SANITARY SIGNIFICANCE OF FAECAL COLIFORMS IN A FISH LANDING CENTRE.

Dissertation Submitted By

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CERTIFICATE

This is to certify that this Dissertation is a bonafide record of the work done by **GISHA C.V.** under my supervision and that no part there of has been presented before for any other degree.

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CONTENTS

1. INTRODUCTION

Fish and Fish Products meant for human consumption must be free from chemical residual substances and faecal microorganisms which might be dangerous to the fish eating community. So chemical and bacteriological examination of fish-landing centre should be regularly and systematically done.

Of all the flesh foods fish is most susceptible to autolysis, oxidation, hydrolysis of fats and microbial spoilage. So it needs various preventive preservative methods. When fishes are harvested far from the processing plants preservative methods must be applied even on the fishing boat itself. Likewise hygienic procedures are equally important because it reduces the spoilage of fish.

The kind and rate of spoilage of fish vary with number of factors that is the kind of fish, the condition of fish when caught and the kind and the extent of contamination. If the contamination may reduce, the spoilage may also reduce. So the bacterial analysis have a primary importance in each processing of the fish during fish-harvesting and in fish-processing centres.

Fish landing area is one of the place where fishes were easily contaminated. Air contamination, washing process, quality of the ice are some of the means by which fishes are contaminated. So the sanitary significance of fish landing area is very important.

Concern about marine faecal pollution has grown steadily in recent years. Damage to marine organisms and ecosystems, deterioration of human health by direct contact with polluted water and consumption of contaminated sea food are among its diverse effects. Associated with these environmental aspects are economic costs and various social implications. Many effects of marine faecal pollution are unknown and the magnitude of those known to occur is often undetermined. Now a days many parts of the polluted marine environment have been examined for indicators of bacterial pollution and the effect of faecal pollution on marine ecology is one of the important line of bacteriological study today.

The environmental quality of fishery harbour is not assessed so far and published reports on faecal bacterial contamination in fishing harbour are very few. It is well known that faecal bacteria has got diversified character which influence the chemical and biological condition of fish. Gore et al. (1979), Chandrika (1983) studied quantitative occurrence of indicator organisms like Coliforms in surface water and sediment samples of the estuary. The nature and source of the faecal pollution was found to be of human origin as faecal index was always above four. In Malabar area, Korapuzha estuary was surveyed for coliform in mussel cultured field by Venkataraman and Sreenivasan (1955). Bacteria in the inshore environment at Mandapam, (Gulf of Mannar) was studied by Velankar (1955).

Incidence of Coliforms and enterococci in natural waters was studied by Sastri et al. (1969) in Bhopal city. Madhur and Ramanathan (1969) and Sen and Ghosh (1970) have indicated many advantageous of using enterococei index in place of Coliform index, while doing analysis of drinking water supply in Calcutta. Studies on indicator organism of faecal pollution in the Cochin back-water include the work of Santhakumari (1966) Gore (1971). Dwivedi et al. (1974) and Chandrika (1986).

Recently it has been emphasized the potential hazards to public health associated with the faecal pollution of coastal areas and another specialised conditions like fisheries harbour by the direct and indirect discharge of raw or partially treated sewage. Sewage contributes considerably to the quantity of faecal microorganisms such as Salmonella, Shigella, Coliforms and Faecal coliforms in water and sediment which are carried with the coastal waters and may give rise to epidemics. It has been shown that sewage polluted water is often a common source of disease in man and animals either directly or indirectly (Crown 1972; Geldreich 1972; Gangarosa et al. 1972;). Apart from pathogenic germs sewage contains great quantities of organic and inorganic nutrients which enhance mass development of pathogenic and nonpathogenic microorganisms in controlled systems. Coliform numbers varies consistently in harbour water according to the degree of organic pollution of water (Kuch 1974; Kuch and Chan 1975). On the other hand the microflora is not frequently inhibited or detroyed by poisonous substances. Thus continued

addition of sewage effluents to sea water environment will result in eutrophication enriching the growth of pathogenic bacteria. Distribution of heterotrophic bacteria related to some environmental factors in Tolo Harbour was studied by Chan K. and C.S.W. Kuch in 1976.

Human pathogenic microflora cannot grow permanently in the marine environment and die off eventually in the sea within a week. But depending on the prevailing conditions various pathogens can survive for a period and remain virulent. Studies on the survival of faecal coliform in the marine environment deal with various factors controlling the death of these organisms (Greenberg 1956; Mitchell 1968). It has been well documented that occurrence of toxic materials as Mercury (Klarmann; 1950), enzyme and metabolic inhibitors (Webb; 1963) and selenate and other conservative pollutant (Ketchum et al. 1952; Orlob, 1956; Jones 1963; Nakamura et al. 1964;) high salt concentration and pH of Sea Water (Carlucci and Pramer; 1960, 1961, Chan and Li 1977), limited nutrient supply (Greenberg, 1956), competition by native microflora (Waksman and Hotchkiss, 1937), the grazing action by protozoa and other Predators (Mitchell and Morris 1969; Engineer and Cooper, 1976) the existence of heavy metals (Johnes 1963) and lytic bacteria in sea water (Gulin et al., 1967 Mirtchell and Morris, 1969) are the major factors contributing to the rapid die off of Coliforms in marine environment. On the other hand, the addition of cysteine (Johanneson, 1957; Carlucci

and Prakner, 1960) and chelating agents (Jones 1963) to sea water increased the survival of Escherichia Coli.

High concentration of Coliform bacteria existed in sediments rather than in the overlying waters (Wesiss 1951, Ritternberg et al. 1958) and that cells of E. coli were capable of utilising the nutrients released from estuarine sediments (Gerba and McLeod, 1976). Furthermore, presence of estuarine sediments has been found to greatly enhance the survival of E. coli in natural sea water under laboratory conditions (Gerba and MacLeod, 1976) as the sea water and mud basically contain most of the nutrients required for the growth of microorganisms. So the pathogens thrive themselves for a certain period after the sea water and sediments have been contaminated by sewage and drainage. With the environmental factors such as favourable pH, salinity and optimum temperature, the microorganism readily develop and rapidly colonize in estuarine and in any aquatic environment. These observations indicate the ability of aquatic environment to support to a limited extent the survival and even growth of Coliform bacteria.

It is well known that the pathogenic microbes produce diseases such as cholera, dysentry, diarrhoea, typhoid, Jaundice, Leptospirosis, Hepatitis B etc. Hence sewage loaded environment may be dangerous source of infection. Salmonella infection however be caused by eating oysters, shell fishes and fishes

harvested from sewage loaded waters. Less frequent are Shigella. Which occur epidemically. Members of the genera Streptococci. Staphylococci, Lactobacilli, Proteus, Psuedomonas and spore forming bacteria also contain fish pathogens. But the number and types will vary with geographic area and state of community health. The nature of sewage treatment and physiological state of microorganism.

Whenever pathogens are present, they are scarce in number when compared to commensal bacteria (as 50% of the faeces of human beings is considered to be the weight of bacteria itself) and this complicates the problem of their detection. So the procedure adopted for testing potability rely on the detection of indicator bacteria which are bacterial parameters of faecal pollution.

The fishery harbour environment with all its fish landings remain as a potential environment for monitoring the bacteriological quality of the fishing landing centre.

Geldreich and Clarke (1966) reported that the occurrence of indicator bacteria signifies the presence of other enteric pathogens in the same environment. In many instances indicators of bacterial pollution signified human pathogenic viruses of faecal origin in Coastal waters (Gerba ands Goyal 1978). Above information initiated a thorough study and a detailed investigation on indicators of bacterial pollution. At

present there is a general lack of information regarding the pathways of bacterial pollutants from sewage and land drainage and their fate in the aquatic environment of Cochin. Hence, the present study was aimed to monitor indicators of bacterial pollutants in a fish landing centre of Cochin and also to investigate the effect of bacterial pollutants on fisheries resources and human health as an essential step towards evolving a scientific basis for protection and management measures.

2. SCOPE OF STUDY

The results of the investigations are presented and discussed under three methods. Under first method quantitative and qualitative study of faecal coliform (E. coli type I) was carried out by isolating them by pour plate method using MacConkey medium from the three samples, collected from the fish landing centres. Identification of the preceeding group with confirmatory test (IMvic test) wherever possible was made with numerous physiological and biochemical tests. Selected cultures were identified (57 Nos) based on their biochemical potential towards different substrates based on the scheme of Edwards and Ewing (1972). Quantitative distributions of faecal streptococci was asscessed using selective medium KF-agar. Faecal index was constructed by constructing the ratio between Escheichia coli (type I) and faecal streptococci.

In Second method most probable number method (MPN - test) was used to detect E. coli. All the samples were inoculated into MacConkey broth which are then incubated at 37^{O} for 1-2 days and examined for acid and gas production. From this positive results, coliforms were further identified by Differential Coliform test using elevated temperature method.

In third method Manja's medium was Prepared and medium with strips are placed in Mccartney bottles and 20ml water sample

was poured into the bottles and results was noted after 10 hours of incubation.

All these methods were used to develop and detect coliform bacteria for ascertaining the occurence of faecal contamination in water, ice and fish. The methods of sanitary analysis by bacteriologist somewhat differ from country to country.

3. HISTORICAL RESUME

Treatises on water bacteriology are concerned with the study of domestic water, swimming pools and sewage. Sea water and fish landing centres and processing industries are rarely considered for various reasons. The fish landing centre presents certain problems of interest to sanitary bacteriologist and students of public health. Out breaks of oyster borne typhoid, gastroenteritis and R factor transferrence and other diseases focused attention on this problem on recent years. Geldreich (1966) published the review of research conducted on Coliform at the cincinnati water research laboratory, cincinnati ohio. Geldreich and Kenner (1969) believed the occurrence of faecal streptococci to indicate the recent faecal pollution. Coliforms are found less resistant to disinfectant than other microbes. The dispersion and disappearance rate of Coliform from a marine outfall in Israel was estimated Gilfath et al. (1970).

Pollution of coastal waters by enteric pathogens has been also studied in estuarine and marine environments by Yosphe-Purer and Shuval et al. (1972); Paeoletti (1964) Brezenski and Russonanno (1969), Brezenski (1971) and Shuval (1972). Gerbra and Schaiberger (1973) found a direct correlation between the amount of rainfall and Coliforms on an ocean bathing beach.

Hashimoto et al. (1974) found the distribution of motile streptococci in faeces of man and animal in river and sea

water in Japan. In 1975, Geldreich published microbiological concepts for coastal bathing waters.

Significant numbers of Coliform bacteria have been noted (Gameson 1975) in sea water adjacent to a colony of nesting gulls. While it is accepted that disposal of some conservative bio-degradable substances to estuaries and the sea should be prevented or restricted. Difference of opinion exists regarding the need for limiting of discharge of degradable wastes (Calvert 1975 and Baalsrud, 1975) and for the regulation of water quality by the imposition of standards (Moore, 1975 and Shuval 1975). Control has been excercised to dispose sewage into the sea in accordance with certain qualitative criteria (Agg, 1975).

Mosley (1975) doubts the potential for infectivity of enteric organisms in the sea, but even the dilution of the infective agents will not prevent the cause of epidemological problems that many subclinical infections result because of small infective doses. Faecal pollution of beaches by 'Indicator bacteria' like Coliforms, E. coli and streptococcus faecalis has been identified and studied by Regnier and Park (1972) Turker (1976) Bonde (1968); Stevenson (1953); Moore (1959); Berger et al. (1963); Shuval et al. (1968); Grunnet et al. (1970) Cohen and Purer (1968) and Tinker (1976) Enteric pathogens like Salmonella have been reported from seafoods of India (Arul James & Iyer, 1972; Joseph Mathen & Iyer; 1976; Nerkar et al. 1975).

Water, sediment and sand from Southern Biscayne Bay were examined over three months period by Buck (1976) for the indicator and potentially pathogenic bacteria and yeasts. High concentrations of Coliform bacteria existed in sediments rather than in the overlying water (Weiss 1951; Rittenberg et al. 1958) and that cells of E. coli were capable of utilising the nutrients released from estuarine sediments (Gerba and Mc Leod 1976). Further more presence of estuarine sediments has been found to enhance greatly the survival of E. coli in natural sea water under laboratory conditions (Gerba and mac Leod 1976). Vasconcelos and Swertz (1976) studied the survival of bacteria in sea water using a diffusion chamber apparatus. An electro chemicalmethod for early detection and monitoring of Coliform in water was analytically designed by Wilkin and Boykin (1976).

Studies on enumeration of bacteria in marine water by Stanfield and Irwing (1977) demonstrated that methods of enumeration are not always equivalent and that a realistic assessment of the value of a method can be made only when the organisms to be recovered have been exposed to an environment capable of stressing or attenuating the bacteria. Robinson and Stanfield (1977) have demonstrated that the variation in total Coliform count can be obtained in marine waters by using different selective media and incubation procedures.

Distribution of viral and bacterial pathogens in a coastal canal community was studied by Gerba et al. 1977) who

detected high concentrations of microbes in sediments of polluted coastal areas. Pathogenic and nonpathogenic bacteria are present in large numbers in bottom sediments and may be released upon resuspension following dredging, boating, storms and other activities (Goyal et al. 1977; Greines; 1975).

Tobin and Dutka (1977) found significant differences between various brands of membrane filters in their ability to recover bacteria, from pure cultures, natural waters or sewage. Apparently they also found that small changes in the incubation tmeperature cannot affect the Coliform count obtained. A high correlation of the Salmonella and enterococci indices was found by Vlodavetes and Kalina (1977), when a study was conducted to isolate Salmonella in the coastal sea water.

Shuval (1978) found that all these pathogens survive long enough and in high enough concentration to lead to the transmission of the disease to man by contaminated shell fish and by bathing in water highly contaminated by fresh sewage. The infleunce of pH, Salinity and organic matter on the adsorption of E. coli and other enteric pathogens in estuarine sediment was studied by Labella and Gerba (1979).

Thapliyal et al. (1972) studied standard plate counts, Coliform and enterococcus densities in various natural waters in the Tarai region (India) and identified ten IMVic types in their source of water supply. They considered enterococci indicator

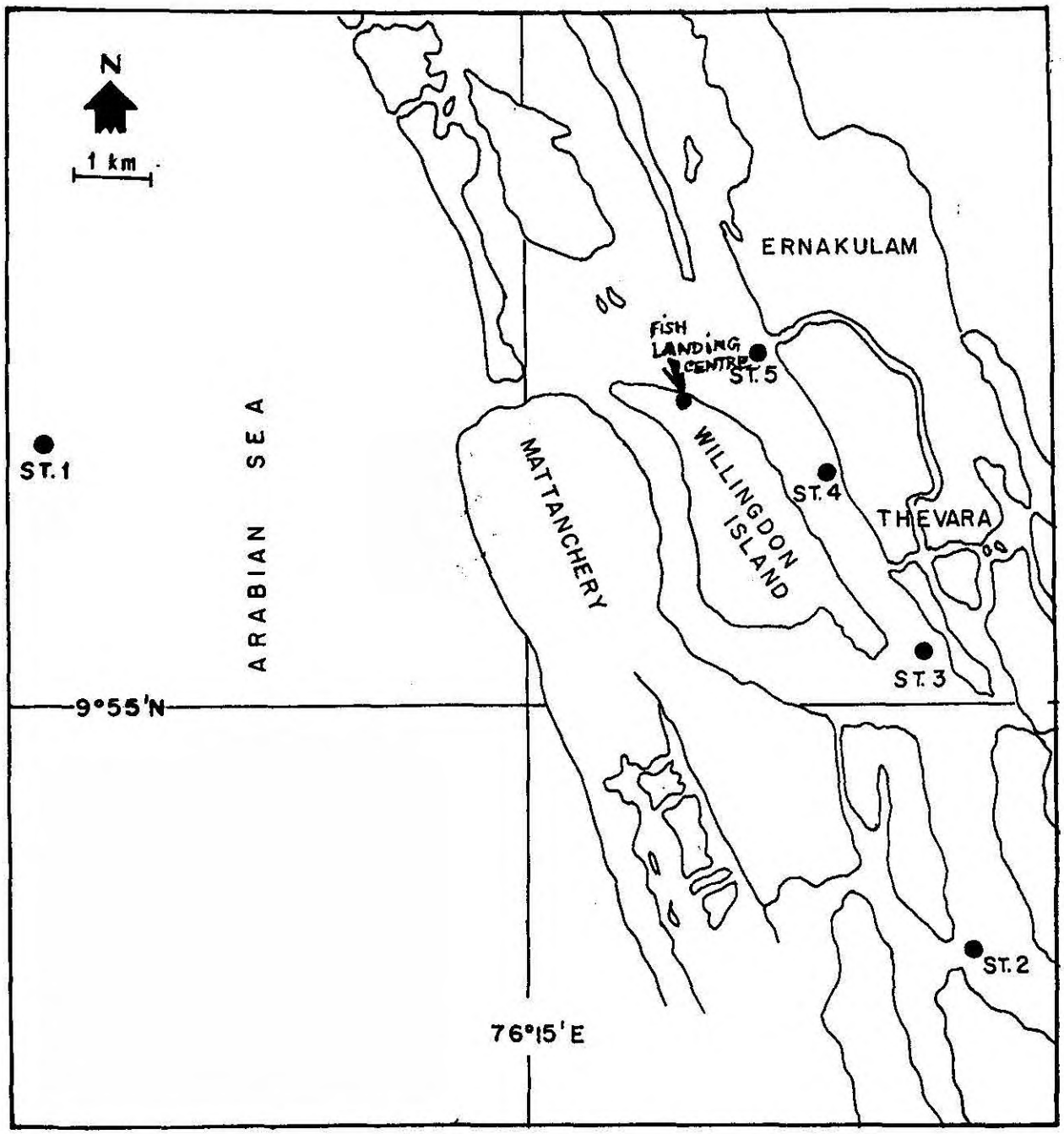
system of greater value than the Coliform count indicating water pollution. Phirke and Verma (1972) worked in river water samples in Delhi and found a linear relationship among the two indicator systems.

Feasibility of using ozone to disinfect sea water use in controlled environment in shrimp culture were done the environmental shrimp culture station at puerto penasco, Mexico (Donald and Lightener 1979). 99.9% of the pathogens were killed within 95 minutes of minimum exposure period with ozone.

In summary, it can be stated that the knowledge of bacterial pollution in marine environment has accumulated slowly from a very late beginning when compared with progress in other areas of the parent discipline. Periodic reports of marine bacteria resulting from for-tuitous discovery interspersed with "Short term" concentration on very special group, mark the general trend of historical development of faecal Coliform ecophysiology.

4. MATERIAL AND METHODS

FIG 1 - FISH LANDING CENTRE - THE SAMPLING STATION


N
1 km
ERNAKULAM
FISH LANDING CENTRE
ST. 5
ST. 1
ARABIAN SEA
MATTANCHERY
WILLINGDON ISLAND
ST. 4
THEVARA
ST. 3
9°55'N
ST. 2
76°15'E

Study Area

Samples such as fish, ice and water were collected weekly from fish Landing Centre, Fisheries Harbour, Cochin (located along $9^{o}5S'$-$10^{o}00'N$ and $76^{o}10'$- $76^{o}20'$) near Mattancherry (Fig. 1). All the samples were randomly collected immediately after fish-landing in between 15.15-15.45 hrs.during the study period of 10th March 1997 - 22nd April, 1997.

Collection of the Sample

Before sampling, conical flask with Swab, forceps were sterilized and made aseptic to prevent contamination. Samples such as fish and ice were collected in sterile plastic bags, while water in sterile conical flask using swab. Water and ice were also collected aseptically in McCartney bottles containing Manja's medium.

Samples were subjected to bacterial analysis within two hours after collection. If there is any delay in the examination of the sample it was stored at a temperature between $0^{o}c$ and $10^{o}c$.

Preparation of the Sample

Fish is taken from polythene bag and placed in a mortar.99ml of sterile distilled water was added and mixed gently

SITE 1 - FISH LANDING CENTRE

SITE 2 - FISH SAMPLE

SITE 3 - ICE SAMPLE

SITE 4 - WATER SAMPLE


3


4

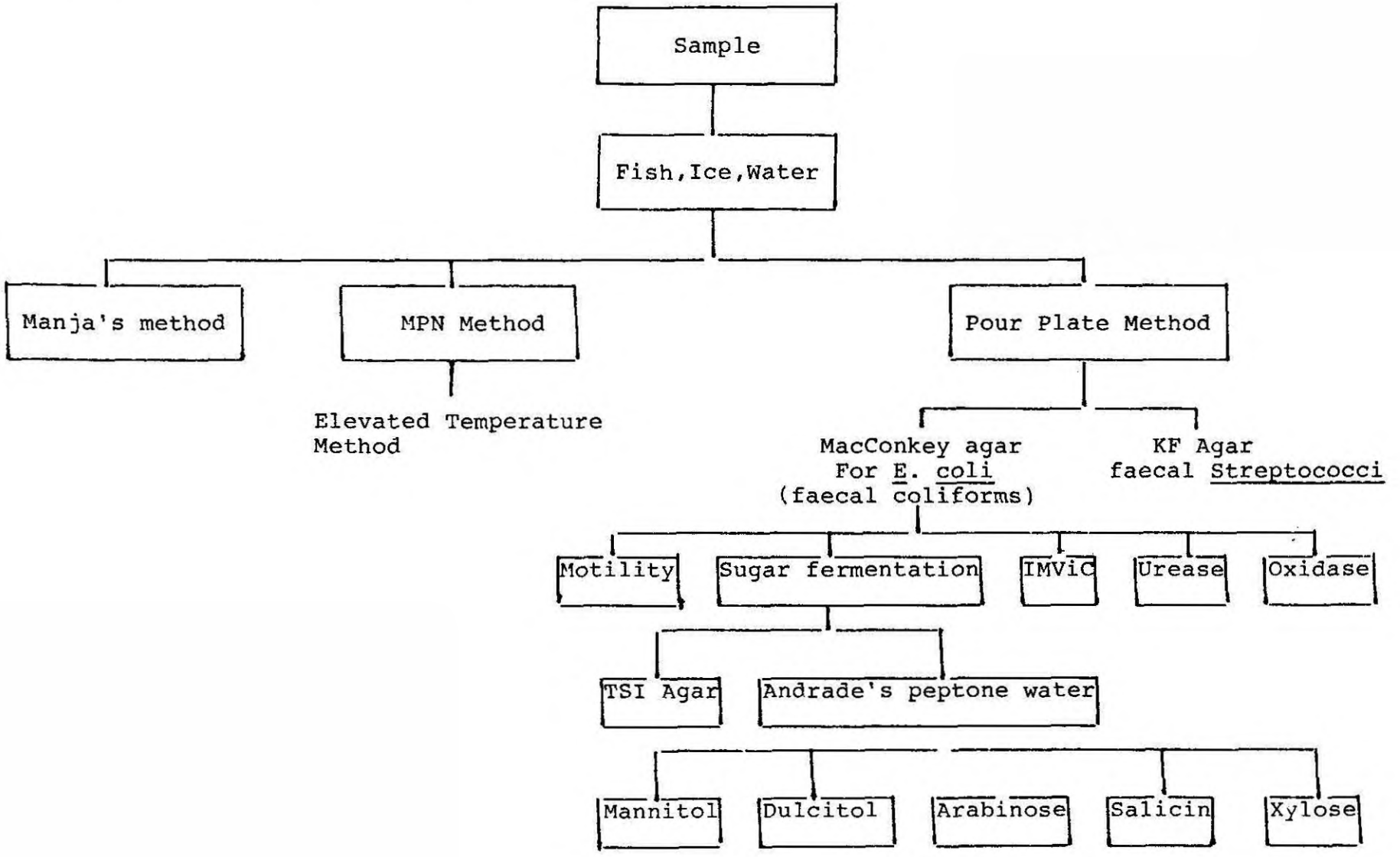
PLATE 5 & 6 - MACCONKEY PLATE
LF AND NLF COLONIES


5


6

Table No. 1

Pattern of analysis of samples from fish-landing centre



using the pestle. From this suspension 20ml water was inoculated into McCartney bottle containing Manja's medium. All the three bottles (Fish, Water, Ice)were placed in an incubater for an overnight incubation at $37^{o}c$.

99ml of Distilled water is added to the swab and mixed thouroughly. Weight of the ice was taken and it is mixed with 99ml sterile distilled water.

The pattern of analysis of fish, ice and water sample from fish landing centre was shown in Table (1)

Quantitative analysis of bacteria

0.25 and 0.5ml of each sample were inoculated into MacConkey agar and KF agar by pour plate method. Incubate at $37^{o}C$ for 24 hours. Colony count were taken from the MacConkey agar isolated colonies were taken and various biochemical tests are done.

KF Agar

Protease Peptone	-	1%
Yeast extract	-	1%
Sodium Chloride	-	0.5%
Sodium glycerophosphate	-	1%
Maltose	-	2%
Lactose	-	0.1%
Sodium Azide	-	0.04%

Bromo-cresol purple	-	15ml/litre
Agar	-	1.5%
Water	-	100ml
pH	-	7.4

The Faecal Coliform - Faecal Streptococci ratio was constructed by using this formula.

$$\frac{\text{Faecal Coliform}}{\text{Faecal Strep}} = \text{Ratio}$$

Interpretation

$\frac{FC}{FS} \geq 4.0$

When the ratio is greater than or equal to 4, it may be taken as strong evidence that pollution derives from human wastes

$\frac{FC}{FS} \leq 0.7$

When the ratio is less than or equal to 0.7, it may be taken as strong evidence that pollution derives predominantly or entirely from livestock or poultry wastes.

$2 < \frac{FC}{FS} < 1.0$

When the ratio is between 0.7 and 1.0, it may suggest a predominance of livestock and poultry wastes in mixed pollution

$1 \quad \frac{FC}{FS} \leq 2.0$

When the ratio falls on values from 1 to 2, it represents a "grey area" of uncertain interpretation. Samples should be taken nearer the source of pollution.

IMViC reactions

IMVic hese tests are used to identify Enterobacteriaceae.

Indole test

The test determines the ability of an organism to produce indole from aminoacid tryptophan. This is tested in a peptone water culture after 48 or 96 hours incubation at 37°c. Added 0.5ml Kovac's reagent and shaked gently. A red colour indicates a positive reaction. Kovac's reagent consist of

Para dimethylaminobenzaldehyde	- 10g
Amyl or isoamyl alcohol	- 150ml
Conc. Hcl	- 50ml

Methyl red test

The test is employed to detect the production of acid during the fermentation of glucose and maintenance of a pH below 4.5 in an old culture. Five drops of 0.04% solution of methyl red are added to the culture in glucose phosphate medium incubated at 30°c for five days, mixed well and read at once.

Voges-Proskauer test (VP test)

This test involves the detection of diacetyl ($CH_3CO.COCH_3$) or its precursors acetoin and 2,3 butonediol formed. In the presence of alkali and atmospheric oxygen, the

small amount of acetyl methyl carbinol present in the medium is oxidised to diacetyl which reacts with the peptone of the broth to give a red colour.

Requirements

Glucose phosphate Peptone broth
5% alpha naphthol in ethanol
40% KOH

Method

The test is performed by adding 0.6ml of alpha napthol and 0.2ml of KOH are added to one ml of glucose phosphate culture of the organism incubated at 30^{o}c for 5 days or 37^{o}c for 48 hours. In a positive reaction, a pink colour appears in 2-5 minutes, deepening to magenta or crimson in half an hour.

Citrate Utilisation test

This test determine the ability of an organism to use citrate as the sole source of carbon.

Requirements

Simmon's citrate medium
Culture to be tested.

Method

One loop full of organism is inoculated into this medium and placed for an overnight incubation. Presence of blue colour indicated the positive result.

Urease Test (Christen-Sen' medium)

Urease producing bacteria reduce urea to ammonia.

Method

Inoculate the slope heavily and incubated at 37^{o}c and placed for an overnight incubation. The medium change in pink colour show the positive result.

I. **Fermentation tests**

Tested in sugar media. Acid production is showed by change in the colour of the medium and the gas produced collects in Durham's tube. Sugars like Mannitol, xylose, Dulcitol, Salicin and arabinose are tested. After the overnight incubation the medium which change in pink colour and gas produced at Durham's tube indicates the positive result.

TSI agar medium

To differentiate Enterobacteriaceae according to their ability to ferment lactose, sucrose and dextrose and to produce H_2S.

Composition

Peptone	-	10g
Tryptone	-	10g
Yeast extrant	-	3g
Beef extract	-	3g
Lactose	-	10g
Sucrose	-	10g
Dextrose	-	1g
Ferrous Sulphate	-	0.2g
Sodium thio sulphate	-	0.3g
Nacl	-	5g
Phenol red	-	0.02 ugm
Agar	-	12
pH	-	$25^{o}c$ (7.4 $\pm$ 02)

The culture may be inoculated over the entire surface of the slant using a young culture as the source of inoculum. Stab inoculations were made.

Yellow colour is seen when medium is acidic due to sugar fermentation and pink colour is seen due to alkaline

nature of the medium when oxidative decarboxylation of proteins take place. H_2S produced is visualised as a black precipitate resulting from the reaction between H_2S produced and ferrous sulphate present in the medium. Gas produced (usually H_2Co_2) is visualised as bubbles in medium or cracking of medium or lifting of the butt.

II. Presumptive Method

a. Multiple Tube Method or Most Probable Number Method)

A method for estimating the concentration of viable organism (usually bacteria) in a suspension. The most probable number (MPN) calculations are used commonly to enumerate, particularly physiological types of microorganisms such as lactose fermenters in Coliform counts. The MPN method gives higher count than the spread method, some times as much as 10 times higher.

Purpose: To familarize with a method applicable to the study of the bacterial content of water with special emphazis upon the identification of the colon-aerogenes group.

Media: MacConkey broth double strength and single strength

Composition

Sodium taurocholate	-	5g
Peptone	-	20g
Sodium chloride	-	5g
Lactose	-	10g
Bromo Cresol Purple (1% solution in ethanol)	-	1ml
Distilled water	-	100ml

MacConkey broth in fermentation tubes with small inverted Durham's tube inside.

Inoculate the water sample as follows

Sample	No. of tubes	Types of medium
10ml	Five(10ml)	Double strength MacConkey
1ml	Five(5ml)	Single strength MacConkey
0.1ml	Five(5ml)	Single strength MacConkey

Inoculated the tubes at 37^{o}c for 18-24 hours and gas production was noted and if negative incubated further 24 hours. Following incubation each tube was examined for presence or absence of growth.

b. **Differential Coli-aerogenes test**

To determine whether the Coliform organisms detected in the presumptive test are E. coli, then further differential test must be applied.

Eijkman test: This depends on the ability of E. coli (type I), to produce gas when grown in MacConkey medium at 44°c.

Requirements

Brilliant Green Bile Broth

Peptone	-	10g
Beef Extract	-	20g
Lactose	-	10g
Brilliant green(0.1%)	-	13ml
Distilled water	-	13ml

Brilliant gree bile broth was prepared with inverted small Durham's tube inside in fermentation tubes.

All the tubes which show positive acid and gas production in the presumptive tests are inoculated into this medium. The tubes are then incubated for 24 hour at 44°c± 0.5°c in a water bath.

c. **By using Manja's method**: This is a very simple method and can use an alternative method for MPN method. The presence of coliform in drinking water is consistently associated with the organism that produce, Hydrogen sulphide. This simple method assess faecal contamination in surface water based on the detection of hydrogen

FIG 2 - CHECKING OF FAECAL POLLUTION - MANJA'S METHOD

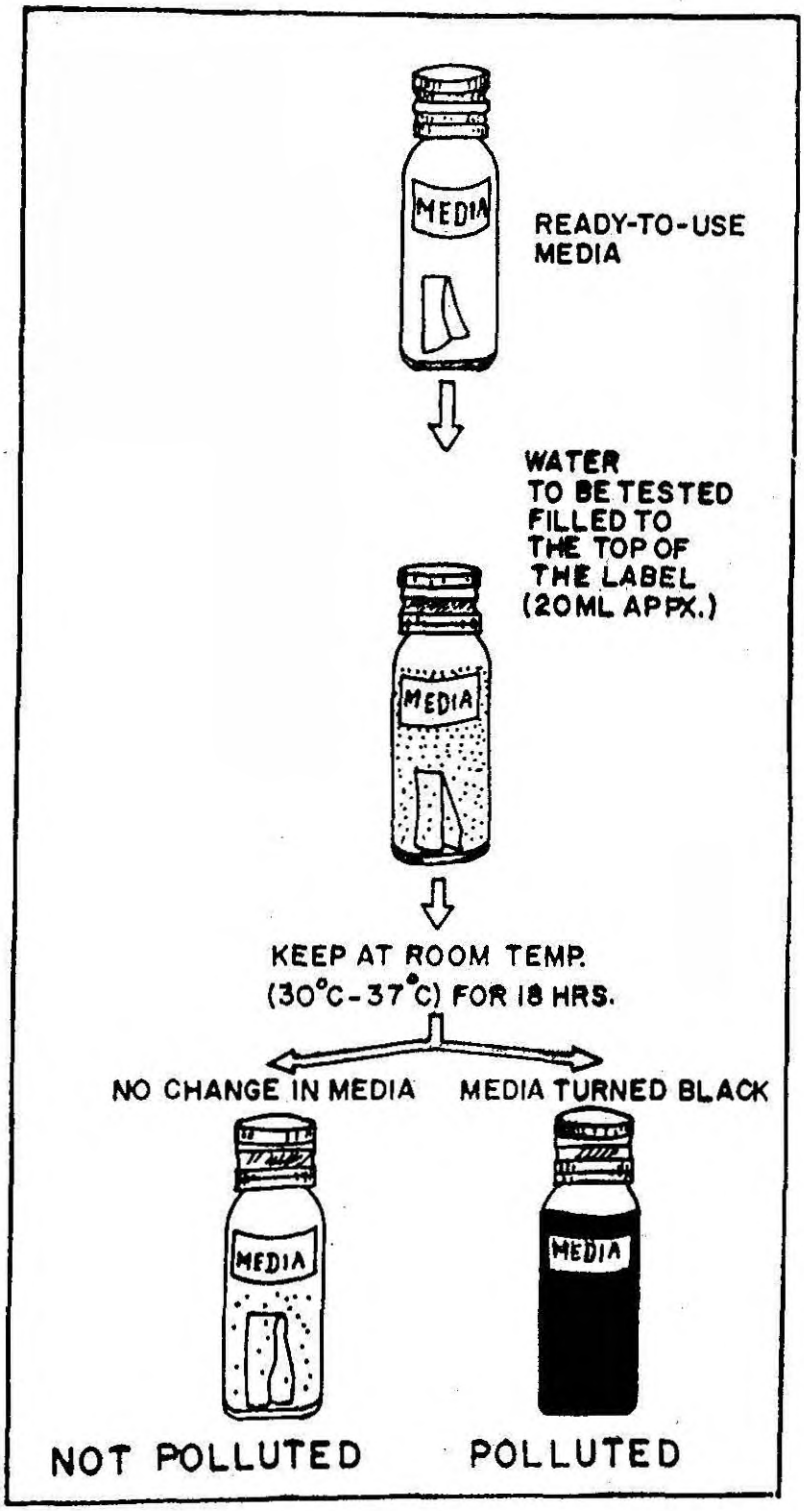


Fig.3 CHECKING OF FAECAL POLLUTION
MANJA, 1986

FIG 7 & 8 - MANJA'S MEDIUM WITH WATER SAMPLE


7

sulphide producing organisms.

Materials: Manja's medium; Filter paper; Mc-cartney bottles

Composition of Manja's medium

Peptone	-	20g
K_2 HPo_4	-	1.5 g
Ferric ammonium citrate	-	0.75 g
Sodium thiosulphate	-	1 g
Teepol	-	1 ml
Distilled water	-	50 ml.

Aliquots of 1 ml of the concentrated medium were absorbed onto folded tissue paper (80 cm^2), which was placed in a McCartney bottle, sterilized and dried at 50^{o}C under sterile conditions. The water samples to be screened for facecal pollutions were placed in the bottles upto a precalibrated mark (20 ml) and allowed to stand at ambient temperature (30-37^{o}C). Faecal pollution is indicated if the contents of the bottle turned black within 12-18 hour, in this case, the water was graded as faecally polluted.

5. RESULTS

Table No. 2

Identification of bacterial strains from fish samples

Name of Bio-chemical tests / Stain No.	Glucose	Lactose	Sucrose	Mannitol	Dulcitol	Arabinose	Salicin	Xylose	Urease	Indole	MR	VP	Citrate	Oxidase	Motility	H_2S	Identified Micro organisms
I F1	+	-	-	+	+	+	-	+	+	-	+	-	+	-	+	+	Citrobacter freundi +-
F2	+	1	1	-	-	-	+	+	+	-	+	-	+	-	+	+	Proteus mirabilis
F3	+	1	1	-	-	-	1	1	-	+	+	-	-	-	-	-	Providencia
F4	+	+	+	+	+	+	-	+	-	+	+	-	-	-	+	-	E.Coli.
F5	-	+	+	+	+	-	-	+	-	-	+	-	-	-	-	-	*Shigella sonnei*
F6	+	-	-	+	-	-	+	+	+	-	+	-	+	-	+	+	Proteus mirabilis
F7	+	+	+	1	1	-	1	1	-	-	-	+	+	-	+	-	Enterobacter
II F1	+	+	+	+	-	+	-	-	-	+	+	-	-	-	+	-	E.Coli.
F2	+	1	1	-	-	1	1	-	-	-	+	+	-	-	-	-	Shigella sonniei
F3	+	+	+	+	+	+	+	+	-	+	+	-	-	-	+	-	E. Coli.
III F1	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-	Klebsiella
F2	+	-	-	-	-	-	+	+	-	+	+	-	-	-	+	-	Edwardsiella
IV F1	+	+	+	+	+	+	+	+	-	+	+	-	-	-	+	-	E.Coli.
F2	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-	Klebsiella
F3	+	-	-	+	+	+	+	+	+	+	+	-	+	-	+	-	Proteus rettgerii
F4	+	1	1	-	-	+	+	+	-	-	+	-	+	-	+	-	Citrobacter freundi
F5	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+	Zymomonas
V F1	+	+	+	-	-	+	-	-	+	-	-	+	+	-	-	-	Klebsiella
F2	-	-	+	-	-	+	-	-	-	-	-	-	+	-	+	-	Enterobacter hafniae
F3	+	-	-	-	-	+	-	-	-	-	-	+	+	-	+	-	Enterobacter cloacae
VI F1	+	+	+	1	-	+	+	+	-	+	+	-	-	-	+	-	E.Coli.
F2	+	+	+	-	-	1	1	1	+	-	-	+	+	-	-	-	*Enterobacter*
F3	+	+	+	+	-	+	+	+	-	-	+	-	-	-	-	-	Shigella
F4	-	-	-	-	-	-	+	1	-	+	+	-	-	-	-	+	Edwardsiella

F = Fish sample
\+ = Acid and gas
\- = Negative no acid and gas
1 = Acid only

Table No. 3

Identification of bacterial strains from Ice samples

Name of tests / Stain No.	Glucose	Lactose	Sucrose	Mannitol	Dulcitol	Arabinose	Salicin	Xylose	Urease	Indole	MR	VP	Citrate	Oxidase	Motility	H2S	Identified Micro organisms
I																	
Ice 1	+	+	+	+	-	+	-	+	+	-	-	+	+	-	-	-	Klebsiella
Ice 2	+	+	+	-	-	+	+	+	-	-	-	+	+	-	+	-	Enterobacter
II																	
Ice 1	+	+	+	+	+	+	+	+	-	-	+	-	-	-	+	-	E.Coli.
Ice 2	+	-	-	-	-	+	-	+	-	+	+	-	-	-	+	+	Edwardsiella tarda
Ice 3	+	+	+	+	-	+	-	+	-	-	-	+	+	-	+	-	Enterobacter acrogenes
III																	
Ice 1	+	+	+	+	-	+	+	+	-	-	-	+	+	-	+	-	Enterobacter hafniae
IV																	
Ice 1	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-	Klebsiella
Ice 2	+	-	-	+	-	+	+	+	+	-	+	-	+	-	+	+	Citrobacter freundi
Ice 3	+	+	+	+	-	-	-	+	-	-	+	+	-	-	+	-	Enterobacter
V																	
Ice 1	+	+	+	+	+	-	-	+	-	-	-	+	+	-	+	-	Enterobacter aerogenes
Ice 2	+	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	Shigella sonnei
Ice 3	+	-	-	+	-	+	-	-	-	-	-	-	+	-	+	-	Enterobacter hafniae
Ice 4	+	-	-	-	-	-	-	-	-	-	+	-	+	+	+	-	Zymomonas

MICROBIAL FLORA ENCOUNTERED IN MacCONKEY AGAR

Selected colonies were isolated based on morphological variations from each plate and maintained on nutrient agar slants for doing various physiological and biochemical tests. By morphological variations and by various biochemical tests isolated cultures were identified based on the scheme of Edward's and Ewing (1972).

Microflora of Fish

Totally 24 colonies were isolated from the fish sample (Table 2) out of which E. coli predominated and formed 20.83% of the total isolates. Enterobacter species was encountered in the intensity of 16.66%. Klebsiella, Shigella and Proteus occurred almost in the same intensity forming 12.5%. 8.33% were Citrobacter and Edwardsiella species. Providencia and Zymomonas were also encountered in one of the fish sample which showed their stray occurrence. Generally quantitative occurrence of enterobacteriaceae in selective media was uniform in all the observations as the sampling frequency was too close to cause variations.

Microflora (Enterobacteriaceae) from Ice

13 colonies were randomly isolated from ice sample for identification (Table 3). In ice sample E. coli was absent

Table No. 4

Identification of bacterial strains from water SWAB

Name of tests / Strain No:		Gluc ose	Lact ose	Sucr ose	Man nitol	Dul citol	Ara bino se	Sal icin	Xyl ose	Ure ase	Ind ole	MR	VP	Cit rate	Oxi dase	Mot ility	H2s	Identified Micro organisms
I	WS1	+	+	+	+	-	+	-	+	+	-	+	-	+	-	+	-	Citro bacter
	WS2	+	+	+	+	-	-	+	+	+	+	+	-	+	-	+	-	Proteus rettgeri
II	WS1	+	+	+	+	-	+	-	-	-	-	-	+	-	-	+	-	Enterobacter hafniae
	WS2	+	+	+	-	-	+	-	-	-	-	-	-	-	-	+	-	Enterobacter hafniae
	WS3	+	+	+	+	-	+	-	-	-	+	+	-	-	-	+	-	E.Coli
III	WS1	+	+	+	+	-	-	-	+	-	-	+	+	+	-	+	-	Serratia
	WS2	+	+	+	+	-	+	-	-	+	+	-	+	+	-	-	-	Klebsiella
	WS3	+	-	-	-	-	-	-	-	-	+	+	-	+	-	+	-	Providencia
	WS4	+	+	+	-	-	-	+	+	+	-	-	+	+	-	-	-	Klebsiella
	WS5	-	-	-	-	+	-	+	+	-	-	+	-	-	+	+	-	Zymomonas
	WS6	+	-	-	+	-	-	-	+	-	+	+	-	+	-	+	-	Proteus rittgeri
IV	WS1	+	+	+	+	-	-	-	-	+	-	-	+	+	-	-	-	Klebsiella
	WS2	+	+	+	-	-	+	+	+	-	-	+	+	+	-	+	-	Enterobacter hafniae
V	WS1	+	+	+	+	+	+	-	-	-	+	+	-	-	-	+	-	E. coli
	WS2	+	+	+	+	-	-	-	-	+	-	-	+	+	-	-	-	Klebsiella
VI	WS1	+	+	+	+	-	-	+	+	+	+	+	+	-	-	+	-	E.Coli
	WS2	+	+	+	-	-	-	-	+	-	+	+	-	-	-	+	-	Providencia
	WS3	+	+	+	+	-	+	-	+	-	+	+	-	-	-	+	-	E.Coli
	WS4	+	+	+	+	-	+	+	+	+	-	-	+	+	-	-	-	Klebsiella
	WS5	+	+	+	-	-	-	+	+	-	-	+	+	+	-	+	-	Enterobacter hafriae

except in one sample. Edwardsiella, Shigella and Citrobacter are also found in one observation. Ice sample was predominated by Enterobacter species 45.15% and the remaining isolates was Klebsiella the intensity of being 15.38%.

Microflora of Water

Totally twenty colonies (Table 4) were isolated for qualitative determination. Of these Klebsiella predominated forming 25% of the total. E. coli and Enterobacter species also formed 20% of the total isolated bacteria Shigella and Zymomonas were rarely isolated from the water samples. Providencia, Proteus, Citrobacter were identified and formed 10% of the total.

Differential Characterisation of bacteria isolated from fish

Table 2 illustrates the biochemical tests performed in all the six observations in the strains of Enterobacteriaceae isolated from fish sample. In the first observation (March 1997) Citrobacter freundi, Proteus mirabilis, Providencia, E. coli and Shigella sonnei were identified. Second observation on the same month E. coli and Shigella sonnei were isolated and in the third observation Klebsiella and Edwardsiella were the organism encountered. In the fourth investigation E. coli, Klebsiella, Proteus rettgeri, Citrobacter freundi and Zymomonas have been observed Klebsiella, Enterobacter hafniae and Enterobacter cloacae were isolated from fifth observation E. coli

Table No. 5

Morphological and biochemical characteristics of bacteria isolated from fish samples collected at Cochin Fish landing centre from March 1997 - April 1997.

Characteristics	Frequency of occurence (%)
Gram stain	100
Motility	75
I. Sugar fermentation	
Glucose (Monosaccharide)	83.33
Lactose (Disaccharide)	75
Sucrose (Disaccharide)	75
Mannitol (Sugar alcohol)	54.16
Dulcitol (Sugar alcohol)	37.5
Arabinose (Mono saccharide)	66.66
Salicin (Glycosides)	66.66
Xylose (Mono saccharides)	75
Indole	37.5
MR	66.66
VP	29.16
Citrate	58.33
Urease	33.33
Oxidase	4.16
H_2S	16.66

Enterobacter, Shigella, and Edwardsiella have been isolated during April 97.

In sewage and receiving waters, all coliform organisms cannot be considered indicators, as some may multiply in nature and most of than do not have a fecal origin, but may belong to nature. For that reason only Escherichia coli (type I) is considered a proper indicator and only that group can be estimated quantitatively with an adequate precision (elevated temp incubation + indole test)

In the present study E. coli (type I) was isolated in all the 4 observations in water and 5 observation in fish samples and in ice samples E. coli (type I) was found in one observation.

Enzymatic reactions

All bacteria isolated from fish samples are gram negative rods, 75% were motile. The international comittee recommended biochemical tests for group differentiation of Enterobacteriaceae (Report 1958). Much use has been made for identification. Metabolism of carbohydrate an anaerobic phenomenon or fermentation usually result in the production of acids. There is a wide difference in the products of breakdown during fermentation. These can be tested for their nature used for their diagnostic purposes.

Table No. 6

Differentiation of Enterobacteriaceae based on IMvic - Tests

Sample	Identified Microorganisms	Typical Reactions			
		Indole	Methyl Red	Voges Proskeur	Citrate
Fish 1.	Citrobacter freundi	-	+	–	+
	Proteus mirabilis	-	+	–	+
	Providencia	+	+	-	-
	E. Coli	+	+	-	-
	Shigella sonnei	-	+	-	-
	Enterobacter species	-	-	+	-
	Klebsiella	-	-	+	+
	Edwardsiella	+	+	-	-
	Proteus rettgeri	+	+	-	+
	Zymomonas		+		
	Enterobacter hafniae	-	-	-	+
	Enterobacter Cloaceae	-	-	+	+

Table 5 illustrates 83.23% of the isolates were capable of fermenting glucose (Monosaccharide). 75% of the isolates were capable of fermenting lactose and sucrose (Disaccharide). Sugar alcohol like Mannitol and dulcitol was fermented by 54.16% and 37.5% respectively. Monosaccharide like Arabinose and xylose was fermented by 66.66% of the organism and 75% of the organism respectively. Glycoside salicin was tested for their fermentation capability and 66.66% was capable of fermenting salicin.

IMVic test

Indole was produced from the amino acid tryptophan by 37.5% isolates. (Table 5) 66.66% of the isolates fermented glucose giving positive reaction in methyl red test - Acetyl methyl carbinol was produced by 29.16% . 58.33% of the isolates were capable of utilising citrate as the sole source of carbon.

Typical results showed by various isolates were given in the table 6. of these four tests the MR and VP are most significant since they indirectly revealed the mode of fermentative sugar break down. Imvic pattern of + + - +, - - - -, - - - + formed 4.16%, 12.55 formed - + - - and 16.6% formed - + - + in the fish sample. 33.33% formed + + - - showing the presence of E. coli and Edwardsiella. The percentage distribution of all Imvic obtained and the organism isolated were shown in Table 8 and 9.

BIO-CHEMICAL

Potential of flora from fish

33.3% were capable of producing ammonia from urea. Very poor oxidase was there in the isolated culture which show the abscence of cytochrome oxidase in the isolates.

H_2S can be produced by all bacteria in small amounts. But definite level of sensitivity of thiosulphate utilisation may be of value in intra group differentiation. In the present study 16.66% were capable of producing H_2S in TSI - reaction. H_2S is usually detected by the black insoluble ferrous salt in the slant.

Bacterial Profile from Ice

All the Enterobacteriaceae isolated from ice are given in Table 3.13 Selected colonies were isolated and subjected to various biochemical tests. Two colonies were isolated from first observation and they were Klebsiella, Enterobacter, E. coli, Edwardsiella tarda and Enterobacter aerogenes are encountered in second observation. Enterobacter hafniae was isolated from the third observation. From fourth observation Klebsiella, Citrobacter freundi and Enterobacter were isolated. The fifth observation harboured Enterobacter aerogenes, Shigella sonnei, Enterobacter hafniae and Zymomonas. Faecal coliform was abscent in sixth observation.

E. coli (type I) was found only in second observation

Table No: 7

Morphological and biochemical characteristics of bacteria isolated from Ice samples collected at Cochin Fish landing centre from March 1997 - April 1997.

Characteristics	Frequency of occurence (%)
Gram Stain	100
Motility	76.92
Fermentation reactions	
Glucose	100
Lactose	69.2
Sucrose	69.2
Mannitol	76.92
Dulcitol	23.07
Arabinose	6.92
Salicin	53.84
Xylose	76.92
Indole	15.38
MR	46.15
VP	46.15
Citrate	61.53
Urease	23.07
Oxidase	7.67
H_2S	15.38

Table No. 8

Bacteria associated with fish samples - Types of IMvic reaction (%) *Enterobacteriaceae*

Table A

From Fish Sample					
Percentage	4.16%	12.5%	16.6%	25	33.33
Pattern of IMvic	+ + - + - - - - - - - +	- + - -	- + - +	- - + +	+ + - -

Table B

From Ice Samples			
Percentage	7.96%	15.38	46.15
Pattern of IMvic	- + + - - + - - - - - +	+ + - - - + - +	- - + +

Table C

From Water Samples					
Percentage	5%	10%	15%	25%	30%
Pattern of IMvic	- + - + - - + - - - - - - + - -	+ + - +	- + + +	- - + +	+ + - -

Table No. 9

Differentiation of Enterobacteriaceae based on IMvic - tests in ice samples.

Sample	Identified Microorganisms	Typical Reactions			
		Indole	Methyl Red	Voges Proskeur	Citrate
Ice	Klebsiella freundi	-	-	+	+
	Enterobacter aerogenes	-	-	+	+
	E. Coli	+	+	-	-
	Edwardsiella tarda	+	+	-	-
	Enterobacter hafniae	-	-	+	+
	Enterobacter freundii	-	+	-	+
	Shigella Sonnei	-	+	-	+
	Zymomonas	-	+	-	+

Biochemical characteristics of Enterobacteriaceae isolated from ice

Table 7 shows that all isolates were gram-negative rods and 76.92% showed motility. All the isolates fermented glucose with acid or acid and gas. 69.2% were capable of fermenting lactose and sucrose. Acid alcohol like mannitol and Dulcitol were fermented by 76.92% and 23.07% of the microorganisms respectively. 53.84% were capable of utilising salicin. Arabinose and xylose were fermented by 69.2% and 76.92% of the isolates respectively.

Imvic - Tests

By using amino acid tryptophan 15.3% were capable of producing Indole. 46.15% isolates were MR and VP positive. 61.53% of the isolates were capable of utilising citrate as the sole source of carbon Table 8 shows that three combinations of Imvic pattern was showed by 7.96% of the organism. The pattern - + + -, - + - -, - - - +. the highest percentage of 46.15% included - - + + in the pattern of Imvic. So Enterobacter aerogenes was predominated in the ice sample. Citrate permease is abscent in E. coli and present in Enterobacter which makes this differentiation (Table 8 and 9).

Table No.10

Morphological and biochemical characteristics of bacteria isolated from water samples collected at Cochin Fish landing centre from March 1997 - April 1997.

Characteristics	Frequency of occurence (%)
Gram stain	100
Motility	75
Sugar fermentation	
Glucose	95
Lactose	85
Sucrose	85
Mannitol	65
Dulcitol	10
Arabinose	45
Salicin	35
Xylose	60
Indole	45
MR	65
VP	50
Citrate	65
Urease	40
Oxidase	5
H_2S	0

Biochemical potential of flora from Ice

23.07% of the isolates were ureolytic converting urea to ammonia. 7.96% were sensitive to cytochrome oxidase reaction 15.38% isolates were capable of producing H_2S.

Bacterial profile from water sample

Bacteria isolated from water sample are given in Table 4. Six observations were made in the present study. Citrobacter and Proteus rettgeri were isolated from the first observation during March 1997. From the second observation Enterobacter hafniae, E. coli were isolated. Serratia, Klebsiella, Providencia, Zymomonas and Proteus rettgeri were isolated from the third observation and Enterobacter hafniae from fourth sample in April 1997, E. coli, providencia, Klebsiella and Enterobacter hafniae have been isolated. E. coli were observed in three samples.

Enzymatic pattern of bacteria isolated from water sample

All are gram negative rods and 75% of the isolates were motile 95% of them were fermented glucose with the production of acid or acid and gas. 85% of the isolates were capable of fermenting lactose and sucrose. Mannitol and Dulcitol are fermented by 65% of the microorganism and 10% of the microorganism respectively. Glycosides like salicin were

Table No. 11

Differentiation of Enterobacteriaceae based on IMvic tests in water sample.

Sample	Identified Microorganisms	Typical Reactions			
		Indole	Methyl Red	Voges Proskeur	Citrate
Water	Citrobacter	-	+	_	+
	Proteus rettgeri	+	+	_	+
	Enterobacter hafniae	-	-	+	-
	E. Coli	+	+	-	-
	Serratia	-	+	+	+
	Klebsiella	-	-	+	+
	Providencia	+	+	-	+
	Zymomonas	-	+	-	-
	P1	+	+	-	+

fermented by 35% of the microorganism. 45% of the total and 60% of the microorganism were capable of fermenting monosaccharides like Arabinose and xylose respectively. (table no:10).

IMVic

45% of the isolates were Indole and MR positive. 50% of them are VP positive. By utilising citrate as the sole source of carbon 65% of the isolates were considered as citrate positive. Four types of IMVic pattern was found in 5% of the total isolates, the combination being - + - +, - - + -, - - - -, - + - +. Predominant Imvic combination was of E. coli (type I) forming 33% of the total isolates. (Table 8 and 11). Enterobacter species combination - - + + formed 2.5% of the total isolates from the water sample.

Biochemical potential of the isolates

Very few organism 5% of them were capable of catalysing the oxidation of reduced cytochrome by molecular oxygen. All of the organism were H_2s negative.

The pathogenic significance of Triple sugar iron agar is not clearly defined. But parr (1933) showed close relationship between anaerogenic E. coli and slow lactose fermenting coliform bacilli to the Salmonella and Shigella

Table No. 12

Cultural Characteristics of microorganisms isolated from Fish samples on TSI agar

Strain		Glucose	Lactose/ sucrose	Gas	H_2S	Identified Organism
I.	F_1	+	-	+	+	Citrobacter freundi
	F_2	-	+	-	+	Proteus mirabilis
	F_3	+	+	-	+	Providencia
	F_4	+	+	+	-	E.coli
	F_5	-	+	+	-	Shigella sonnei
	F_6	+	-	-	+	Proteus mirabilis
	F_7	+	+	+	-	Enterobacter species
II.	F_1	+	+	+	-	E.coli
	F_2	+	+	+	-	Shigella
	F_3	+	+	-	-	E.coli
III.	F_1	+	+	+	-	Klebsiella
	F_2	+	+	+	-	Edwardsiella
IV.	F_1	+	+	+	-	E.coli
	F_2	+	+	+	-	Klebsiella
	F_3	+	-	-	-	Proteus retggeri
	F_4	+	+	-	-	Citrobacter freundi
	F_5	+	+	+	-	Zymomonas
V.	F_1	+	+	+	-	Klebsiella
	F_2	-	-	-	-	Enterobacter hafniae
	F_3	+	-	-	-	Enterobacter cloacae
VI.	F_1	+	+	+	-	E.coli
	F_2	+	+	+	-	Enterobacter species
	F_3	+	+	+	-	Shigella
	F_4	-	-	-	+	Edwardsiella

Table No. 13

Cultural Characteristics of microorganisms isolated from Ice samples on TSI agar

Strain		Glucose	Lactose/ sucrose	Gas	H_2S	Identified Microorganisms
I.	I_1	+	+	+	-	Klebsiella
	I_2	+	+	+	-	Enterobacter species
II.	I_1	+	+	+	-	E. coli
	I_2	+	+	+	-	Edwardsiella tarda
	I_3	+	+	+	-	Enterobacter aerogenes
III.	I_1	+	+	+	-	Enterobacter hafniae
IV.	I_1	+	+	+	-	Klebsiella
	I_2	+	-	-	-	Citrobacter freundi
	I_3	+	+	+	+	Enterobacter
V.	I_1	+	+	+	-	Enterobacter aerogenes
	I_2	+	+	+	-	Shigella Sonnei
	I_3	+	-	-	-	Enterobacter hafniae
	I_4	+	+	+	-	Zymomonas

Table No. 14

Cultural Characteristics of microorganisms isolated from Water samples on TSI agar

Strain	Glucose	Lactose/ sucrose	Gas	H_2S	Identified Microorganisms
I. WS_1	+	+	+	-	Citrobacter
WS_2	-	+	+	-	Proteus rettgeri
II. WS_1	+	-	-	-	Enterobacter hafniae
WS_2	+	-	-	-	Enterobacter hafniae
WS_3	+	+	+	-	E. Coli
III. WS_1	+	+	-	-	Serratia
WS_2	+	+	+	-	Klebsiella
WS_3	+	-	-	-	Providencia
WS_4	+	+	+	-	Klebsiella
WS_5	+	+	+	-	
WS_6	+	+	+	-	Proteus rettgeri
IV. WS_1	+	+	+	-	Klebsiella
WS_2	+	+	+	-	Enterobacter hafniae
V. WS_1	+	+	+	-	E.coli
WS_2	+	+	+	-	Klebsiella
VI. WS_1	+	+	+	-	E.coli
WS_2	+	+	+	-	Providencia
WS_3	+	+	+	-	E.coli
WS_4	+	+	+	-	Klesbsiella
WS_5	+	+	+	-	Enterobacter hafniae

groups. TSI agar in the present investigation does not eliminate all saccharose fermenting strains of Proteus and Paracolons. The present study H_2S producing forms were very less - four in fish sample and one in ice and H_2S producers are completely abscent in water samples. (Table 12, 13, 14)

Bacteria are the classical monitors of faecal pollution in the aquatic environment but many eg. E. coli and coliforms are restricted in their usefulness to areas near the source of pollution.

Faecal index was constructed to find out the nature source and origin of faecal pollution. The ratio between the number of E. coli (type I) to the faecal streptococci will form the faecal index. The number of E. coli (type I) in the selective macConkey media was determined by its morphological appearance and confirmed by various biochemical tests. The enumeration of faecal Streptococci was also done in the selective KF media.

Faecal index of fish

E. coli type (I) was 5 and Streptococci was 14 in number in the first observation. In the second observation the faecal index was 0.7. None of the E. coli (type I) can be isolated from the third observation. So faecal index could not be constructed. In fourth sample faecal index was 0.3 which

Table No. 15

Colony Counts on MacConkey agar and K.F. agar and faecal index of Fish, Ice and Water samples.

Sample No Collection	Colony Count on Mac Conkey agar			Colony Count on KF agar			Fecal Index		
	Fish	Ice	Water	Fish	Ice	Water	Fish	Ice	Water
1	5	0	300	14	1	5	0.3		60
II	20	4	300	28	1	23	0.7	4	13.4
III	0	0	300	7	2	23			13.4
IV	9	0	300	26	1	28	0.3		10.72
V	0	0	300	0	0	300			1
VI	4	0	300	9	0	23	0.4		13.4

indicated origin of faecal pollution from animal source. E. coli (type I) and faecal Streptococci was abscent in fifth sample. The ratio of four E. coli and nine faecal Streptococci gave 0.44 as faecal index in sixth sample. Faecal index 0.4 indicated again nature and origin of faecal pollution is from animal source. (Table 15)

Faecal index of Ice

E. coli (type I) was absent in the first observation but a single colony of faecal Streptococci was encountered. In the second observation 4 colonies of E. coli were isolated but single faecal Streptococci was only encountered. So the faecal index was found to be four which indicated again the nature of the origin and source of faecal pollution from human origin. In the fourth observation E. coli was abscent and only one faecal Streptococci was recorded. In the fifth and sixth observation none of the organism could be isolated.

Faecal index of water sample

In all observations total colonies were countless, so E. coli was not isolated. Five faecal Streptococci was isolated in the first observation 23 colonies were found in the second, third and sixth observation. In the fifth observation colonies were countless. So faecal index was constructed in water samples to assess the nature and origin of faecal pollution from human origin.

Table No.16

Qualitative Detection of faecal coliforms in ice fish and floor water SWAB samples from Fish - Landing Area - Fisheries Harbour

Observation	MPN method			Elevated temperature method			Manja's method		
	1	2	3	1	2	3	1	2	3
SAMPLES	Fish	Ice	Water	Fish	Ice	Water	Fish	Ice	Water
I	1800+	13	1800+	1800+	13+	130	+	+	+
II	1800	250	1800	350	80	1800	+	+	+
III	1800	80	1800	50	25	1800	+	+	+
IV	1800	550	1800	1800	225	1800	+	-	+
V	1800	250	1800	250	17	250	+	+	+
VI	350		1800	350	2	1800	+	+	+

II MPN - TEST RESULT

Fish Sample:- In most probable number test the organism were found countless in all samples except the sixth sample. The colony count from Mccardy's probability tables showed a maximum and 1800 in first five observation. In the last observation the count was found to be only 250.

The MPN - test is relatively non-specific since, many bacteria can grow at 37°C in lactose broth with acid and gas production Eijkman's test was much specific of primary enrichment of E. coli if inoculated in Glucose broth and incubated at 44°C. The slight elevation of incubation temperature eliminates Enterobacter aerogenes and other organisms selectively permitting the growth of E. coli.

In differential coliform test (Eijkman's test) great variability was observed in the colony count. The first and fourth sample showed the count as 1800. The most probable number of bacteria in second and sixth observation was found to be 350. Second and fifth observation showed the count as 50 and 250 respectively.

Ice sample:- In the presumptive test Fig (16) (MPN test) first observation showed the count of 13. Second and fifth observation showed the count as 250. The third observation showed the least number, that is 80 and 550 was the highest count, observed in the fourth collection.

In Eijkman test great variability was observed in their colony count. 55, 80, 25, 225, 17 and 2 were the most probable number found in first, second, third, fourth, fifth and sixth observations respectively.

From water sample

In the presumptive test all the observations showed the highest number that is countless (1800).

In differential Eijkman's test the same positive (countless) results were observed in second, third, fourth and sixth sample. The first observation showed the count of 130 and fifth showed the count of 250, the fifth sample exceeding the limits of 180/100 ml.

III **Manja's method**

All the three sample of fish, ice, and water showed the positive results in all observations, indicating the faecal pollution of fish, ice and water. The medium was turning black immediately after pouring the samples. Only in ice samples the H_2S production was slow and black colour formed indicated Hydrogen Sulfide production indicating the anaerobic bacterial intensity.

Manja's method [SIGNIFICANCE]

It is a simple method for assessment of contamination based on detection of H_2S producing organisms. Presence of

coliforms is consistently associated with organisms that produce Hydrogen sulfide (H_2S). Enteric bacteria such as Salmonella, Proteus, Citrobacter and some strains of Klebsiella also produced H_2S.

Water samples turning black in Manja's medium were graded as unsatisfactory. Faecal pollution was indicated if the contents of bottles turned black within 12-18 hrs. In the present study all water samples and fish samples were turned black within 2-4 hrs. only in the case of ice sample it took 8-10 hrs for turning black in Manja's medium. All samples are considered unsatisfactory in the present investigations, whenever manja's medium was used for investigation.

The following genera of Enterobacteriaceae were isolated from MacConky medium. E. coli, Shigella, Klebsiella, Proteus, Citrobacter, Enterobacter Edwardriella, Providencia and Zymomonas are the organisms isolated based on Edwards and Ewings (1972) which might have produced H_2S in the medium.

6. DISCUSSION

When fish is caught from natural waters away from land masses, microbial flora mainly consists of bacteria which are harmless to human being. Handling after catch in fish-Landing centres introduces contamination of varying degrees.

Pathogenic bacteria that may cause contamination of fishery products are Coliforms, Escherichia coli, Faecal Streptococci, Staphylococci, salmonella, Vibrio and others.

Coliform organisms particularly E. coli and faecal Streptococci are indicative of faecal contamination. Primary habitat of E. coli is the intestinal tract of man. It is reported that one gram of faeces contain 10^7 to 10^9 E. coli/gm. Like E. coli the primary habitat of Faecal Streptococci is the intestinal tract of man and animal. It is reported that 10^3 to 10^5 numbers of Streptococci are present in one gram of faeces.

E. coli or faecal Streptococci are absent in offshore waters but their presence is reported in near-shore waters. Apart from this, the water gets contaminated by direct contact with faecal matter. Fish caught from sewage contaminated water will have more of those organisms. Flies and insects act as a source for contaminating the product with these organisms. Since their presence is an indicative of faecal contamination, there is

every likelihood that the product might be contaminated with other pathogenic organisms.

Salmonella is present in the gut of infected persons and warm-blooded animals. A true incidence of human disease arising from faecal contamination of fish, ice or water is impossible to determine. An insight into the problem is necessary as this pathogen or indicators of pathogen will deteriorate the quality of fish by creating variations in morphological appearance, colour and texture of the muscle etc. Transmission through the contaminated water supplies most serious source of infection and was responsible for massive epidemics for enteric diseases.(particulary Typhoid and Cholera). Today these disease are almost unknown for most part of the world although cholera has recently reappeared in some places like Alappuzha district and in Mediterranian countries. Their eradication was achieved primarly by appropriate sanitary controls. An essential part of this operation was "The development of bacterial methods for ascertaining the occurrence of faecal contamination in water and fish and other food stuffs".

It is seldom possible to isolate enteric pathogens directly from contaminated water or fish because they are usually present in small numbers. To demonstrate faecal contamination it is sufficient to show the presence of bacteria specific inhabitants of intestinal tract even though they may not be the agent of disease. The bacteria that can be used as indices of

such contamination or faecal streptococci and E. coli. APHA has designated the following six indices to denote the faecal contamination.

1. Coliforms - Total coliforms
2. Escherichia coli - Faecal coliforms
3. Faecal Streptococci
4. Salmonella
5. Staphylococci - body pollution indicator
6. Clostridia etc.

The methods of sanitary analysis developed by bacteriologist differ some what from country to country.

In the presence study E. coli type (I) and faecal Streptococci employed as a faecal tracer. The fish landing centres were periodically monitored for a period of two months during March and April 1997. Totally six observations were made and all the samples were subjected to bacteriological investigation within 18-30 hrs of collection.

Pathogens adsorbed to sediment possess danger by adhering to fish catch during trawling and resuspension of sediments which result in the release of adsorbed bacteria to overlying water thus causing hazards to human health (Sayler et al 1975); Gore et al (1979) found lower number of indicator organisms in sediment sample than water sample. In the present study higher number of faecal coliforms were recorded in fish

Table 17

Tolerance Limits for Pollutatns in Surf Zone Subject to Effluent Discharges

Characteristics	Tolerance Limits for			
	Shellfish culture and salt manufacture	fish culture	Water	Navigation
1. pH value	6.5 to 8.5	6.5 to 8.5	6.5 to 9.0	6.5 to 9.0
2. Free Ammonia (as N) (mg/l Max	1.2	1.2	no limit	no limit
3. Dissolved oxygen (mg/l Min	40% saturation value or 3 mg/l whichever is higher	-	3 mg/l	2 mg/l
4. Phenolic compounds mg/l Max	0.1	0.1	-	-
5. Insecticides, pesticides,herbicides fungicides mg/l	+	+	-	-
6. Arsenic (as As), mg/l Max	0.01	0.01	-	-
7. Floating material	No visible floating material of sewage of industrial waste origin			
8. Colour and odour	No noticeable colour or offensive odour			
9. BOD for 5 days at 20°C mg/l Max	5	-	5	-
10. Bacterial count, Coliform organisms, MPN per 100 ml Max	2500	-	2500	-
11. Suspened solids	No visible suspended solids of sewage or industrial waste origin.			
12. Blotary test	No less than 90% test animals shall survive in 96 hr test			
13. Mercury mg/l Max	+	-	-	-

samples. The ability of Enterobacteriaceae to utilise nutrients from sediments is shown by Hendreich and Morison (1966). Gerba and Macleod (1976) also observed E. coli to survive and grow in natural sea water when nutrient-rich sediment was present.

In the present study water sample harbourd E. coli (type I) in first second, third and sixth observations (Table 4) indicating the ability of E. coli to survive in the terrestrial environment in ice and also in fish. This is also supported by Geldreich (1972) that coliform can survive in bottom deposits of aquatic environment for several weeks before they die also support the above theory. Vandonsel and Geldreich (1971) examined wide variety of sediments and found that total coliforms, faecal coliforms, streptococcus concentration were hundred to thousand times higher in sediments than in overlying water.

Indian standards Institution has recommended tolerance limits for bacterial pollutants to the effluent discharge. 2500 total coliform per 100 ml of water for bathing, recreation, shell fish culture and salt manufacture (Table 17). This value has exceeded in almost all the samples in all the observations in the present study.

Totally 57 isolates have been isolated identified and their biochemical potential were analysed and classified based on the scheme of Edwards and Ewing (1972). Nine cultures were

FIG 3 - ENTRY OF MICRO ORGANISM INTO A WATER SOURCE

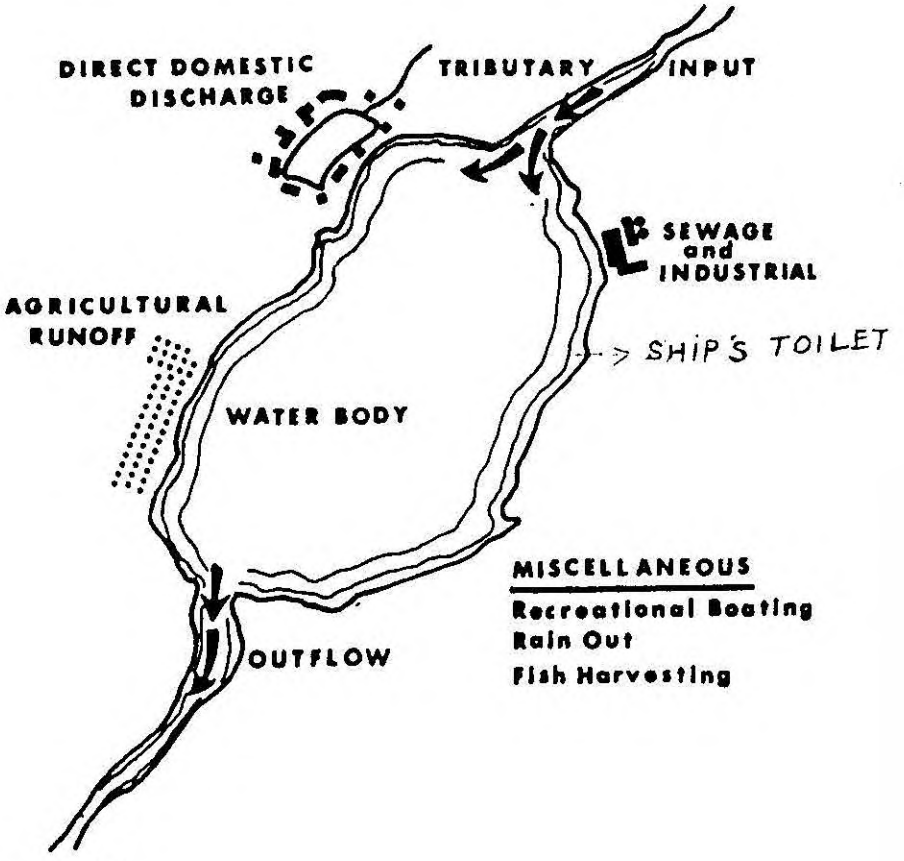

DIRECT DOMESTIC DISCHARGE
TRIBUTARY INPUT
SEWAGE and INDUSTRIAL
AGRICULTURAL RUNOFF
SHIP'S TOILET
WATER BODY
MISCELLANEOUS
Recreational Boating
Rain Out
Fish Harvesting
OUTFLOW

FIG. 3.

considered as E. coli as they are E. coil (type I) giving + + - - in IMVic test. The occurrence of Streptococci faecalis (Table 15) was predominent in fish and water sample. Fish sample harbourd 28 faecal Streptococci in the first and twenty four faecal streptococci in the second observation. Faecal Streptococci in water samples in all the five observations was the maximum being 28 per gram. Geldreich and Kenner (1969) found two species of Streptococci in water which indicated a very recent form and animal waste contamination. The transport of faecal indicator organism like E. coli and Streptococci faecalis from various sources into a water bodies is given in figure 3.

Fish sample harboured E.coli in the first, third and sixth observation. E. coli occured 21.73% of the observation totally made. 90% of the mussel sample in a purification tank contained nine E. coli/ml of tissue (Reynolds 1968) whereas 97% of the oyster from common oyster purification plants contained no E. coli in 5 ml of tissue (Wood 1963).

Morphological and biochemical characteristics of Enterobacteriaceae of fish sample (Table 2 and 5) showed they were very good in Saccharolytic activities, 83.33% fermented glucose 80% ferment lactose and sucrose 60% ferment. Mannitol Arabinose and Salicin and 75% ferment Xylose. 50% of the isolate were IMVic positive 20% produced H_2S from thiosulphate (TSI agar). The biochemical potential has enhanced and supported the survival of E. coli. Chandrika (1984) obtained more counts of

E. coli during monsoon months whenever the biochemical potential was found more. Carlucci and Pramer (1951) Mitchell (1968) has revealed the 'die off' of coliform was controlled by a variety of factors including toxicity, high salt concentration predation, competition by native microflora and limited nutrient supply Shewan (1962) recorded Salmonellosis resulting from the ingestion of fish from the Nile valley, South America and the Great Lakes in central Africa which was due to Salmonellas and Shigellas from newly caught fish. All these results showed the ability of the environment to support the enterobacteriaccae.

In ice samples (Table 2) 13 colonies were randomly isolated and identified and E. coli (type I), Edwardsiella, Citrobacter, Shigella and Zymomonas were the flora identified. Shewan (1965) found surface flora of fish stored in chilled sea water differed from that of fish stored in melting ice having a gradual build up during storage of the more anaerobic components of the original flora. Even if trimethylamine oxide reducers are present their metabolism has been diverted into other pathways.

Ice is considered as potential source and danger because typhoid bacillus can remain viable for several months in ice. Salmonella spp. can tolerate continuous low temperature in ice better than alternate freezing and thawing. According to Frankland (1894) ice from various lakes in the vicinity of Berlin contained from a few to as many as 14,400 bacteria per ml. Jensen (1943) found Achromobacter, Aerobacter, Bacillus,

Cellulomonas, Chromobacterium, Flavobacterium, Micrococcus, Proteus, Psuedomonas, Serratia and Sprillum apart from E. coli.E. coli remained viable for long periods when frozen in distilled water at -16°-40°and -79°. Ice provided beneficial solid surface for increased bacterial activity for adsorption of nutrients Trihydrol, a form of water in ice, may stimulate the growth and activity of bacteria. While ice cannot be relied upon to prevent the spoilage of fish or other flesh foods, it has a preservative value. According to TARR and SUNDARLAND (1940) ice prepared from water treated with 0.1% either benzoic acid or sodium nitrite is relatively effective for preserving fish fillets and other fleshes of fish. Jensen (1943) reports that chlorine water ice azochloramide ice, sodium propionate ice and other kinds of germicidal ice show promise for various purposes.

E. coli (type I) in ice samples occurred only in the second observation indicating the faecal pollution of the water used for making ice. Enterobacteriaceae, other than E. coli (type I) were present in all the observations. J.J. Licciardells and Pentremont (1987) found reduction in total aerobic count and inactivation of Pseudomonas putrefaciens in ice. But no inactivation of (Table 7) Enterobacteriaceae was seen in the present study in ice.

In IMVic test 15.38% were indole producers 53.84% were fermentative in M.R.reaction, 53.84% were capable of producing acetyl methyl carbinol in Voges-Proskauer reaction.

84.61 were able to use citrate as sole carbon source indicating absence of E. coli (type I) in ice samples.

In water samples out of the 20 isolates studied in detail (Table 4) 25% formed E. coli (type I). 20% were Enterobacter aerogenes. Van Donsel and Geldreich (1967) recorded very high levels of naturally occurring fecal coliforms and faecal Streptococci in storm water pollution in cincianati Ohio. Indicator bacteria contributing to faecal pollution of fresh or decaying Salvinia molesta debris was studied by Gore (1977) which supported both types of pollution during monsoon and post-monsoon months. The faecal pollution was entirely from animal source during monsoon months (March - July 1977) whereas in the present study only in fish sample animal faecal pollution was recorded high in fecal streptococci numbers.

Macconky Agar indicated countless lactose fermenters (Table 2) in all the observations while Faecal Streptococci was countless only in the 5th observation but in 2nd, 3rd and 6th observation the Faecal Streptocci was only 23. In the fourth observation only 28 Faecal Streptococci was isolated (Table 15). When Lactose fermenting forms were countless and assuming that a petriplate can accommodate 300 colonies in a 10 cm diameter plate Faecal index was constructed and was found above 4 in all the observations except the fifth observation where the nature and origin of faecal pollution was from warm-blooded animal source

(Table 15). Weibel et. al (1964) found that pollutional characteristics of urban-run-off was due to non-human origin.

Faecal index was 0.7 in second observation in fish sample which indicated the nature and source of pollution from animal source. At the same observation faecal index of ice was 4 and above 4-indicating the origin and nature of faecal pollution from human source (Table 15). In all the other observation the faecal index was found to be of animal origin. This shows Faecal Streptococci was recorded very high in all the samplings during these premonsoon months but the colony size of Streptococci in KF agar was (Table 15) punctiform in nature.

Out of the 57 isolates subjected to identification based on (Edwards and Ewings 1972) IMvic tests, the following genera were identified E. coli, Proteus, Providencia, Enterobacter, Citrobacter, Edwardsiella, Klebsiella, Shigella etc. (Table 9,10,11). On the basis of these data the microflora found in the samples has no apparent relationship.

IMvic tests are essential for final distinction between Eschericlia coli and Enterobacter aerogenes. E. coli according to IMVic test in the present study was very high in fish sample forming 28.96% (Table 8,A) whereas Enterobacter aerogenes formed 21.73% in pattern of presentation of IMVic. In water E. coli (type I) was 25% and in ice it was completely absent whereas Enterobacter aeorgenes formed 23.09% (Table 8 c) and in water samples formed 20% of the total (Table 8c)

Based on IMVic tests only ice samples are considered satisfactory except in 5th observation where it was found contaminated with E. coli type I (Table 3)

Based on Manja's medium all fish, ice and water samples were considered unsatisfactory in the present study. Warm, dry, clear conditions prevailed, generally during the March and April '97 months and the enhanced temperature might have favoured the multiplication and occurrence of coliforms and H_2S producing forms in the Manja's medium in 2-5 hours of incubation time.

Out of the 3 methods used IMVic and pour plate method in selective media MacConkey gave reliable results compared to Manja's medium as it was so sensitive to anaerobes and coliforms and more suitable only to drinking water than other natural water testing.

Faecal pollution of the fish-landing centre is a situation difficult to control the source of contamination. So protection must be secured through knowledge of the characteristics of the contamination and application of this knowledge to safeguard fish-landing centres.

7. SUMMARY

1. An ecological survey of indicators of water pollution and related organism and their sanitary significance was carried out during March and April 1997 in a Fish landing centre, Fisheries Harbour Cochin, $9^{o}55'$ - $10^{o}N$ and $76^{o}10'$ - $76^{o}20^{o}E$, to determine the faecal index, and its ecological significance. Totally six observations were made for the purpose in these pre-monsoon months.

2. All reagents, media used were from Hi-media and all Standard methods were followed in the methodology.

3. A taxonomic study was done using strains isolated from Fish, ice and water samples using selective MacConkey medium by pour plate method and applying the scheme of Edwards and Ewing (1972). The following conclusions were made.

4. Catching, handling, processing, storage, marketing and the way the product is prepared may affect the transmission of microbial disease through fish products and indicate inferior water quality used for ice preparation etc.

5. The effect of contamination on consumers include bacterial infection and intoxications, parasitic diseases, intoxications due to accumulated chemical poisons or biotoxins, allergic reactions, responses of undetermined etiology, offensive flavour, causing nausea or more acute illness due to tainting of the product.

6. Effect on non consumer include occupational diseases such as secondary bacterial skin infection, allergic reactions etc.

7. Two types of pollutants like sewage and bio-active wastes have a psychological impact on fishermen even though there is no physical harm to the fishermen, his gear or his catch.

8. Faecal streptococci was always predominant in all the sampling done but the size of the colony was always punctiform in KF agar.

9. Much emphasis was given to quantitative and qualitative occurrence of Escherichia. Coli and faecal Streptococci to know their occurrence, distribution and nature and origin of faecal pollution by constructing the faecal index.

10. E. coli (type I) occurred in 9 observations indicating the faecal pollution of the Fish-landing centre.

11. Faecal index constructed showed the nature and origin of faecal pollution is of animal origin as the ratio constructed between faecal coliform and faecal Streptococci ranged between 0.7 - 1.

12. Out of 57 isolates subjected to identification by IMVic tests and other biochemical tests the following genera were identified the microflora occurring most frequently were E. coli, Proteus, Providencia, Enterobacter, Citrobacter, Edwardsiella, Klebsiella, Shigella etc.

13. The closer relation of faecal coliform growth and survival to that of Salmonella and Shigella is the reason why the faecal coliform group was taken as the indicator of choice in the present study in assessing the faecal pollution.

14. A true incidence of human enteric disease arising from faecal contamination of fish, ice or water is impossible to determine. An insight into the problem is necessary as these pathogens or indicators of pathogen will deteriorate the quality of fish by creating variations in morphological appearance, colour and texture of the muscle etc.

Faecal pollution of the Fish landing centre is a situation difficult to control the source. So protection must be secured through knowledge of the characteristic of contamination and application of this knowledge to safe quard fish landing centres.

8. REFERENCES

AGG, A.R. 1975. Criteria for marine waste disposal in Great Britain In:Marine Pollution and Marine waste disposal (ed. E.A. Pearson and E.D.F. Frangipane, Pergamon Press: 75-83).

ARUL JAMES and **K.M. IYER**, 1972. Studies on isolation of Salmonella from sea foods. Part A. Comparison of enrichment and selective media for recovery of Salmonella from fishery technology: 9-115.

BAALSRUD, K. 1975. *The case for treatment In:* Discharge of sewage from sea out falls. Ed. A.L.H. Gameson, Pergamon Press: 165-172.

BERGER, B.B., EMIL. C., JENSEN, HARVEY LUDWIG, HAROLD ROMER and **MAURICE, A. SHAPIRO.** 1963. Coliform standards for Recreational Waters. *Journal of sanitary Engineering* division of American society of Civil Engineers 89:57.

BONDE, G.I. 1968. Studies on the dispersion and disappearance phenomena of enteric bacteria in the marine environment, Rey. Int. Ocean and Med:IX: 17-43.

BREZENSKI, F.T. 1971. Estuary water quality and Salmonella. Proceedings on national special conference of disinfectation of American Society of Civil Engineers, New York.N.Y.

BUCK, J.D. 1976. Pollution microbiology of Biscayne beaches, Fla. Science, 39(2) 111-20.

CALVERT, J.T. 1975. The Case against treatment In: Discharge of sewage from sea out falls Ed. A.L.H. Gameson, Pergamon Press: 173-176.

CARLUCCI, A.F. Scarpino and D. Pramer, 1961. Evaluation of factors affecting survival of Escheri-chia. coli in sea water V.studies with heat and filter-sterilised sea water. Appl. Microbiol. 9:400-404.

CHANDRIKA, 1983. Studies on the Ecophysiology of some heterotrophic and marine environments of Kerala. Ph.D. Thesis.

CHANDRIKA, 1986. Use of clams as sentinel organisms for monitoring faecal pollution. National Seminar on Mussel Watch 13-14 Feb 1986. 20, Page 168.

CHANK and C.S.W. KUCH, 1976. Distribution of heterotrophic bacteria related to some environmental factors in Tolo Harbour Int. J. Ecol. Environ. Sci. 2(1) 47-57.

CIANDE E. ZOBELL, 1946. Marine Microbiology, Chronica Botanica, Waltham Mass, U.S.A.

CRAWN, G.F. 1972. Microbiology - water borne out breaks. J. Water. Pollut. Control Fed 44: 1175-1182.

DWIVEDI, S.N. P.S. GORE, R.M.S. BHARGAWA and S.R. RAJGURU, 1974. Feasibility report on the development of a tourist resort at Siridaon beach, Goa, N:1.0 report.

EDWARDS, P.R. and **EWING, W.H.** 1972. Identification of Enterbacteriaceae, Burgers pub-Co. Minneapolis, Mimnesota.

ENGINEER, R.M. and **COOPER, R.C.** 1975. Role of bacteria and protozoa in removal of E. Coli from estuarine waters. Appl. Environ. Microbiol, 31 758-63.

GAMESON, A.L.H. 1975. Experience on the British Coast; In Marine Pollution and Marine Waste disposal (ed. E.A. Pearson and E.D.F. Frangipane) PP 387-339. Perganson Press.

GANGAROSA, E.J. T.V, BENNETL, C. WYATT, P.E. PIERCE, J. OLARTE, P.M. HERNANDES, V. VAZQUEZ and **D. BESSUDO.** 1972. An epidemic associated episome. J. infect Dis. 126: 215-218.

GELDREICH, E.E. 1966. Sanitary significance of faecal coliforms in the environment. Federal water pollution control Administration Publ. WP - 20-3.

GELDREICH, E.E. 1972. Water-brone pathogens. In water pollution microbiology Edited by R. Mitchell PP 207-241. Wiley-Inter Science, New York.NY.

GELDREICH, E.E. 1975. Microbiological criteria concepts for coastal bathing waters. Ocean ugt. 30:225.

GELDREICH and CLARKE; 1996. Applied Microbiology 14:429.

GELDREICH, E.E. and **B.A. KENNER.** 1969. Concepts of faecal streptococci in steam pollution, J. water Pollut. Contr. Fed. 41:R336-R352.

GERBA, C.P. and J.S. McLEOD. 1976. Effect of sediments on the survival of Escherichia. Coli in marine waters Appl. Environ. Microbiol 32: 114-120.

GERBA, C.P. GOYAL S.M., SMITH E.M. and MELNICK, J.L. 1977. Distribution of viral and bacterial pathogens in a coastal canal community. Mar. Pollut. Bull-8, 279-282.

GERBA, C.P. and McLEOD, J.S. 1976. Effect of sediments on the survival of Escherichia. coli in marine waters, Appl. envir. Microbiol, 32: 114-120.

GILATH, Ch. BLIT, S. and MELTZER, M. 1970 Dispersion and disappearance rate of enteric organisms from a marine out fall. Israel, Atomic Energy Commission. 77 PP.

GORE, P.S. 1971. Some observations on the bacterial flora of the beach sand and beach sea water. Environ. Hlth Vol. 13, No.2, 115-119.

GORE, P.S. O. RAVEENDRAN and R.V. UNNITHAN. 1979. Pollution in Cochin Back Waters with reference to Indicator Bacteria Indian. J. Mar. Sci. Vol. 8, No.1, PP 43-46.

GOYAL, S.M. GERBA, C.P. and MELINICK, J.L. 1978. Prevalence of human enteric viruses in Coastal Canal Communities.J. Wate. Pollut. Control. Fed, 50: 2247-2256.

GREENBERG, A.E. 1956. Survival of cateric organisms in sea water Publ. Hlth Rep, Wash; 71:77.

GRIMES, D.J. 1975 Release of sediment bound faecal colifom by dredging, Appl. Microbiol. 29:101-111.

GRUNNEK, K., A. GUNDSTREP and G.J. BONDE 1970. Isolation of Salmonella from a polluted marine environment, technique and enrichment media. Review on International oceanography of mediterranean, 17:165.

HASHIMOTO, H. NOBORISAKA, R. and VANAGWA, R. IRVING. 1974. Distribution of motile streptococci in faeces of man and animal in river and sea water-Jap. J. Bacteriol. 29. 387-396.

IRVING, T.E. 1997. Preliminary Investigations on the effects of particulate matter on the fate of sewage bacteria in the sea. WRC Technical Report TR 55.

JONES, G.E. 1964. Effect of chelating agents on the growth of Escheri chia. coli. in sea water. J. Bacterial 87:483-99.

JOSEPH, A.C. CYRIAC KALHE and T.S.G. IYER. 1976. Internal Circular Central Institute of Fisheries Technology, Cochin.

LA BELLE, R.L. and GERBA C.P. 1979. Influence of pH, salinity and organic matter on the adsorption of enteric viruses to estuarine sediment. Appl. envir. Microbiol. 38, 93-101.

MATHUR, R.P. and RAMNATHAN, K.N. 1966. Significance of enterococci as pollutions indicator. Env. Hlth; 8, 1-5.

MITCHELL, R. 1968. The effect of water movement on lysis of non-marine microorganisms by marine bacteria.Sarsia 34, 236-266.

MITCHELL, R. and **MORRIS, J.C.** 1969. The fate of intestinal bacteria in the sea. In Advances in Water Pollution Research (Edited by S.H. Jenkins) Pergamson Press London.

MOORE, B. 1959. Sewage contimination of Coastal bathing waters in England and wale Committee on bathing beach Contamination of the Public Health laboratory service, Journal of Hygiene (London) 57:435.

MOORE, B. 1975. The case against microbial standards for bathing beaches. In discharge of sewage from sea out falls (ed A.L.H. Gameson) PP. 103-110 Pergamon Press.

MOSLEY, J.W. 1975. Epidemiological aspects of microbiol standard's for bathing beaches. In discharge of sewage from sea out falls ed. A.L.H. Gameson), PP. 85-93. Pergammon Press.

NERKER, D.P. N.F. LEWIS and **V.S. KITMIA.** 1975. Symposium on Fish Processing In India 13-14, February CFTRI Mysore.

ORLOB, G.T. 1956. Viability of sewage bacteria in sea water, sewage Ind wastes, 28: 147-67.

PAOLETHI, A. 1964. Microorganisms Pathogens dans Kc Milen Marin Poll. Marin. par les Micro org et les products petrolierns, symposium Monaco; 135.

PARR Bact. Rev. 3 No.1 1939.

PHIRKE, R.M. and **VERMA S.R.** 1972. "Significance of Enterococci as indicators of stream Pollution".Indian Four-Environ. Health (India) 14, 328.

REGIER, A.P. and **R.W.A. PARK**, 1972. Faecal pollution of our beaches - How serious these situation. Nature, 239:408.

RITTENBERG, S.C. MILTWER, T. and **ILVER, D.** 1958. Coliform bacteria around three marine sewage artfalls, Limnol, oceanogr. 3, 101-108.

REYNOLDS, No. 1968. Bacteriological standards for mussels. Pub. Hlth Inspector, September 1968. 524.

SANTHA KUMARI, R. 1966. Some bacterial characteristics of the Cochin Back water. Curr. Sci. 35-310.

SASTRI, C.A; K.M. ABOO and **M.N. RAO.** 1969. Incidence of coliforms and enterococci in natural waters, Environ. Health. 11:32-40.

SAYLER, G.S. J.D. NELSON, Jr., A. JUSTICE and **R.R. COLWELL.** 1975. Distribution and significance of faecal indicator organisms in the upper Chesapeake Bay. Applied Microbiol; 30: 625-638.

SEN, R. and **S.N. GHOSH.** 1970. A comparison of the enterococci index with index as indication of faecal Pollution of water, Indian. J. Med. Res. 58, PP. 1164-1168.

J.M. SHEWAN, 1962. The Bacteriology of fresh and spoiling fish and some related chemical changes. Recent advances in Food Science, Haw Worth adn Mull Leitch J. eds. Published by Butter Worths London.

J.M. SHEWAN, 1965. Bacteriology of Fish stored in chilled sea water. Meeting on fish Techn. Fish Handling and preservation. Scheveninger, 1964, OECD, Paris 1965. P.95.

SHUVAL, H.N. COHEN and **Y. YOSHE PURER**. 1968. The dispersion of bacterial pollution along the Tel Aviv Shore, Review on Internat; ional oceanography of Mediterranena 9:107.

SHUVAL, H.I. 1975. The case for microbial standards for bathing beaches. In discharge of sewage from sea out falls (ed. A.L.H. Gameson) PP. 95-100 Pergamon Press.

STANFIELD, G., ROBINSON. J.A. and **STANFIELD J.P.** 1977. Investigation into membrane filteration procedures for determining the total coliform population to sea water samples WRC Technical Report 7R 57.

STEVENSON, A.H. 1953. Studies of bathing water quality and health. Journal of American Public Health Association 43:529.

THAPLIYAL, D.C. 1972 "The bacteriological quality of Tarai Water" Indian Journ. Environ. Health (India) 14, 88 1972.
Water Poll. Abs; 45-1972.

TINKER J. 1976. New Scientist, 70 Coastal de la Mierda (988):238.

TOBIN, R.S. and **DUTKAB, B.J.** 1977. Comparison of the surface structure, Metal Binding and faecal coliform Recoveries of nine membrane filters. Appl. Envor. Microbiol. 34. 69-79.

VAS-CONCELS and SWARTZ, 1976. Survival of bacteria in sea water using a diffusion chamber apparatus, In situ. Applied Environ. Microbiol; 31 913-920.

N.K. VELANKAR. 1957. Bacteria isolated from sea-water and marine mud off Mandapam. (Gulf of Mannar and Pal. Bay) Indian.J. Fish. Vol. 4.1)

VENKATARAMAN. R. and **SREENIVASAN, A.** 1955. A mussel Pollution at Korapuzha Estuary (Malabar) with an account of certain coliform types. Indian. J. Fisheries, 2:34-324.

VLODAVETS and KALINA. 1977. Salmonella in the Coastal Sea waters; Methods of study, ecology and correlation with the indicator microbes; zh. Microbial, Epidemol, Immunobiol. 54(12) 33-39-1977.

WAKSMAN, S.A. HOTCHKISS MARGARET and **CAREY, CORNELIA, L.** 1933 Marine bacteria and their role in the cycle of life in the sea. II Bacteria concerned in the cycle of nitrogen in the sea bind 65 137-65.

WEUSS, C.M. 1951. Adsorption of E. coli on river and Estuarine silt, sewage Ind. wastes. 23, 227-237.

WILKIN, J.R. and **BRYKIN, E.H.** 1976 Analytical notes electrochemical method for early detection and monitoring of coliforms. Water technology/Quality 257-263.

WOOD, P.C. 1963. The sanitary control of molluscan shell fish. Some observations on existing methods and their possible improvement. *ICES C.M., Shell fish committee*, Doc. No.24, 2 PP (mimeo).

VAN DONSEL, D.J. and **E.E. GELDREICH**, 1971. Relationship of Salmonella to faecal coliforms in bottom sediments. Water Res., S: 1079.

YOSPHE PURER.Y and **H.I. SHUVAL**. 1972. Salmonella and bacterial indicator organisms in polluted coastal waters and their hygienic significance-marine pollution and sea Life Fishing News (Books) Ltd.; London: 574.