INTRODUCTION

1.1 PREAMBLE

The coastal areas of any nation are a valuable resource for food and recreation. During recent years the use of marine and estuarine ecosystems for the disposal of sanitary and industrial wastes has increased greatly. The self purification power of the sea is overloaded with the increasing input of wastes. As a result, these aquatic ecosystems are getting eutrophied giving ample chances for the survival and growth of sewage borne microorganisms. They include a large number and variety of infectious and toxigenic agents such as viruses, bacteria, fungi, protozoan and metazoan parasites and biotoxins produced by several species of bacteria and dinoflagellates. In tropical situation, the range of hazard is wide including infections arising directly from water contact or from consuming uncooked or under-cooked sea foods.

The range of presence of bacterial indicator organisms such as total coliforms, faecal coliforms, Escherichia coli and faecal streptococci in an aquatic
environment determines the extent of sewage pollution and portrays the suitability of the environment for recreational or fishing activities. Disease outbreaks due to *Salmonella*, *Vibrio*, *Shigella*, *Leptospira*, *Pasteurella*, *Mycobacterium* and enteropathogenic *E. coli* through the use of polluted estuarine and coastal environment for food and recreation has increased. Therefore in determining water quality primary emphasis is given to health hazards. Through harvesting fish and prawn from polluted waters, disease outbreaks can be initiated by pathogenic microorganisms of autochthonous to the habitat or by the allochthonous ones brought in through the sewage input.

*Vibrio parahaemolyticus* has been recognised as an estuarine bacterium, which is responsible for numerous out-breaks of seafood-borne gastroenteritis. Besides, it has been found to infect fish and prawns.

Cochin, a major commercial and industrial city (seafood landing and exporting centre also) on the south-west coast of India, supports a population of about 6.5 lakhs. The sewage from the Cochin city and neighbouring areas find their way into Cochin backwater which is the northern end of the Vembanad lake. The six major rivers emptying into Vembanad lake also brings with them large amount of enteric pathogens along with silt and sewage. This water body forms
a good nursery and fishing ground for finfish and shellfish. Also many commercial fish and prawn hatcheries and culture farms have been established to raise commercially esteemed species around the backwater system. Hence, it is appropriate to monitor faecal bacteria and pathogenic bacteria like *V. parahaemolyticus* in this environment to understand the ecology, annual cycle and level of faecal coliforms and *V. parahaemolyticus* associated with freshly harvested seafoods.

### 1.2 REVIEW OF PREVIOUS WORK

#### 1.2.1 Distribution of faecal indicator bacteria

From time immemorial, contamination of water and food with human faeces were considered to be dangerous. The use of bacteria as an indicator of water quality originated in 1880 when von Fritsch described *Klebsiella pneumonia* and *K. rhinoscleromatis* as microorganisms of human contamination. In 1885, Escherich found out a bacterium of human faecal origin, *Bacillus coli* and postulated that such microorganisms could be used to indicate faecal contamination of water. He put forth *Bacillus coli* as an indicator of faecal pollution and at the beginning this was used in the sanitary evaluation of water bodies. Within two decades, these coliforms were isolated from the intestinal contents of fresh water fishes (Amyot, 1901;
Johnson, 1904). Subsequent studies confirmed that coliform bacteria are not usually associated with the normal intestinal flora of fish and hence their presence in fish indicate recent faecal contamination (Gibbons, 1934 a,b). It was also suggested that fishes, if they acquire pathogenic organisms, could become carriers of such bacteria and pollute distant unpolluted areas (Geldreich and Clarke, 1966).

The use of streptococci as an indicator of water quality could be traced back to 1900 (Houston, 1900). He reported that faecal streptococci were present in polluted water and absent in unpolluted water. For half a centuary interest in the field of streptococcal indicators was slowed down. However in 1950s a renewed interest was seen (Hajna 1951; Allen et al., 1953; Litsky et al., 1955; Slantz and Bartley, 1957). With development of KP Streptococcus agar and broth (Kenner et al., 1961), studies on streptococcal indicator were accelerated. A detailed review on streptococcal indicators has already been made earlier (Kenner, 1978; Kibbey et al., 1978).

Geldreich and Kenner (1969) proposed a faecal coliform to faecal streptococci ratio in differentiating human faecal pollution from faecal pollution of other animal origin. In the faeces of man faecal coliform to faecal streptococci ratio was always greater than 4 while in other farm animals, cats, dogs and rodents this ratio was below 0.7. Though this concept was
originally designed for streams, a number of workers applied this to estuarine and coastal waters with varying degrees of success (Sayler et al., 1975; Carney et al., 1975; Gore et al., 1979a,b).

1.2.1.1 Estuarine and coastal waters

Studies on bacterial indicators in coastal waters were initiated in 1950 (Stevenson, 1953; Moore, 1954; Nusbaum and Garver, 1955; Orlob, 1956; Moore, 1959) and significance of coastal bacterial pollution was recognised subsequently. As a result, numerous reports on the distribution of bacterial indicators in estuarine and coastal waters of different countries have appeared. These reports also included survival studies of these bacterial indicators in coastal waters and critical evaluations of them in indicating the presence of pathogenic microorganisms.

Extensive studies on the distribution of indicator bacteria in coastal waters of Texas were carried out (Gerba et al., 1977; Goyal et al., 1977). Seasonal occurrence and distribution of bacterial indicators and pathogens in Chesapeake Bay (Sayler et al., 1975; Carney et al., 1975) and the bacteriological pollution of the Long Island Sound were also worked out in detail (Bireley and Buck, 1975; Dudley et al., 1977). Similar works on Californian coast (Kim, 1975),
Biscayne Bay beaches (Buck, 1976), coastal waters of Puerto Rico (Grimes et al., 1984) and New York Bight (Babinchak et al., 1977) were also reported from USA.

The bacteriological quality of Canadian coastal recreational waters were assessed by Robertson (1984). Hashimoto et al. (1976) and Yoshikura et al. (1980) studied the distribution of faecal indicator organisms in coastal sea waters of Fukuyama and Osaka respectively in Japan. Similar studies from Shizuoka region were also reported earlier (Ogawa, 1973).

Bacterial pollution of the Bristol Channel (Ware et al., 1972), estuaries of Mersey (Dillon and Sellers, 1984) and River Lagen (Parker et al., 1979) were studied in United Kingdom. Investigations on bacterial indicators in Italian coastal waters were many (Lombardo, 1973; Parvis et al., 1975; Boeddu et al., 1977; Izzo et al. 1983, Volterra et al., 1985). Similarly, there were a number of reports from French coast also (Leclerc, 1971; Moreau et al., 1971; Oger et al., 1974; Breittmayer and Gauthier, 1978). Yoshpe and others had done extensive studies on the hygienic quality of coastal waters of Israel (Yoshpe and Shuval, 1972; Yoshpe, 1981, 82).

Reports on the distribution of bacterial indicators in coastal environment of other countries include Burgess (1974).
from Australia; Papadakis (1972) from Greece; Kush (1974)
from China; Sales (1976) from Chile; Fernandez (1973) from
Venezuela; Owens (1978) from Malaya; Thayib and Suhadi (1979)
from Indonesia; Hirn et al (1980) from Finland; Kim and Chang
Pinon and Pijck (1972) and Yde et al (1980) from Belgium coast.

1.2.1.2 Association with seafoods

Isolation of coliform bacteria from fishes dates
back to the beginning of this century (Amyot, 1901; Johnson,
1904). They were isolated from marine fishes (Gibbons, 1934 a,b)
and oysters and oyster waters (Perry and Bayliss, 1936).
Retention of E. coli in the intestine of trout upto fourteen
days after giving food and water dosed with E.coli was reported
(Glantz and Krantz, 1965). In an elaborate study on the
occurrence, distribution and persistence of coliforms, faecal
coliforms and faecal streptococci in the intestine of various
fishes, Geldreich and Clarke (1966) came to the conclusion that
the composition of the intestinal flora is related to the level
of contamination of water and food in the environment.
Microbiology of aquatic organisms are influenced to a great
extent by their feeding habits. Because of their bottom
dwelling and detritus (rich in microorganisms) feeding nature,
they contain high loads of microorganisms than planktivore
fishes (Natarajan et al., 1979).
Most of the microorganisms associated with freshly harvested seafoods are eliminated by chlorination and other treatments during processing. Hence from the commercial and public health point of view extensive works on bacterial indicators were done in processed seafoods (Larkin et al., 1956; Fujiwara et al., 1972; Nickerson and Pollak, 1972; Chang et al., 1975; Baross et al., 1977; Wood et al., 1983; Greenwood et al., 1985). Informations on bacterial indicators associated with live or freshly harvested estuarine and coastal organisms are mostly confined to filter-feeding organisms such as shellfishes. Because of their unique feeding mechanisms, they accumulate large number of microorganisms including pathogens during their feeding process (Cabelli et al., 1970; Plusquellec et al., 1983; Timoney and Abston, 1984; Kelly and Dinuzzo, 1985). Bacterial indicators associated with shellfishes were reported from USA (Zapatka and Bartolomeo, 1973), UK (Ayers, 1975, Al-Jebouri and Trollope, 1981), Canada (Bernard, 1973; MacLean, 1978), China (Leung et al., 1975; Morton and Shortridge, 1976), Nigeria and Ghana (Ottunola et al., 1983), Florida (Hood et al., 1983), Chile (Tello et al., 1970), Tokyo (Kakubo et al., 1978) and Italy (Volterra et al., 1984).

Studies on bacterial indicators and specific pathogens in freshly harvested finfishes were reported from various geographic locations. Andrews et al. (1977) surveyed three
hundred and thirty five fresh and three hundred and forty-two frozen samples of catfish (*Ictalurus punctatus*) for faecal indicators and pathogens such as *Salmonella*, *Arizona*, *Shigella* and *Edwardsiella*. Faecal coliform values for 70.7% of fresh and 92.4% of frozen samples were 400 organisms per g. 132 raw foods and 593 ready-to-eat foods were evaluated by Miskimini *et al.* (1976) for the presence of faecal indicator bacteria and food borne pathogens like *Staphylococcus* aureus, *Clostridium perfringens* and *Salmonella*. Significant corelations were found between the pathogens and the indicator ranges. Different genera of enteric bacteria present in kidneys and intestines of 192 carp (*Cyprinus carpio*) and 49 white suckers (*Catostomus commersoni*) were examined by Souter *et al.* (1976). They found that *Proteus* and *Enterobacter* were the dominant genera. *Enterobacter* was found to be dominating among the coliform flora of migrating Sockeye salmon, *Oncorhynchus nerka* (Strasdin and Dubetz, 1974). Alimentary tracts of teleosotei fished along the coast of Bari were found to harbour differing levels of *E. Coli* (Marano *et al.*, 1974). Bacterial densities in total plate counts, coliforms and *V. parahaemolyticus* in *Chromis notatus* were found to be higher in August and September (Ahn and Hwang, 1975). Nuhi and Khorasanii (1981) investigated faecal coliform and faecal streptococci densities in four species of fishes captured from Amir - Kolayeh Lagoon.
1.2.1.3 Environmental factors influencing the distribution and growth of coliforms.

Survival and growth of coliform bacteria in estuarine and coastal waters are influenced by a number of physicochemical and biological factors. Extensive works have been carried out on the response of coliforms to these factors.

Solar radiation was reported to have detrimental effect on coliform bacteria (Gameson and Saxon, 1967; Gameson et al., 1971, 73; Gameson and Gould, 1975; Chamberlin and Mitchell, 1978; Grigsby and Calkins, 1980; Fujioka et al., 1981; Kapuscinski and Mitchell, 1981; 83; Fujioka and Narikawa, 1982). This effect was primarily attributed to the UV fraction of the solar radiation. Mccambridge and McMeekin (1981) and Rhodes and Kator (1984) were of opinion that the decline of E. coli in estuarine waters were greater when microbial predators and solar radiation act together than their independent action.

In estuarine and coastal waters, coliforms are acted upon by protozoan predation (Roper and Marshall, 1978; McCambridge and McMeekin, 1979, 80a) and viruses parasitic on bacteria (Carlucci and Pramer, 1960 b). The removal of E. coli from estuarine water by lytic bacteria and indigenous protozoa were reported (Enzinger and Cooper, 1976; Roper and Marshall, 1977).
McCamberge and McMeekin (1980 b) observed that protozoan predation on Salmonella typhimurium and E. coli had an optimum temperature of 15-20°C whereas bacterial predation increased with temperature. Mitchell (1971) described the imbalances induced in autochthonous microbial predators by the entry of allochthonous microorganisms.

Pike et al (1970) reported that mortality of coliform bacteria increased with temperature. McFeters and Stuart (1972) also observed a sharp decline in E. coli population when temperature was increased from 5 to 25°C. Similar effects of temperature on coliform bacteria were also reported (Faust et al; 1975; Alton and Rakhno, 1979a; Oragui and Mara, 1983 and Rao and Boopathy, 1985).

Carlucci and Pramer (1960 a) reported that sea water usually has a pH of 8.0 and it does not favour survival of E. coli. McFeters and Stuart (1972) observed that optimum pH for survival of E. coli was between 5.5 and 7.5 and they rapidly declined below and above these values.

The detrimental effects of salinity on coliforms and E. coli were already reported (Carlucci and Pramer, 1960 a, Pike et al., 1970; Faust et al., 1975; Anderson et al., 1979). The degree of injury was proportional to salinity.
Faust et al (1975) observed survival of *E. coli* cells directly proportional to dissolved oxygen concentration. Freshwater isolates of *E. coli* showed a negative relationship with dissolved oxygen (Hanes et al., 1964).

Availability of nutrients was an advantage for coliforms in nullifying the adverse effects of some environmental factors. Nutrients like phosphorous and nitrogen, and organic substances of sewage origin were reported favouring survival and growth of *E. coli* (Carlucci and Pramer, 1960 a). However, Moebus (1972 a) observed that in synthetic and filter sterilized natural sea water the inactivation of coliform bacteria increased by addition of ZoBell's broth, peptone or glucose. He postulated that actively metabolizing cells are sensitised much faster than resting cells. Burke and Baird (1931), Vaccaro et al (1950), Orlob (1956), Savage and Hanes (1971) and Bethoux and Montegut (1976) were of opinion that addition of organic nutrients decreased bactericidal effect of sea water.

The response of coliforms to an environmental stress also depends on the duration and intensity of the stress. Scarpino and Pramer (1962) observed a linear relationship between death of *E. coli* in sea water and time. Similar observations were also reported by Bianchi et al (1976) and
Anticoliiform activity of *Skeletonema costatum* was reported by Sieburth and Pratt (1962). During 1969 and 1970, the antibacterial actions of North Sea water were found to be positively correlated with the life cycle of several diatom species (Moebus, 1972b). Acrylic acid produced by certain unicellular algae were inhibitory to coliforms (Brown *et al.*, 1977). In the Gulf of Finland, Hirn *et al.* (1980) did not find any significant relationship between phytoplankton and coliform bacteria.

Survival of coliform bacteria associated with bottom sediment was investigated extensively (Grimes, 1975; Simmann and Rheinheimer, 1975; Gerba and McLeod, 1976; Babinchak *et al.*, 1977; Chan *et al.*, 1979; Edenborn and Renwick, 1981; Hood and Nes, 1982; Izzo *et al.*, 1983; Volterra *et al.*, 1985). Sediments with their micro-environment provide protection from predator organisms. An inverse relationship was found between grain size and bacterial density (Chan *et al.*, 1979).

Other factors related to the survival of coliforms in estuarine and coastal waters are suspended particulate matter (Moebus, 1972c; Bitton and Mitchell, 1974; Faust *et al.*, 1975; Ogawa, 1977a,b) and pollution of various origin.
1.2.2 Distribution of Vibrio parahaemolyticus

History of V. parahaemolyticus can be traced to the "Shirasu food poisoning" which occurred in Osaka prefecture, Japan on October 21, 1950. Bacteriological investigation of this outbreak was carried out by Fujino and he isolated a hemolytic, fat, rod shaped and bipolar staining bacterium which he named as Pasteurella parahaemolytica (Fujino et al., 1953). Another food borne outbreak occurred on August 21, 1955 at Yokohama National Hospital involving 120 cases. Takikawa isolated a halophilic bacterium as the etiological agent and named it as Pseudomonas enteritis. Later, this was found to be very similar to Pasteurella parahaemolytica. Through the years, the nomenclature and systematic position of this bacterium underwent several modification and Sakazaki et al (1963) proposed the name V. parahaemolyticus on the basis of several morphological, cultural and biochemical characters.

After the discovery of V. parahaemolyticus in 1950 (Fujino et al., 1951) extensive works on the clinical, epidemiological and ecological aspects of the bacterium were
carried out in Japan. For about one and a half decade these works were mostly confined to Japan because *V. parahaemolyticus* initiated gastroenteritis was thought to be a problem confined to Japan and the Far East. However, in the years after 1966 interest on the study of *V. parahaemolyticus* was aroused in a number of countries and this resulted in accumulation of a vast body of literature from ecological to molecular aspects of this bacterium. The distribution of *V. parahaemolyticus* to estuarine and coastal environment and in association with seafoods has been reviewed by Natarajan *et al* (1978), Abraham (1981) and Nair (1981). Reports on this bacterium in coastal waters after 1980s are reviewed here.

1.2.2.1 Estuarine and coastal waters

During summer season *V. parahaemolyticus* was isolated in large numbers from Chesapeake Bay water and sediment (Colwell *et al*., 1981) and a high correlation was observed between salinity and population size of this species. Larson *et al* (1981) also isolated *V. parahaemolyticus* from Danish coast during summer months. Occurrence of this bacterium in marine organisms, water and sediment were reported from Kenya (Binta *et al*., 1982) and Egypt (El-Sahn *et al*., 1982). Other reports from coastal water bodies were from
Julu Harbor (Kim and Oh, 1982), Puget Sound (Weagant and Kaysner, 1982); Nova Scotian coastal waters (Robertson and Tobin, 1983); Rhode Island (Watkin and Cabelli, 1985), Jakarta Bay (Molitoris and Joseph, 1985) and Japan (Shinoda et al., 1985).

1.2.2.2 Association with seafoods

Among the one hundred and seventy seafood samples examined in Lebanon, Abdelnoor and Roumani (1980) isolated *V. parahaemolyticus* from two fishes and one crab samples. This bacterium was found to be pathogenic to a snail, *Biomphalaria glabrata* (Ducklow et al., 1980). Population of *V. parahaemolyticus* in Malaysian shrimp was estimated through various stages from catch to frozen product (Cann et al., 1981) and it varied from nil to $4 \times 10^4/g$. 4.5% of seafish and 5.5% of shellfish collected from Kenya were positive for *V. parahaemolyticus* (Binta et al., 1982) 88% of the hemolymph of the blue crab *Callinectes sapidus* collected from Galveston Bay contained various *Vibrio* spp and their density varied from $10^3$ to $10^5$ per ml. *V. parahaemolyticus* was the most prevalent of the pathogenic *Vibrio* spp and it was detected in 23% of the hemolymph samples (Davis and Sizemore, 1982). Low levels of *V. parahaemolyticus* were observed in sea urchin, clams and wedge shells collected along Egyptian
coast (El-Sahn et al., 1982). Cells of *E. coli* were depurated faster than *V. parahaemolyticus* and *V. harveyi* by the hardshell clam (Greenberg et al., 1982). In the Long Island oysters tested 12 of 36 samples contained *V. parahaemolyticus* at a range of 3.6 to 23 cells per gram (Tepedino, 1982). Growth of *V. parahaemolyticus* in opened and unopened Sydney Rock oysters were studied by Eyles et al. (1985). An outbreak of gastro-enteritis and wound infection were reported by Nolan et al. (1984) and in another instance a kanagawa negative strain of *V. parahaemolyticus* was isolated from a wound infection (Johnson et al., 1984).

### 1.2.2.3 Effect of physico-chemical parameters on *V. parahaemolyticus*

Effect of physico-chemical parameters such as temperature (Matches et al., 1977; Goldmintz et al., 1973; Johnson and Liston, 1973; Johnson et al., 1973; Thomson and Thacker, 1973; Bradshaw et al., 1974; Goatcher et al., 1974), salt concentration (Nelson and Potter, 1976; Ro and Woodburn, 1976) and various proteins (Beuchat and Jones, 1979) on growth and survival of *V. parahaemolyticus* in various kinds of seafoods and tissue homogenates were reported. Vanderzant and Nickelson (1972), Ermolina and Shikalov (1975) and James (1983) also studied the interaction of temperature, $pH$ and NaCl concentration on growth and survival of this bacterium in seafoods or tissue.
homogenates. However, the effect of environmental factors on the growth and survival of *V. parahaemolyticus* in semisynthetic media are limited. In the light of the present study, the review is restricted to reports on the effect of environmental factors on *V. parahaemolyticus* in semisynthetic media such as nutrient broth or tryptic soy broth.

Growth of *V. parahaemolyticus* is adversely affected by lower and higher temperatures. Asakawa (1967) reported that inactivation of this bacterium was higher at -10°C than at -20°C whereas survival was more at 0°C. NaCl has a protective effect on survival of this bacterium of lower as well as higher temperatures (Covert and Woodburn, 1972; Beuchat, 1973, 74, 75; Jackson, 1974). Beuchat and Worthington (1976) observed a change in the ratio of saturated to unsaturated fatty acid in *V. parahaemolyticus* when temperature was increased. Membrane damage took place when these bacteria were exposed to 2°C (van den Brock and Mossel, 1977).

Growth of *V. parahaemolyticus* was observed at pH 4.8 at 5°C (Beuchat, 1973). At pH 7 the organism exhibited least sensitivity to heat treatment (Goldmintz, 1974; Beuchat, 1975). Formation of lateral flagella was inhibited under an alkaline pH whereas monotrichous flagellation was not affected (Kimura et al., 1979).
Ions usually have a protective effect on *V. parahaemolyticus* when exposed to extremes of temperature tolerance (Covert and Woodburn, 1972; Beuchat, 1973, 74, 75). In experimental studies with sodium, potassium and lithium ions, this bacterium did not grow in the absence of sodium ions (Rottini *et al.*, 1974) but Palasuotheram (1981) did not find any such specific sodium ion requirement. Magnesium and potassium ions were found to assist in recovery from thermal injury (Heinis *et al.*, 1977). Similarly, recovery of chill-stressed *V. parahaemolyticus* was enhanced by the presence of magnesium and iron salts (Lin and Beuchat, 1980).

Among the biological agents acting against *V. parahaemolyticus* in estuarine and coastal waters, *Bdellovibrio* is the most prominent one. These are not host specific and act on other *Vibrio* spp also. (Miyamoto and Kuroda, 1975). From eight of nine sampling stations this parasite was isolated in Chesapeake Bay (Williams *et al.*, 1980). Among the marine bacterial flora *Vibrio* and *Pseudomonas* were found to be highly affected by *Bdellovibrio* (Horie and Kobayashi, 1981). Apart from the *Bdellovibrio*, marine phytoplankton also were found to inhibit growth of *V. parahaemolyticus* in coastal waters (Nakayama and Ohno, 1981). Goatcher and Westhoff (1975) observed repression of this bacterium by a *Pseudomonas* sp.
Growth of *V. parahaemolyticus* was found to be inhibited by irradiation (Matches and Liston, 1971), glycerine (Chun *et al.*, 1972), distilled water (Lee, 1972), water activity (Beuchat, 1974, 1975), hydrostatic pressure (Schwarz and Colwell, 1974), spices and organic acids (Beuchat, 1976; Robach and Hickey, 1978), and iodophor (Gray and Hsu, 1979; Chandramohan *et al.* 1980). Chitin was reported to be a major factor favouring the growth of *V. parahaemolyticus* in estuarine and coastal waters (Kaneko and Colwell, 1975).

### 1.2.2.4 Antibiotic and metal sensitivity of *V. parahaemolyticus*

A number of reports on the antibiotic sensitivity pattern of *V. parahaemolyticus* are available. Sakazaki *et al.* (1963) observed that among a large number of *V. parahaemolyticus* strains tested, all were sensitive to tetracycline and chloramphenicol. The concentrations of these antibiotics were 100 and 40 μg/disc respectively. In the studies of Chatterjee *et al.* (1970) this bacterium was found sensitive to tetracycline, chloramphenicol, streptomycin, kanamycin and polymyxin-B. Apart from these antibiotics Sanyal *et al.* (1973) found sensitivity of this bacterium to neomycin and gentamycin also. *V. parahaemolyticus* isolated from gastroenteritis cases and sea foods in Jakarta when subjected to antibiotic tests (Bonang *et al.*, 1974) exhibited sensitivity to doxycycline,
tetracycline and bac-

erythromycin and josamycin. Joseph (1974) reported the most inhibitory action of chloramphenicol, gentamycin, nalidixic acid and tetracycline on *V. parahaemolyticus* and the highest resistance to ampicillin.

In clinical and epidemiological studies on *V. parahaemolyticus* in Calcutta, Sircar *et al* (1976) and Sen *et al* (1977) reported gentamycin and chloramphenicol as most effective against this bacterium whereas ampicillin, kanamycin and streptomycin were ineffective. Apart from chloramphenicol, Kaneko and Colwell (1978) observed high sensitivity of *V. parahaemolyticus* to furacin, furoxone, neomycin, novobiocin and kantrex. James (1983) reported chloramphenicol and dihydrostreptomycin as most effective against this bacterium. Karunasagar and Karunasagar (1985) reported resistance of *V. parahaemolyticus* to 0/129 compound.

Bacterial interaction with various metals have been reported from different countries. Bacterial resistance to mercury and their role in mercury transformations were reported (Olson and Cooper, 1974; Walkar and Colwell, 1974; Olson *et al.*, 1979; Gauthier *et al.*, 1985). Kurata *et al* (1977) observed high incidence of nickel tolerant bacteria in water and sediments of the sea of Aso and they attributed this to the industrial waste containing high concentrations of nickel.
dumped into this area. Transmission of arsenic resistance in Enterobacteriaceae by conjugation and through phage was reported (Smith, 1978). In a comparative study of media, for their ability to neutralize the bacteriostatic effect of silver, Tilton and Roseberg (1978) found out that tryptone glucose agar and tryptic soy agar were more neutralizing than eosin methylene blue agar. In chlorine free water distribution systems, low levels of copper were found to injure majority of coliform bacteria (Domek et al., 1984). Copper was reported to be more toxic to Vibrio alginolyticus in anaerobic culture than aerobic culture (Schreiber et al., 1985). Role of bacteria in reduction of copper toxicity by formation of complexes were reported (Rho, 1984). Apart from this, there are a number of reports on association of antibiotic resistance with heavy metal resistance (Allen et al., 1977; Austin et al., 1977; Marques et al., 1979, Devanas et al., 1980; Sjogren and Port, 1981; Calomiris et al., 1984; Pujol et al., 1980; Timoney et al., 1978; Kadri and Salem, 1985).

1.2.3 Indian works on faecal indicator bacteria

Importance of bacterial indicators has been recognized in India as early as 1939 (Raghavachari and Iyer, 1939). However, such studies were mostly confined to freshwater habitat, clinical environment and processed foods. Due to
increasing urbanization and discharge of untreated and primarily treated sewage into estuarine and coastal waters, there developed a basic need for the study of the bacterial indicator organisms of sewage origin in estuarine and coastal waters of India.

1.2.3.1 Estuarine and coastal waters

Bacterial pollution studies in inshore waters of Kerala and Madras were carried out by Sreenivasan (1964). The coastal fishing villages of Madras coast was commented as a source of faecal pollution and highlighted the role of tidal water in the flushing of the estuary (Azariah and Subramaniam, 1982). Sastry et al. (1969) observed that enterococci were not detected in the absence of coliforms and Sen and Ghosh (1970) found enterococci index superior over coliform index in assessing water quality.

Incidence of pollution in coastal waters of Bombay was reported to be of recurring nature and the primary source of this pollution was raw or improperly treated sewage (Dwivedi and Abidi, 1977). A low level of bacterial pollution was noticed in Mandovi and Zuari estuarine waters at Goa (Row, 1981). Coliform densities in these waters ranged between 0 and 1100/ml. Raveendran et al. (1978) conducted a seasonal study on faecal
pollution of Cherai beach and on the basis of FC/FS ratio
the faecal pollution was found to be from non-human source.
Gore et al. (1979 a,b; 80) also reported faecal pollution
of Cochin backwater and a few beaches in Kerala. In a six
month period study in Cochin backwater, Lakshmanaperumalsamy
et al. (1981) reported the occurrence of faecal indicator
bacteria, *Salmonella*, *Staphylococci*, *Pseudomonas*,
*V. parahaemolyticus* *Aeromonas* and *Clostridium*. From an
ecophysiological point of view, Chandrika (1983) demonstrated
the seasonal variations of faecal indicator bacteria at
several stations in Vembanad lake and other estuarine systems
in south-west coast of India.

1.2.3.2 Association with sea foods.

Venkataraman and Sreenivasan (1953) studied the
coliform and streptococcal groups of bacteria occurring
in the intestinal tract of various fishes. Quantitative
and qualitative studies on the bacterial flora of fresh
sardines and marine fishes and prawns were reported by
Karthiayani and Iyer (1967, 75). Different IMViC types of
coliforms were isolated by Iyer and Pillai (1971) from
various processed fishery products. Rao and Gupta (1978)
isolated enteropathogenic *E. coli* from marine fishes
along the coast of Kakinada. This was the first reported
case of enteropathogenic *E. coli* in sciaenids and cat fish from India. The significance of faecal indicator bacteria in seafood was reviewed and preventive measures were suggested by Iyer (1979). High incidence of faecal coliform and *E. coli* in finfish and shellfish from Vellar estuary was reported by Sivakumar *et al* (1980) and Lakshmanaperumalsamy *et al* (1986). Occurrence of *E. coli*, faecal streptococci, and coagulase positive staphylococci in *Perma indica* cultured at Vizhinjam was reported by Pillai (1980). In Tuticorin area, Durairaj *et al* (1983) observed a low incidence of faecal coliforms, and absence of *Salmonella*, *V. cholerae*, coagulase positive staphylococci and faecal streptococci.

1.2.4 Indian works on *V. parahaemolyticus*

Though *V. parahaemolyticus* was described as an etiological agent of seafood borne gastroenteritis in Japan as early as 1951 (Fujino *et al.*, 1951), the first clinically confirmed cases with this bacterium from India was reported in 1970 (Chatterjee *et al.*, 1970; Neogy *et al.*, 1970). This was followed by a series of clinical reports in the succeeding years (Sakazaki *et al.*, 1971, Chatterjee and Neogy, 1972).
Chatterjee and Sen, 1974; Deb et al., 1975; Sircar et al., 1976 and Huq et al., 1979). Apart from seafoods, transmission of *V. parahaemolyticus* mediated through flies was reported by Chatterjee et al. (1978). In a six month period observation, 2 out of 74 gastroenteritic cases admitted in the GB Pant hospital, Port Blair were reported to be due to *V. parahaemolyticus* (Lall et al., 1979).

The mechanism of pathogenicity and toxin production in *V. parahaemolyticus* was explained (Bhattacharya et al., 1971; Guhathakurta et al., 1978). When the production of hemolysin was established, attention was branched off to isolate and purify the hemolysins. Serine and glutamic acid were found to be essential for the production of hemolysin and a chemically defined medium was described for production of hemolysin (Karunasagar, 1981). Further a factor present in lysed erythrocytes was reported to bring down the 50% lethal dose considerably in mice (Karunasagar et al., 1984). They also demonstrated the same effect with ferric ammonium citrate and manganous sulfate.

Experimental and human volunteer studies were conducted on selected strains of *V. parahaemolyticus*. Sasmal et al. (1973), while making comparative studies on biochemical aspects of different *Vibrio* spp., demonstrated differences in amounts of RNA, DNA, polysaccharides and total amino acids between *V. cholerae* and *V. parahaemolyticus*. Though kanagawa
negative strains of *V. parahaemolyticus* were occasionally isolated from gastroenteritic cases. Sanyal and Sen (1974) in their human volunteer studies demonstrated the kanagawa negative strains were unlikely to produce gastroenteritis in man. In survival studies of *V. parahaemolyticus* in sterile and nonsterile coastal and sea water *V. parahaemolyticus* survived longer in sea water than in coastal water, and this was attributed to low level of biological and chemical pollution in sea (Sinha and Doctor, 1983).

Antibiotic sensitivity on clinical isolates of *V. parahaemolyticus* were conducted by Sanyal et al. (1973) and Sen et al. (1977). All isolates were found to be sensitive to streptomycin, tetracycline, chloramphenicol, neomycin, kanamycin, gentamycin and polymyxin - B (Sanyal et al., 1973) while in a later study gentamycin and chloramphenicol were found to be effective (Sen et al., 1977). Among 1787 isolates of *V. parahaemolyticus* tested, 36 numbers were found to be resistant to 0/129 compound (Karunasagar and Karunasagar, 1985).

Apart from the clinical and experimental fields, studies on *V. parahaemolyticus* had further extended to aquatic environments and products of aquatic origin. These ecological studies were mostly confined to Calcutta and Porto Novo environments. Soon after the first clinically confirmed
reports on *V. parahaemolyticus* from Calcutta, this bacterium was isolated from cold blooded animals and non-marine fishes around this city (Chatterjee and Neogy, 1971a, 1972b; Sarkar et al., 1985). It was also detected in slime and gut of prawns and in seawater off Nagapattanam (Chandrabose and Chandrasekaran, 1976). In a survey conducted at Calcutta environment 57% of crabs, 35.2% of pomfrets, 32.5% of shrimps and 28.8% of tangra were contaminated with *V. parahaemolyticus* (De et al., 1977). Isolation of this bacterium in freshwater plankton from this area was also reported (Sarkar et al., 1983). Ecological variability was found among *V. parahaemolyticus* serotypes isolated from hydrobiological by dissimilar aquatic environments (Nair et al., 1985a).

Occurrence of *V. parahaemolyticus* in Porto Novo environment was reported (Manavalan et al., 1977). Detailed investigations on the presence of the bacterium in this environment were reported in the succeeding years. In detritus feeders the incidence of *V. parahaemolyticus* was high (56.3%) (Natarajan et al., 1979 a). In planktivore fishes, the incidence of *V. parahaemolyticus* was high in gills while in other groups, it was in faecal samples. A similarity of 94.6% was observed between freshwater and estuarine strains of *V. parahaemolyticus* in characterization experiments (Natarajan et al., 1979b). Studies on the distribution of this bacterium and allied vibrios in backwater and mangrove biotopes at Porto Novo showed that a large percentage of animals harboured this pathogen and their
survival was enhanced through sediments.

Reports on the ecology of *V. parahaemolyticus* in Porto Novo environment were made (Abraham *et al.*, 1980; Nair *et al.*, 1980a,b; Natarajan *et al.*, 1980b,c; Abraham, 1981; Nair, 1981) and the significance of *V. parahaemolyticus* in seafood industry was highlighted by Natarajan *et al.* (1980 b, c). James (1983) reported incidence and level of *V. parahaemolyticus* in freshly harvested and market samples of commercially important fin fishes and shellfishes in and around Cochin. In his studies, 45.1% of fresh samples and 43.7% of market samples were positive for *V. parahaemolyticus*. He also reported higher incidence of this bacterium in shellfishes than in fin fishers. Survival of *V. parahaemolyticus* during various processing conditions were also exemplified by him.

A few reports on isolation of this bacterium are also available from Bombay (Bandekar *et al.*, 1982; Joshi *et al.*, 1985) and Mangalore coast (Karunasagar and Mohankumar, 1980). Direct plating on TCBS agar was reported to be superior to MPN technique when *V. parahaemolyticus* counts in the sample were high (Venugopal *et al.*, 1985).
1.3 OBJECTIVES OF THE PRESENT INVESTIGATION

Microbiological studies on the incidence, behaviour, activity and ecological implications of marine micro-organisms, particularly microbial pathogens in coastal waters and estuaries exhibit the increasing concern and awareness of environmental impacts on health and wealth. Marine microbiologists have been active in investigating on the distribution, kinds of organisms and their activity in the environment. However, informations on the effect of environment on the ecology or on the distribution (spatial/temporal) of microbial community and competition among groups inhabiting the ecosystem are sparse. Estuarine environment are complex with respect to diversity of habitats, variation in physico-chemical parameters and contamination by terrestrial bacterial species.

Being the organisms of public health significance, ecological studies on total coliforms, faecal coliforms, faecal streptococci, *E. coli* and *V. parahaemolyticus* have great relevance as studies of these types would provide a wealth of information to environmentalists and to fishery industry. In order to evaluate the status, role and significance of potentially hazardous bacterial species in natural environment,
it is necessary to monitor the ecology of such organisms systematically in relation to physico-chemical parameters.

A survey on relevant literature would reveal that most of the investigations are confined to qualitative distribution of faecal coliforms and *V. parahaemolyticus*. However, very few quantitative studies on these bacteria have been carried out. Except Colwell and collaborators in Chesapeake Bay and Thompson and Vanderzant (1976) in Galveston Bay, no extensive ecological studies on faecal coliforms and *V. parahaemolyticus* over extended period of time in a predetermined site are carried out.

Ecology of indicator bacteria present in water and sediment off Cochin backwater, southwest coast of India, has been carried out (Gore et al., 1979a; Chandrika, 1983). Ecology and annual cycle of *V. parahaemolyticus* in Porto Novo coastal zone, southeast coast of India were carried out (Abraham, 1981; Nair, 1981). Literature reviewed in sections 1.2.3. and 1.2.4 show that to date no attempt has been made to study the ecology of both faecal indicator bacteria and *V. parahaemolyticus* in an estuarine system in India. The distribution and extent of survival of micro-organisms in an ecosystem depend on various environmental factors including antagonism and pollutants. Information on a) annual cycle of faecal
coliforms and *V. parahaemolyticus* b) effect of temperature, pH and salinity on the growth of *V. parahaemolyticus* individually and collectively and c) heavy metal and antibiotic sensitive/resistant *V. parahaemolyticus* in an estuarine ecosystem are not available. Hence, it is imperative to conduct a detailed investigation to bridge the prevailing gap in our knowledge on the ecology, annual cycle and relationship of faecal indicator bacteria and *V. parahaemolyticus* in Cochin backwater, a tropical estuarine ecosystem. The objectives of the present study were thus drawn out as follows:

1) To monitor the population dynamics of bacterial indicators such as total coliforms, faecal coliforms, *E. coli* and faecal streptococci in water, sediment, zooplankton, fish and prawn over a period of one year.

2) To monitor the population dynamics of the seafood borne pathogen *Vibrio parahaemolyticus* and allied organisms in the above mentioned samples.

3) To ascertain the influence of the hydrobiological parameters on the seasonal distribution of the above mentioned indicators and pathogen at three selected stations in Cochin backwater.
4) To confirm identity with the aid of reference cultures and to outline the characteristics and intergroup relationships of *V. parahaemolyticus*.

5) To study the effect of various physico-chemical parameters (individually and collectively) on growth of *V. parahaemolyticus*.

6) To find out the sensitivity/resistance pattern of *V. parahaemolyticus* to various antibiotics and heavy metals.

1.4 DISCRIPTION OF THE STUDY AREA

Vembanad lake is one of the largest tropical estuary in the south-west coast of India (9°28' and 10°10' N and 76°13' and 76°30' E). It spreads over an area of about 300 Sq.km and has a length of about 90 km. Vembanad lake has two permanent openings into Arabian sea, one at Cochin and another at Azhicode. The major six rivers emptying into this backwater are Muvattupuzha, Manimala, Meenachil, Pamba, Achancoil and Periyar. During monsoon season these rivers bring large quantities of flood waters carrying nutrients and silt.
At Cochin, Vembanad lake joins with Arabian sea through a narrow opening of about 450 m width. The depth of the channel varies from 6 m to 14 m. Bottom of the Cochin backwater is generally muddy. The major rainfalls of this area are from south-west and north-east monsoons, the total amounting to Ca 300 cm. Of this more than 75% of the fall accounts from south-west monsoon. Air temperature of this region varies between 25 and 35°C. During monsoon months large quantities of the water fern *Salvinia auriculata* spreads over the backwater. Mixed semidiurnal type of tides are present through out the year.