occurrence of *Escherichia coli* and other coliform indicators and *Salmonella* were studied by Gerba et al. (1977); and Gore et al. (1980) established a relationship between them. Reports are available on extensive studies on the distribution of indicator bacteria on coastal waters of Texas (Gerba et al. 1977; Goyal et al. 1977). The quantitative presence of indicator organisms like coliforms in surface water and sediment samples of the Cochin estuary was studied by Gore et al. (1979). In a critical evaluation of coliforms and their disease causing capability, Guerrant et al. (1976) and Back et al. (1980) established that coliforms not only cause gastroenteritis but act as pathogens of certain other types of infections in human and other animals.

Yoshikura et al. (1980) studied the distribution of faecal indicator bacteria in the coastal sea waters of Osaka in Japan. More or less similar works were done in the Shizuoka region of Japan (Ogawa 1973) and in the coastal waters of Puerto Rico (Grimes et al. 1984). The bacteriological pollution of coastal recreational waters and backwaters of Canada were
investigated by Robertson (1984). Studies on bacterial indicators and their role in monitoring the intensity of pollution were reported by various investigators. (Breittmayer and Guathier 1978; Bell et al. 1980; Bockemuhl et al. 1986; Wright 1986)

The hygienic quality of environmental waters is usually assessed by analysing water samples for concentration of indicator bacteria such as coliforms of faecal origin (Fujioka and Narikawa 1982). Rivilla and Gonzalez (1988), isolated faecal indicator bacteria from mineral waters while Rosenberg and Hernandez-Duquino (1989) isolated the same from springs and even from bottled mineral waters.

Reports are available on the bacterial indicators in coastal environment from several parts of the world. Geldreich and Clarke (1966) from Ohio USA; Papadakis (1972) from Greece; Pinon and Pijak (1972) and Yde et al. (1980) from Belgium; Fernandez (1973) from Venezuela; Burgess (1974) from Australia; Oger et al. (1974) from England; Carney et al. (1975) from Maryland, USA; Sales (1976) from Chile; Goyal et al. (1977) from the United states; Owens (1978) from Malaysia; Thayib and Suhadi (1979) from Indonesia; Hirn et al. (1980) from Finland; Velescu (1982) from Romania; Kim (1983) from Korea; Plusquellec et al. (1983) from France and Bhattacherjee et al. (1988) and Daniel and Rajamani (1992) from India, are among the selected ones.
The assessment of micro biological standards of water include the regular monitoring of faecal indicators (Anon 1989) as well as aerobic heterotrophic bacteria, total coliforms, *Pseudomonas aeruginosa* and *Legionella* (Anon 1990). But it was proposed (Leclerc *et al.* 1989) that, the occasional presence of different coliforms need not represent the actual faecal contamination, since they might be present in the soil and enter the source from there and it would be indicative of only the inadequate natural filtration or insufficient protection of springs. Incidents of high FC count in well water in places of better sanitary and sewage clearness facility, indirectly indicate the contamination of coliforms even in underground watersources. In shallow well, the chances of faecal pollution are high and the contamination may be from the near by septic tanks, sewage pits etc.

Kimura (1992) reported a higher heterotrophic and faecal coliform count in estuaries than the open sea. Close proximity and the suitable conditions in the estuary might be the reasons for the higher count. The presence and use of different classes of bacteriophages, enteroviruses and *Clostridium perfringens* as indicators, in natural waters were studied in detail (Ahammed 1993) Briski *et al.* (1993), reported that mountain brook water which contain very small number of microbes were getting polluted with FC during its flow downwards.
A 30% guideline violation of faecal pollution was noted in a bacteriological examination of river water in St. Clair River in Sarnia, Ontario, Canada (Marsalek et al. 1994).

2.4 QUALITATIVE DISTRIBUTION OF HETEROTROPHIC BACTERIA

Fin fish and shell fish constitute the major habitat of both Gram negative and Gram positive bacterial flora in the aquatic environment.

Fantham (1907) and Dimitroff (1926) observed Spirochaetes associated with shell fish. Berry (1916) and Greiger et al. (1926) reported the presence of Proteus, Pseudomonas and Alcaligenes in oysters. The major groups of bacteria loaded by Mexican Gulf shrimp were Acinetobacter, Micrococcus, Pseudomonas and Bacillus (Williams et al. 1952). Klebsiella sp. were found to be a prominent cause of nosocomial infections and were often found in the human bowel and in a variety of environmental situations (Duncan and Razzell 1972; Knittel 1975; Naumura and Seidler 1978; Kumar and Arora 1994).

Karthiayani and Iyer (1975) noticed a predominance of Gram negative, rod like organisms belonging to Achromobacter, Pseudomonas and Vibrio associated with lobsters, prawns, sardines and seer fish, and these bacteria made up the major share of population in natural waters (Schwaller and Schmidt-Lorenz 1980; Quevedo-Sarmiento et al. 1986) Vibrio sp. was found to be the dominant among the flora. In addition,
Aeromonas, Acinetobacter, Alcaligens members of enterobacteriaceae, Bacillus, Pseudomonas and Micrococcus also were contributed to the bulk. Observations suggested that Gram negative bacteria in general, can sustain sub lethal injury in coastal and inland surface waters (Bissonnett et al. 1975; Kaper et al. 1977; Presswood and strong 1978; Dutka et al. 1979).

Cann (1977 a; b) reported a very lower incidence of Pseudomonas in the tropical prawns (Penaeus spp and Metapenaeus spp) from the Gulf of Thailand and Parapenaeopsis spp from the Straits of Malaca. Pseudomonas, Vibrio, Salmonella, Shigella, Proteus and Enterococcus (Kehr and Butterfield 1943) and Escherichia coli (Janssen 1970; Hejkal et al. 1983) were identified in fish intestine. Lawton and Morse (1980) noted down that Clostridium botulinum, Clostridium tetani, Staphylococcus aureus, Erysipelothrix musiopathie, Streptococci, Shigella and various serotypes of Salmonella and Vibrio parahaemolyticus (Janssen 1970) survived and multiplied in the gut, muscle and various other fish tissues.

Prieur (1981 b; 1984) reported that microflora mainly associated with bivalve molluscs were Gram negative fermentative rods identified very often as Vibro and these bacteria were more abundant in the body of bivalves than in the surrounding water. Prieur (1981 b) also showed that the bacterial population of the digestive tract of mussels differ from that of the surrounding water. Plusquellec et al. (1983) mentioned that this selective effect may be linked to the presence of specific digestive enzyme system.
This effect would amplify the relative resistance of bacteria to various factors in the environment (Geldreich and Kenner 1969)

Pellett et al. (1983) identified *Pseudomonas aeruginosa* as a water borne pathogen. The presence of Gram positive *Cocci* in mineral waters has already been reported by Schwaller and Schmidt-Lorenz (1980); Quevedo - Sarmiento et al. (1986), Mavridou (1992), Ogan (1992) and Hunter (1993). Fuhrmann et al. (1984) isolated *Citrobacter freundii* and Sugita et al. (1985) identified *Pseudomonas* and members of enterobacteriaceae from the fish *Cyprinus carpio*.

Surendran et al. (1989) found *Pseudomonas*, *Vibrio*, *Acinetobacter*, *Moraxella*, *Micrococcus* to be the major groups in *Oreochromis mossambicus*. Instances of isolation of obligately anaerobic cellulolytic, mesophilic, rod shaped, spore-forming Gram positive *Clostridium* from municipal solid wastes were reported (Benoit et al. 1992). Viable *Listeria* cells could be isolated from the gut of King Salmon, *Onchorhynchus tshawytscha* (Bremer and Cooke 1993). Singh and Kulshrestha (1994) isolated and identified several numbers of enteropathogenic *Escherichia coli* from molluscs and other sea foods.

A survey of *Vibrio cholerae* non-01 in surface waters and sediment was carried out in the aquatic environment of Hiroshima city (Ciira et al. 1991). Of the 72 samples, 49 (68.1%) were positive for the
organism, while in sediment samples, 17 of 47 (36.2%) were positive. The incidence of the organism was higher in summer both in water and sediment samples. A two year long detailed investigation of the potential enteric pathogens like *Pseudomonas aeruginosa*, faecal *Escherichia coli*, Non-01 *Vibrio*, *Aeromonas hydrophila*, *Plesiomonas*, *Vibrio fluoiatis*, *Proteus mirabilis*, *Vibrio algenolyticus* and *Klebsiella pneumoniae* in the estuarine waters of Hugli in India by Chatterjee *et al.* (1991) pointed out a potential risk in fishing and recreation in the waterbody.

*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* were isolated from swimming pool (Daniel and Rajamani 1992). Bordalo (1994) reported that in brackish water situations, *Escherichia coli* formed in average 82% of the total coliforms. However the percentage ranged between 47.1-88.5.

It has been observed that vibrios could tide over different unfavorable hydrographic conditions by getting lodged in sea foods. (Kaneko and Colwell 1978, Anand *et al.* 1981) and were isolated from bivalves and sediment samples (Broek *et al.* 1979; Garcia and Antillon, 1990). Episodes of outbreak of cholera are not uncommon in India (Asha *et al.* 1992). Vibrios were identified from endemic places as the etiologic agents concerned with several diarrhoeal and systemic diseases in man (Zafari *et al.* 1973; Back *et al.* 1974, Dakin *et al.* 1974; Oliver *et al.* 1982; Singh *et al.* 1986; Janda *et al.* 1988; Deb *et al.* 1990; Fule *et al.* 1990; Pathak *et al.* 1993b; Ghosh *et al.* 1994).
Vibrios have been reported to be associated with severe gastroenteritis after consumption of raw oysters (Carnahan et al. 1994) and were abundantly present in fishes and shell fishes (Sanjeev and Stephen 1992), Gulf coast oysters (Cooke and Ruple 1989); Giant tiger prawn (Penaeus monodon) (Liu et al. 1994); Kuruma prawns (Penaeus japonicus) in Japan (Ishimura et al. 1995).

A high occurrence of Vibrio sp in water during summer was recorded in several investigations (Bashford et al. 1979; Kaper et al. 1979; 81; Lee et al. 1982, West and Lee 1982; Colwell 1984; Rhodes et al. 1986; Joshi and Rajput 1992; Anuradha et al. 1993, Ueda et al.1993; Velammal et al. 1994). Watkins and Cabelli (1985) reported that nutrient loaded by sewage stimulate the growth and proliferation of vibrios in polluted water and sediments and this would provide the most conductive ecological niche for the halophile (Nair 1981; Xu et al. 1983). On Screening different sea foods in coastal waters, it was found that 34.7% of bivalves, 23.8% of crabs, 14.5% of prawns and 14.2% of fin fish accumulated Vibrio parahaemolyticus (Aiyamperumal et al. 1994).

Motile Aeromonads are an ubiquitous component of the aquatic environment (Neilson 1978; Altwegg et al. 1990; Ramteke et al. 1992;93; Okpokwasili 1991) and were considered as normal inhabitant of the intestinal tract of fish (Ojala 1968; Trust and Sparrow,1974; Burke et al. 1984 a; b; Gray 1984). They are identified as a significant water borne bacterial

Though initially considered opportunistic, Aeromonas sp. are now emerging as primary pathogen in a variety of human gastroenteritis, wound infections etc. (Davis et al. 1978; Ljungh et al. 1981; Morgan et al. 1985, Hobbs 1992; Hudson 1992; 93; Sujatha and Rao 1993; Buncic and Panin 1994; Yambot and Inglis 1994). Aeromonas hydrophila, Micrococcus variance and Pseudomonads were isolated from ulcers of Epizootic Ulcerative Syndrome in fishes (Pradhan et al. 1991; Bright Singh et al. 1994; Joseph and Carnahan 1994).

2.5 ACCUMULATION AND DEPURATION OF BACTERIA IN SHELL FISH

Bivalves filter large quantities of bacteria from the surrounding water and sediment. Goverin et al. (1994) reported that when a water stream with heavy urban sewage was passed through a system of mussel collectors, a sharp decrease of 44% of heterotrophs and 44-48% of intestinal bacteria in the running water occurred.

The mechanism of bacterial concentration (uptake) and elimination by shell fish are not exactly known. Bacteria in suspension, in ambient waters are trapped in mucus on the gills, mantle and labial palp surfaces.
then transported by cilia into the labial palp, where, sorting occurs into rejected components (pseudofaeces) and compounds for ingestion (Perkins et al. 1980). The efficacy of purification is related to the rate of oyster pumping and feeding (Rowse and Fleet 1984).

Polluted oysters are purified by allowing them short periods of natural pumping and feeding in tanks of decontaminated sea water. During this time contaminants are eliminated as part of the faeces (Furfari 1976; Fleet 1978; Willington 1982). An appropriate reduction of E. coli was noticed when a pond reared fish was put under starvation condition in tanks of same water (Fattal et al. 1992). Burkhardt et al. (1992) evaluated the relative elimination rates of a diverse group of indicator microorganisms from the hard shell clam, Mercenaria mercenaria (Linneaus) and the eastern oyster, Crassostrea virginica (Gmelin). Haven et al. (1966) reported that naturally contaminated oysters, 4 h after being placed in a depuration plant showed a ratio of 27:1 faecal and 37:1 total coliforms in faeces as compared to pseudofaeces; some of the bacteria might be digested or killed while traversing the gut and even higher proportion of coliform bacteria were probably shunted through it.

Perkins et al. (1980) reported that healthy pumping oysters may have the capacity to inactivate or digest significant numbers of coliforms without obvious defecation occurring. Also it was showed that if oyster’s shells are held shut, with the oyster in or out of water, FC levels will
decline more slowly in the first 72 h than in oysters which are open and pumping (Haven et al. 1978). *Vibrio vulnificus* was found in oysters as a result of filtration of the bacteria from aea water rather than, active multiplication in oysters (Kelley and Dinuzzo 1985).

Naturally contaminated shell fish can eliminate FC in 48h to levels below most market standards over a wide range of environmental conditions. MPN enumeration of shell fish depurated for 48h yielded a median values of <18 FC/100 g of oyster (*Crassostrea virginica*) with <10% of the samples exceeding 78 FC/100 g. (Perkins et al. 1980). Short term (1 h) and long term (3 days) elimination of lower and higher densities of 5 bacteriae - *Klebsiella pneumoniae, Aeromonas hydrophila*, *Escherichia coli, Enterococcus faecalis* and *Staphylococcus epidermidis* by Protists were measured in a fresh water system (Iribérrí et al. 1994)

Shell fishes especially the bivalves can filter considerable volumes of water (Jorgenson 1960), and are particularly suited to retain pathogenic bacteria (Majori et al. 1977; Pellegrino et al. 1977); and the quantity of bacteria accumulated in this manner is proportional to the actual number present in the surrounding water (Cabelli et al. 1979).

The mean values (MPN/100g) of FC associated with the mussel, *Perna perna* was reported to vary between 1,100-44,000 and in mussel growing water it ranged between 18-3,300 (Matte et al. 1994). Thus, the
count in the gut was significantly higher than that in water, indicating the bivalve's holding capacity. Higher levels of FC represented as *E. coli*, derived from culture and applied to oysters under laboratory conditions were not eliminated below 50 FC/100g in 48 h (Haven and Morales - Alamo 1966; Presnell *et al.* 1969). However, such relationships are not likely to be relevant to the naturally occurring situations in estuaries.

There are cases of FC values higher than 130/100g of shell fish even after 72 h depuration (Heffernan and Cabelli 1971; Huntley and Hammerstrom 1971). Regarding the depuration time, Wood (1976) mentioned that bacteria do not penetrate the tissues, instead, they only remain in the alimentary tract or on gill and mantle surfaces and can be readily removed as shell fish are relaid in clean water. Devlin and Neufeld (1971) and Metcalf *et al.* (1973) observed that depuration rates under semi-controlled tank conditions were faster than those in estuaries even when the estuarine waters contain very low or undetectable level of coliforms. Metcalf *et al.* (1973) also found that *Salmonellae* were eliminated faster than FC, in oysters held in estuary. Janssen (1974) however found long residence time in oysters artificially contaminated with higher levels of *Shigella flexneri*, *Francisella tularensis* and *Salmonella typhimurium*.

Efficiency of the clam *Meretrix casta* in concentrating bacterial pollution indicators was reported by Chandrika (1986). Also it was reported
that clams may be used as bio-indicators of faecal pollution and is preferable for testing the water quality.

Effects of environmental factors such as temperature, dissolved oxygen, salinity, turbidity, flow rate and food availability have been examined for several shell fish, like *Crassostrea virginica* and *Mercenaria mercenaria*. Influence of these factors on uptake and elimination of coliforms by the soft shell clam, *Mya arenaria* was studied by Arcisz and Kelley (1955), Erdman and Tennant (1956) and Goggins *et al.* (1964). Appreciable drops in depuration rates occurred below the dissolved oxygen level of $0.018 \times 10^2 \text{ml} \cdot \text{l}^{-1}$ in oysters (Haven *et al.* 1978). Turbidity did not affect depuration rates at turbidity levels as high as 69.4 mg/L in Gulf of Mexico oysters (Presnell *et al.* 1969), 25 mg/L in New England clams (Cabelli and Heffernan 1971) and 77 mg/L in Chesapeake Bay oysters (Haven *et al.* 1978).

When salinity and rate of flow of water had remarkable influence on depuration (Presnell *et al.* 1969), hardly no variation was noticed by food concentration (Haven *et al.* 1978). Extensive observations were made by Goggins *et al.* (1964) regarding the influence of flow-rate of environmental water on depuration activity. They reported that there was an adverse effect of low rates below 1 gal / min per bushel on depuration activity.
Temperature also had a remarkable influence on depuration subjected to the type of shell fish and its physiological state. When there was a lowering of temperature below the range of optimum physiological activity, a decline in pumping and filtration and thereby an inhibition of accumulation of coliform bacteria, but apparently not an inhibition of elimination or inactivation took place. Thus, a net loss of coliform bacteria from shell fish occurred. (Cabelli and Heffernan (1971); Haven et al. (1978). Presnell et al. (1969) observed that Gulf coast oysters (Crassostrea virginica) depurated FC remarkably in 48 h at the range of 16.3 - 28.7°C. Similar observations were obtained by Piel et al. (1974) also. Crassostrea gigas are known to be purified best at temp below 150°C (Korringa 1976; Ayres 1978).

The elimination of Salmonella charity and Escherichia coli from the Sydney rock oyster, Crassostrea commercialis was examined during commercial purification of oysters under different water temperatures and salinities. (Rowse and Fleet 1984). Both organisms were rapidly eliminated at 18-22°C. Purification was effective but slower at 24 - 27°C and incomplete and inconsistent at temperatures below 170°C. The oysters suffered stress and were not effectively purified at water salinities of 15 - 20 °/00 but were rapidly purified at 32-47 °/00 salinity. Winter-harvested and summer-harvested oysters were purified similarly in water at 18-22 °C and 32 - 36 °/00 salinity.
2.6 OBJECTIVES AND SCOPE

The practical conditions necessary for control and prevention of shell fish-borne infections are now well established. However the complete information regarding the bacterial flora-indigenous and contaminated-associated with the bivalved shell fish especially of the black clam Villorita cyprinoides var. cochinensis is yet to be obtained.

The intensity of aquatic bacterial pollution may be estimated by employing clams as monitors. Due to its filter feeding habit, it may concentrate large number of bacteria including pathogens in their body especially in the gastro intestinal tract. The flora are not affected by the internal secretions and are protected from the unfavourable environmental conditions outside. Thus, the clams may act as reservoirs of bacteria including pathogens.

A review on the available literature clearly indicates the lack of sufficient knowledge on the bacterial flora associated with Villorita cyprinoides var. cochinensis (Hanley), in the back waters of Kerala. Hope the present piece of work may fill up the lacuna. The main objectives of the study may be summarised as follows.

1. To estimate quantitatively and qualitatively, the Total heterotrophic bacteria (THB), Total coliforms (TC) and Faecal coliforms (FC) associated with the gastro intestinal tract of the black clam Villorita cyprinoides var. cochinensis, water and sediment at three geographically and ecologically different locations in Vembanadu lake, the largest
tropical estuary and brackish water system in the south west coast of India. This will enable one to determine the intensity and composition of the bacterial flora of clams held under natural conditions in ecologically different locations in the lake.

2. To study the influence of environmental factors such as temperature, pH, salinity and dissolved oxygen on bacterial flora in the gastro intestinal tract of Villorita cyprinoides, water and sediment at the 3 stations.

3. To study the intensity and extent of faecal and sewage pollution and to evaluate the influence of proximity of the main land mass on degree of faecal pollution in water. Periodical enumeration of the total coliforms and faecal coliforms may provide the seasonal variation in faecal contamination of water and sediment in the back water and the connected rivers and rivulets.

4. To determine, whether regular bacteriological analysis of the clam Villorita cyprinoides present in the backwater is a satisfactory method of evaluating and monitoring, in respect of sensibility and consistency, the intensity of bacterial contamination of the backwater through animal faeces, agricultural wastes and urban sewage.

5. To substantiate the role of the black clam in the sporadic out break of epidemics in aquatic and terrestrial environments.