A COMPARISON OF THE BACTERIA AT BAKOVEN BAY AND HOTTENTOTSHUISIE AS INDICATORS OF MARINE POLLUTION FROM A SEWAGE OUTFALL.

by

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ABSTRACT

A comparison is made of the bacterial populations at two geographically similar near-shore marine sites. The difference between the sites is that one is influenced by a raw sewage outfall. Sea-water samples were drawn directly by means of a hand held all-glass bacteriological sampling device. Sea-water samples were analysed for a total coliform count, presumptive E.coli type I and a total bacterial count on a marine agar medium. A proportion of the isolates on the total count plates were subjected to a number of physical, biochemical and antibiotic tests and classified into genera. Bacteriological differences between the sites were shown by the coliform tests. Total count isolates and in particular the pseudomonads also showed bacteriological differences between the sites. It is indicated that these organisms could possibly be used to demonstrate stressed conditions in near-shore marine environments resulting from sources other than sewage contamination.

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C O N T E N T S

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INTRODUCTION

Marine Pollution has become a problem of widespread concern. Various methods of monitoring the long-term effects of pollution including low-level pollution are being investigated to an increasing extent to prevent a situation arising where eventual restoration costs are prohibitive. Over-intensive use of natural areas can have deleterious effects. For example liquid organic wastes can result in organic overloading of aquatic systems causing oxygen depletion in the system and consequent ecological disturbance. Riverine disposal of raw sewerage serves to disperse pathogenic bacteria.

Grindley (1972) and Oliff (1976) have described the extent and possible effects of using the oceans as the eventual sink of man's refuse. It is concern for the ability of the oceans to adequately cope with ever-increasing effluent loads that has led to the recent controversies regarding ocean dumping.

Man's expanding resource utilisation has taken him into the most production areas of the world's oceans, the coastal zones, and these shallow waters are experiencing his intervention via waste discharges, thermal and radio-active pollution, dredging, coastal construction and mining (Inman and Brush, 1973). Lee and Rooth (1973) believe that the water quality of coastal bays and estuaries is a function of the particular water body's ability to remove

pollutants / ....

pollutants, that is the impact of discharged pollutants is dependent on their residence time in a particular body of water. Water quality is also a function of the amount of pollutant discharged. Riddle (1976) agrees that the removal of pollutants from an area is dependent on the lateral mixing rate and on the residual drift currents.

Inman and Brush (1973) support these views. They state that while the surf zone characteristically has high mixing rates, the coastal zones are beginning to show water quality deterioration due to receiving man's biological and chemical wastes, as wind- and wave-induced surface currents tend to produce circulation patterns that favour the retention of particulate material near the coast. Waldichuk (1973) concludes thus that international control of pollution such as dumping at sea, continental run-off, atmospheric emissions, effluent discharges by pipeline and other shore-based activities can only be achieved by effective environmental management via collaborative international marine pollution research and monitoring. Brummage (1973) agrees that the in-shore pollution load must be controlled and warns that with the increasing scale of human activity it is fallacious to regard the sea as an infinite sink.

Although control of wastes in the marine environment dates back to 1926 (Lee and Rooth, 1973), the pollutant that has attracted most attention in recent years has been oil and associated petroleum products (Atlas and Bartha, 1973a). While studying petroleumlytic bacterial population

densities / ....

densities in different Pacific Ocean watermasses, Seki et al (1974a) obtained increasing petroleumlytic bacterial counts in increasingly oil-contaminated waters. Atlas and Bartha (1973b) reported similar findings in Raritan Bay and these authors quote in support of their results work by ZoBell (1969) and Mironov (1970).

Biological indicators of water pollution are today being widely researched. Buikema et al (1974) employed Rotifers as monitors of heavy metal pollution. Similarly, Bryan and Hummerstone (1973) have successfully used brown seaweed as an indicator of heavy metal pollution particularly of manganese and zinc in an estuary. This study also showed the roll of brown seaweed as a measure of the seasonal variation of heavy metal pollution. Sparks et al (1972) and Cairns and Sparks (1971) established a respiratory response of Bluegills as indicative of zinc pollution. Cairns and Messenger (1974) and Staufer et al (1974) studied the effects of thermal loading in aquatic systems on snails and fish distribution respectively. Cairns et al (1972) utilised the optical spatial filtering of diatoms when studying diatoms as indicators of water pollution.

A bacteriological survey of the distribuion and occurrence of coliforms and pathogenic indicators of pollution in near-shore coastal waters along a section of the Natal Coast has been described by Livingstone (1968). After subsequent data analysis, Livingstone (1969) developed a bacteriological method of appraisal for sewage contaminated coastal

waters / .....

waters. Values were applied to the quality indicators *E. coli* type I, *Ascaris* and *Taenia* ova, coagulase and mannitol positive staphylococci, salmonellae and shigellae and salinity. Values were weighted according to the pollution significance of each indicator. The sum of the values ascribed to each of the quality indicators for a particular sea-water was rated on a scale of I to IV classes which classified the sea-water quality. A class I water was classified as a clean water and a class IV water polluted.

BACTERIA AS ENVIRONMENTAL MONITORS

Bacterial indicators of water quality have long been recognised (APHA, 1971). It is, however, the traditional physical and chemical parameters such as pH, BOD_5, DO, temperature, turbidity and chemical composition which have formed the basis for water quality standards.

Bacteriological standards for drinking water (SABS, 1971) have been laid down, and in certain countries recreational or bathing waters today have statutory bacteriological limits (Cabelli and McCabe, 1974). Oliff (1976) quotes that an often used sea-water quality standard is 1000 total coliforms per 100 ml. Bacteriological monitoring in these standards, however, serves only as an indication of faecal pollution. Considerable research effort is currently being expended to determine the most suitable parameter or particular parameters which effectively describe the environmental condition. Experimentation in the food industry (Hunter, pers. comm.; Breyer-Menke, unpub.) indicated that bacterial observation by a particular direct microscopic technique of certain canned food products proved more accurate and rapid in predicting food spoilage than chemical monitoring of the products.

This experience and the fact that bacteria have specific requirements stimulated this investigation into the role of bacteria as pollution indicators.

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In this study an attempt is made to evaluate the possibility of using bacteria as indicators of low-level pollution from a marine sewage outfall. It is thought that if the bacterial population in the proximity of the outfall is compared to that not influenced by the outfall, all other conditions such as temperature, wave action and meteorological conditions being as closely similar as possible, anticipated differences between the populations will indicate the influence of the outfall. These differences would expectedly show as species differences between the populations or differences between the relative number of the species in the populations or, more probably, both.

Having chosen a sewage outfall as the polluting feature, allows for comparisons to be drawn between this site and a reference site on the basis of their coliform populations. Coliforms are the traditional monitor for faecal contamination and in this study formed the foundation for comparing the bacterial populations at the study sites.

Bacterial population analysis could be affected as a result of long-shore currents introducing bacteria from points outside the study zone. This effect is lessened in this study by not culturing spore-forming organisms which could have been transported for very large distances. The possibility of bacterial contamination between the sites is limited by the dilution effect.

Samples / .....

Samples should be drawn as near to simultaneously as possible. Simultaneous sampling would nullify local effects such as total bacterial count variance which could vary considerably over a matter of hours at any one site as a result for example of wind-induced increased wave action churning more sediment and bacteria into the water column.

SAMPLING SITE SELECTION

3.1 CHOICE OF SAMPLING AREA

As the microbiological procedures were to be conducted in the laboratory, one of the prime considerations in the choice of sampling sites was access. It was felt that despite using cool boxes during sample transport no more than 2 hours should elapse between drawing the first sample and the initiation of bacteriological analysis in the laboratory. APHA (1971) recommends that coliform studies should be conducted not longer than 1 hour after the sample has been drawn or after 8 hours if the sample is iced.

Initially sampling to depths of between 10 m and 15 m some distance behind the surf zone was to be undertaken. Access to this point could most easily be achieved using a ski-boat.

Both the abovementioned considerations were met by joining an existing research programme on the kelp-bed ecosystem established on the western side of the Cape Peninsula (Fig. 1).

3.2 SPECIFIC SAMPLING SITES

In choosing the specific sampling sites, these had necessarily to approximate each other as closely as possible in respect of climatic and physical conditions.

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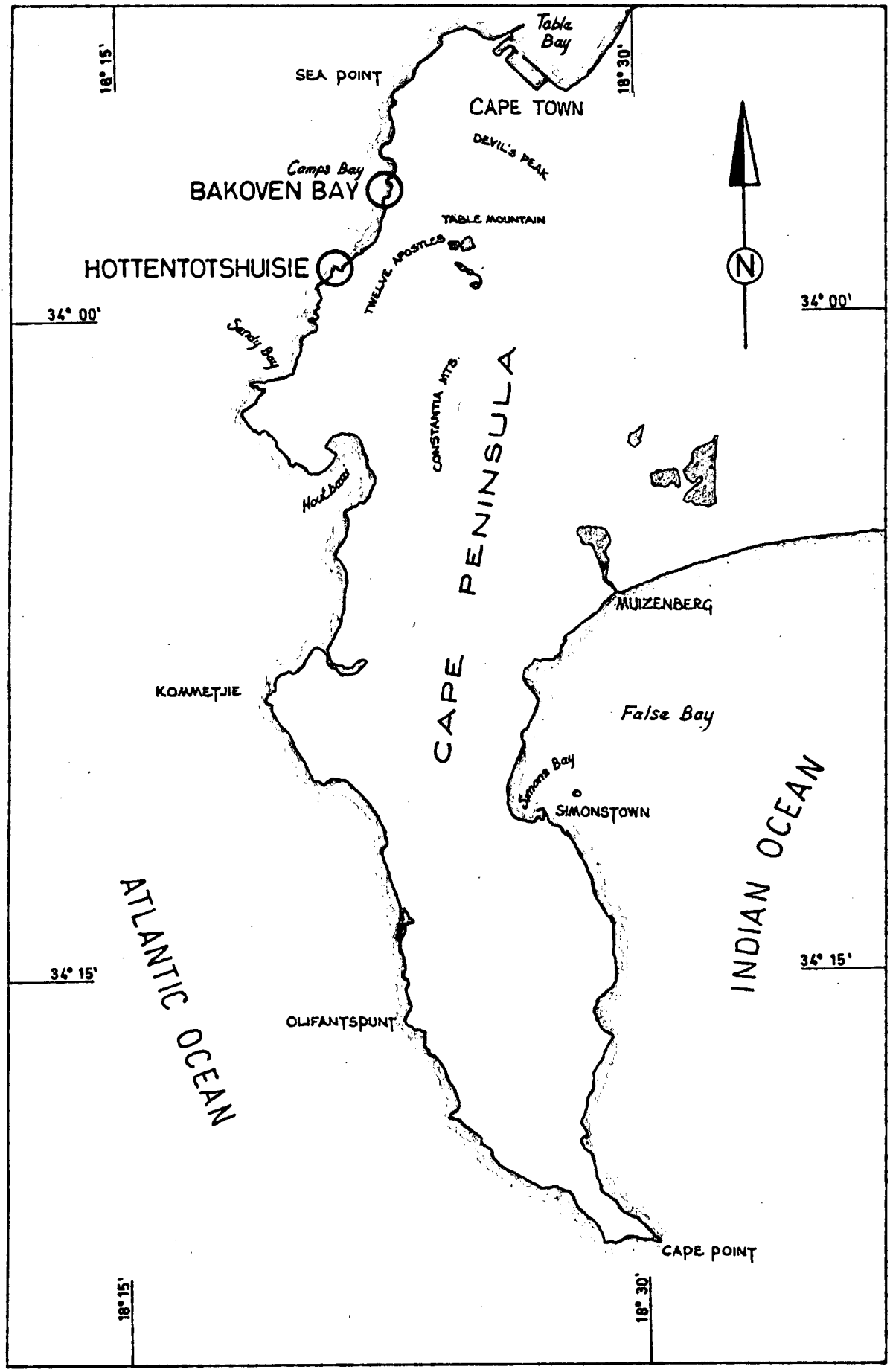

FIG. 1: LOCALITY PLAN.

It was thought that such factors as the prevailing wind and weather conditions, temperature and wave-action could affect the bacterial load in the water column and that these extraneous influences on the bacterial counts between the sites should be limited where possible.

The prevailing summer winds in the Western Cape are south-easterly and these strengthen upwelling of the cold Benguela current due to the off-shore water transport resulting from wind-induced drift. Two effects of this are the good mixing achieved and the fact that on-shore water temperatures are lower than the off-shore water temperatures, the reverse of what occurs in winter. During the winter months north-west winds prevail on the Western Cape, the isotherms tend to be parallel to the water surface and strong temperature stratification can occur. This even close to the wave-line and despite the strong surges which are prevalent in winter. Good vertical mixing of the water does nevertheless occur in strong winds.

3.2.1 Bakoven Bay Sampling Site

Bakoven Bay was chosen as the most suitable site which included a sewage outfall. This Municipal outfall discharges the raw domestic sewage of the Camp's Bay

residents / .....

residents of the City of Cape Town. At the time of this study this outfall although still in use was being replaced. The outfall pipe was in disrepair, and raw sewage was being discharged on the rocky shoreline at a point where the water was 3 m to 4 m deep. The schematic diagram (Fig. 2) below shows the study site.

Strong marine algal growth occurred on the ocean floor at this site despite the poor sunlight penetration limited by the turbidity caused by the discharged sewage.

Two sampling points were chosen at this site, one (A) approximately 5 m from the outfall and the second (B) approximately 50 m from the outfall, with a large rock sited between the outfall and B (Fig. 2).

These two sites were chosen to give some empirical idea of bacterial die-off and dilution if these occur. It was also felt that although the structure of the bacterial population between A and B may differ, greater bacterial similarity should exist between A and B than between B and C (the reference site).

3.2.2 Hottentotshuisie Sampling Site

The sampling point (C) in Fig. 3 was chosen as the reference site. This site was approximately 4,5 km from the polluted site (sampling points A and B) and largely remote from the city's influences such as

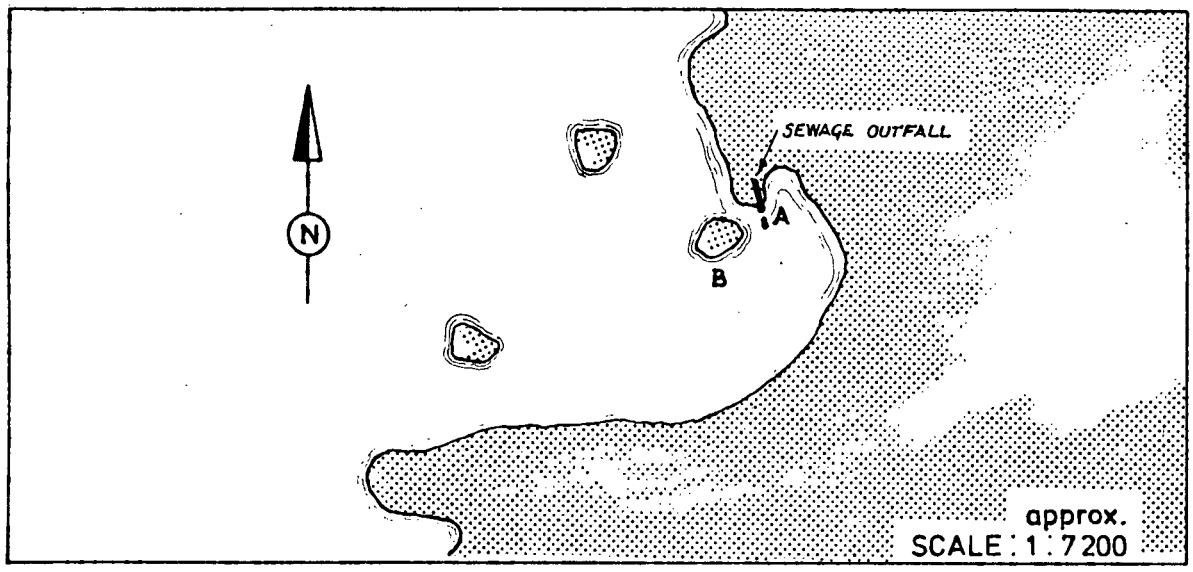

FIG. 2 : SAMPLING STATIONS A AND B AT BAKOVEN BAY

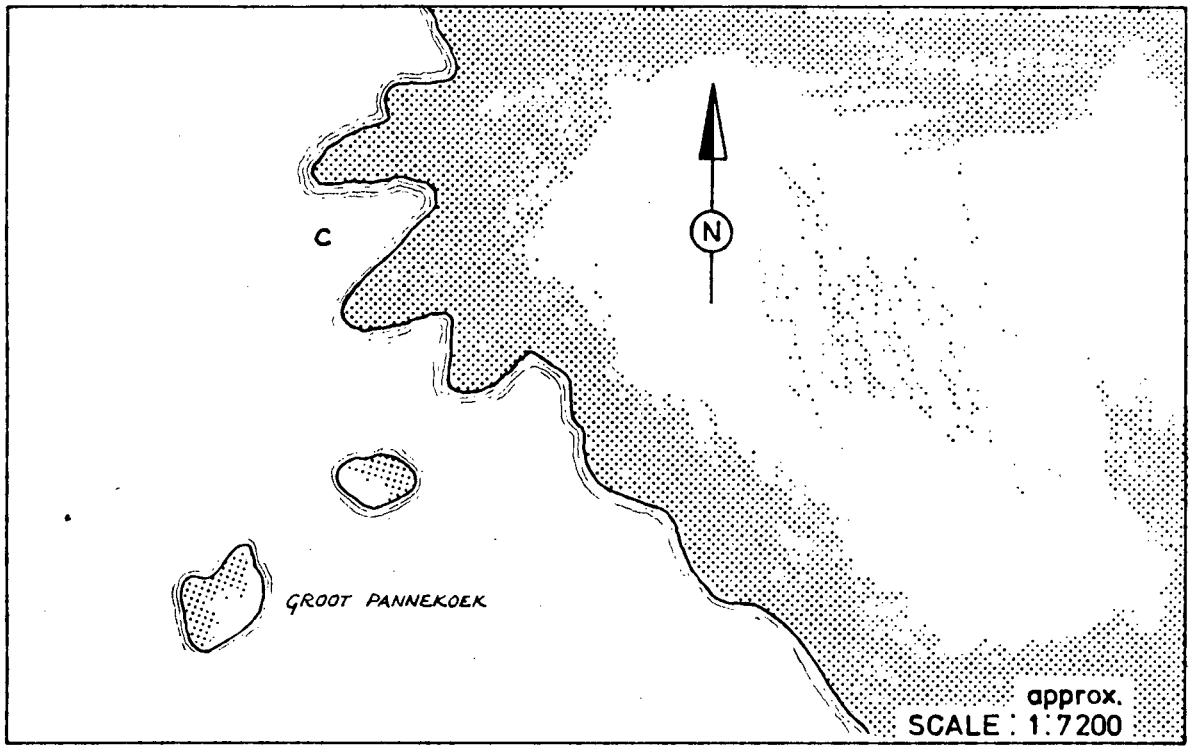

FIG. 3 : SAMPLING STATION C AT HOTTENTOTSHUISIE.

urban run-off. There is no agricultural activity in the on-shore vicinity of the sampling site and rain water run-off would not be expected to contain fertiliser or pesticide leachings.

One difference between C and A in particular is that sunlight penetration at C is usually good. Sunlight penetration is temporarily reduced when strong vertical mixing occurs as sediment is then churned into the water column.

3.3 DETERMINATION OF THE VARIATION ABOUT THE SAMPLING POINTS

The initial experimentation was planned to investigate two aspects of bacterial distribution. Firstly, any differences in the vertical distribution of the bacteria in the water column had to be determined. Secondly, the spatial distribution of the bacteria over the site at different water depths was determined. This work was carried out at Hottentotshuisie and conclusions drawn from these results presumed to apply at both sampling sites.

The three sampling points having been determined, further experimental work was conducted as described in section 6.

SAMPLER DESIGN

Many different samplers suitable for various types of marine sampling have been designed (ZoBell, 1946; Kriss, A.E., 1963; Gunkel and Rheinheimer, 1972; Sieburth, 1965; Ruppert, 1972; Williams, 1969 and Clasby et al, 1972). Most of the remote water sampling devices are designed to operate at depth (Williams, 1969; Sieburth et al, 1963a). Sieburth (1963b) and Seki et al (1974b) have designed remote samplers for use in shallow waters. All these sampling devices rely on taking snap samples which are statistically questionable (Chermisinoff, 1974).

Remote samplers have other deficiencies in that they are usually unsuitable for use from a small boat, expensive, difficult to sterilise and transport and contamination from the upper water layers can often occur. For these reasons it was decided that snap samples using a hand-held sampler and utilizing SCUBA equipment would be the most practical and reliable means of sampling in this programme. Furthermore, a number of samples could be drawn at one time without great inconvenience.

The required sampler would have to fulfill the following criteria :

(i) easily steam sterilized;

(ii) easily opened to draw a sample at depths of up to 20 m; similarly must withstand the expanding pressure effect on rising from 20 m to surface;

(iii) easily / ....

(iii) easily handled underwater;

(iv) sufficiently robust to withstand shocks of transport in a small boat in heavy seas;

(v) inert material which would not contaminate the sample must be used in sampler construction.

4.1 BRASS BACTERIOLOGICAL SEA-WATER SAMPLER

Initially four brass samplers were made. The marine brass tubing used in the construction of each sampler was approximately 5 cm long of internal diameter 18 mm. Each brass tube was threaded internally and fitted with a stopcock with a teflon washer to prevent leaking at each end.

Each brass tube and its respective stopcocks were identified so that the same combination was used in each instance. They were also notched to ensure the stopcocks were always tightened equally on re-assembly of the sampler after cleaning.

Suspecting that some metal migration from the sampler to the sample might occur, two of the samplers were internally coated with Desicote (a silicone-based inert coating by Beckman Instruments). The samplers were standardised, sterilised in an autoclave at 121 C for 20 minutes and cooled overnight in a refrigerator prior to being transported in a cool bag to the sampling site

at / .....

at Hottentotshuisie.

One each of the coated and uncoated samplers was used to draw a surface water sample and this was repeated at a depth of approximately 10 m. Unfortunately the surface sample in the coated sampler was lost.

The interval between the time the samples were drawn and returned to the laboratory was 3 hours which is thought to have had an adverse affect on the samples. One in twenty and one in two hundred dilutions of the samples were filtered through 0,45 gridded Millipore filters. The filters were placed on sea-water Nutrient Agar and incubated for 4 days at 21 C. The surface sample gave a count of 250 bacteria/100 ml and the coated and uncoated 10 m samples 3 200 bacteria/100 ml and 800 bacteria/100 ml respectively. This discrepancy seemed too large to be explained by natural variation and it was decided to investigate the metal pick-up of the sample during transport.

The brass samplers and a screw-topped glass bottle were steam sterilized for 5 minutes at 121 C and filled with sea-water on cooling. Copper and zinc levels in the samples were measured after 5 minutes and 2 hours using flame ionisation atomic absorption. The results are given in Table I.

TABLE I / .....

TABLE I

Atomic Absorption Analyser Readings for Copper and Zinc Concentrations in Sea-water contained in Brass and Glass Bacteriological Samplers

Sampler	Copper (A.A. reading)		Zinc (A.A. reading)	
	5 min.	2 hr.	5 min.	2 hr.
Glass Bottle	0,008	0,012	0,017	0,014
Brass Sampler 1	0,096	0,605	0,621	off-scale
Brass Sampler 2	0,120	0,885	0,676	off-scale
Brass Sampler 3	0,114	0,733	0,502	off-scale
Brass Sampler 4	0,136	0,823	0,522	off-scale

Although the atomic absorption equipment was not standardised the very marked differences in metal pick-up between the brass samplers and the glass bottle was sufficient to indicate that despite their greater fragility glass samplers would be preferable to the brass samplers.

4.2 GLASS BACTERIOLOGICAL SEA-WATER SAMPLER

Glass samplers of volume approximately 50 ml. were decided upon. Similar in design to the brass prototypes, the glass samplers were constructed by welding a 6 mm all glass, high vacuum stopcock to either end of a borosilicate glass tube approximately 12,5 cm long and 5 mm in diameter (internal diameter 18 mm) (Fig. 4).

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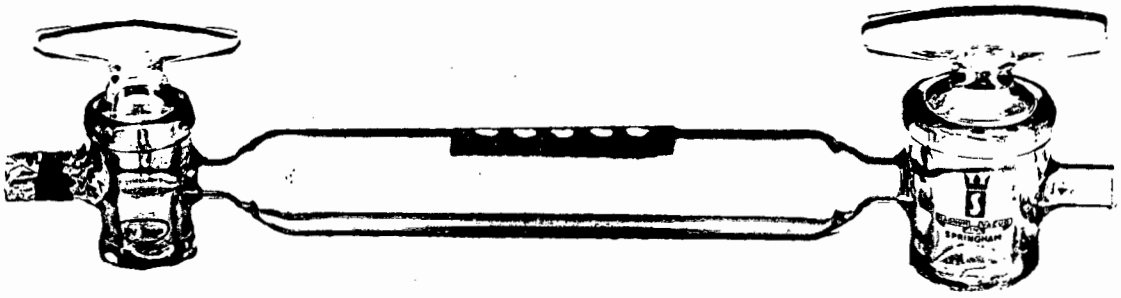

FIG. 4 : GLASS BACTERIOLOGICAL SEA-WATER SAMPLER

Five samplers were made, each one identified by an attached strip of linen water resistant automobile tape with a number of punched holes to allow for identification.

4.2.1 Calibration

The volume of each of the glass samplers was determined by filling each of them with unfiltered, aged sea-water at 20 C and 100 kPa and emptying the sampler into a dry 50 ml measuring cylinder. This procedure was repeated five times. The reason for accurately knowing the volume of the samplers is that a 1 in 2 dilution of the sea-water sample was immediately obtained in the laboratory by emptying the contents of a sampler directly into an equal amount of diluent which reduced the number of manipulations and therefore the time elapsed between the sea-water sample being drawn and plating of the filtered sample.

4.2.2 Copper / ....

4.2.2 Sample Pick-up of Copper and Zinc using Glass Bacteriological Sea-water Sampler

One glass sampler was measured for metal pick-up against the brass samplers. The samplers were cleaned washed and dried. Two of the brass samplers was re-coated with Desicote and allowed to dry for 24 hours. Thereafter all the samplers were steam sterilised at 121 C for 15 minutes.

Prior to filling the samplers with unfiltered, aged sea-water, the atomic absorption equipment was standardised using standard copper and zinc solutions. The calibration curve obtained is given in Appendix 1.

The samplers were filled with sea-water and the sea-water in the samplers analysed for copper and zinc pick-up after 2,0 and 3,0 hours (Appendix 2). The concentrations of copper and zinc in the samples were obtained from the calibration curve (Appendix 1) and are recorded in Table II below.

These results show that while the Desicote coating reduces the metal pick-up of the brass samplers, no metal pick-up occurred in the glass sampler.

TABLE II / ....

TABLE II

Copper and Zinc Concentrations in Sea-water contained in Brass and Glass Bacteriological Samplers

(a) Copper

Sampler	0h (ppm)	2h (ppm)	3h (ppm)
Brass Sampler 1*	0,4	5,3	7,3
Brass Sampler 2	1,4	8,1	9,3
Brass Sampler 3*	1,0	5,8	7,3
Brass Sampler 4	1,1	6,8	7,2
Glass Sampler	0,1	0,1	0,2

(b) Zinc

Sampler	0h (ppm)	2h (ppm)	3h (ppm)
Brass Sampler 1*	0,7	3,2	3,8
Brass Sampler 2	1,1	3,8	4,0
Brass Sampler 3*	0,8	2,0	2,7
Brass Sampler 4	0,9	2,1	3,1
Glass Sampler	0,1	0,1	0,1

* Sampler internally coated with Desicote

EXPERIMENTAL DESIGN AND BACTERIAL IDENTIFICATION

Traditionally bacteria as indicators of water pollution have centred on the presumptive E.coli test for potable water supplies. It was decided in this programme that total coliform and presumptive E.coli tests would be undertaken, including occasional E.coli type I (i.e. coliforms of undoubted faecal origin) confirmatory tests.

Bacterial water quality analysis based on the traditional indicator bacteria has only been applicable to the sanitary quality of water. Utilising bacteria as indicators of coastal stress necessitates a broad approach to quality assessment. It is most unlikely that a single bacterial species would be suitable as an all-embracing pollution indicator. In order therefore to use bacteria as indicators of marine pollution as a whole rather than only as a health hazard, the nature and number of organisms within the bacterial population must be analysed. It is the differences that exist between bacterial species in their requirements for growth and multiplication that indicates their usefulness as biological indicators in marine quality assessment, as each species or group of bacteria will respond differently to its environment. Because of the large number of different bacteria present in nature it is unlikely that a particular bacterium will undergo mutational changes to adjust to a modified environment, but rather that it will be superceded by a bacterium whose requirements make it better equipped to exist in that changed environment. This is the benefit of a

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wide bacterial analysis in pollution monitoring. However, bacterial identification is time-consuming, and it is impractical to identify all the bacterial species in a monitoring procedure.

In this programme, apart from the coliform analyses, part of the aerobic, heterotrophic bacterial population was cultured in order to attempt to relate the structure of this population to that of the coliform counts. The total, aerobic, heterotrphic bacterial count was obtained by culturing the bacterial samples on a suitable nutrient media. A number of randomly chosen colonies were sub-cultured, obtained in pure culture and subjected to a number of bacteriological tests (physical, chemical and biochemical) based largely on the determinative scheme for the identification of certain genera of Gram-negative bacteria as designed by Shewan et al (1960) and incorporating the vibriostat test described by Shewan et al (1954).

MATERIALS AND METHODS

6.1 SAMPLING

Samples for bacteriological analysis were drawn using the glass samplers described in 4.2.

Prior to steam sterilisation at 121 C for 15 minutes the stopcocks on the samplers were opened, a few drops of distilled water were injected into each sampler the open ends of which were then plugged with non-absorbent cotton wool and each end enclosed with aluminium foil. After sterilisation, the stopcocks were closed. The practice of adding a sodium thiosulphate crystal to the sampler (State Health Dept., pers. comm.) in instances where sample waters contain residual chlorine was not adhered to. It would have been difficult with resultant contamination of the sample a strong possibility to introduce a crystal or sterilised solution of sodium thiosulphate into the sampler after it had been sterilised. Furthermore, as the sewage was untreated, the residual chlorine in the waste-water would likely have been neutralised by the organic sewage fraction.

A foam cool box was used to transport the samplers between the laboratory and the sampling sites.

Prior to the samplers being used they were cooled in a refrigerator overnight. Samples were drawn by opening

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the stopcock at each end of the sampler after removing the aluminium caps and the cotton wool plugs. Contamination by surface bacteria when the samples were drawn at depth was minimised by the presence of the aluminium caps as these were only removed on reaching the sampling depth. On being returned to the laboratory, the ends of the glass samplers were rinsed with sterile, distilled water before the sampler contents was drained into a pre-sterilised, screw-topped glass bottle.

The samples were always drawn behind the surf zone apart from those taken at A where, at times, depending on the weather conditions, samples were drawn in the surf zone. During the initial experiments, single samples were taken, but subsequently duplicate samples were taken. The time elapsed between the first sample being drawn and dilution in the laboratory was never more than 2 hours.

A disadvantage of the samplers is that all the air is excluded from them when a sample is drawn which could result in lowered bacterial counts. Particularly at the site of high organic loading, dissolved oxygen depletion within the sample during transportation could well increase bacterial die-off in the sample. For this reason samples were always drawn last at A and the elapsed time between sampling and dilution was never more than 45 minutes. Later in the programme when only

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surface samples were taken, sterile, screw-topped glass bottles in which an air-filled headspace was maintained were often used.

6.2 DILUTION

Serial dilutions were made in pre-sterilized, pre-cooled, aged sea-water contained in screw-topped, glass bottles. Serial decimal dilutions were made by transferring 10 ml or 20 ml of the sample or diluted sample into a subsequent bottle containing 90 ml or 180 ml respectively using 10 ml pipettes which had been sterilized in an oven at 180 C for 3 hours.

Accepted aseptic techniques were followed while conducting the dilutions, although, in this instance the likelihood of airborne coliforms or halotolerant bacteria contaminating the samples must be small.

6.3 FILTERING AND CULTURING

The filtering of the samples prior to culture followed a standard procedure. The membrane filter technique used was principally the same as that employed by the Natal Regional Laboratory of the NIWR of the CSIR.

The following membrane filter procedure was adopted : A stainless steel Millipore 100 ml filter-holder was used in conjunction with a 500 ml filter flask. Vacuum was drawn via a trap and cotton wool filter using an

Edwards / .....

Edwards High Vacuum Pump. The filters used were pre-sterilized, 47 mm, black-gridded, 0,45 µ S-pak Millipore filters in conjunction with pre-sterilized absorbent pads dispensed via Millipore pad dispensers. Where sea-water Nutrient Agar plates were used, the filters were laid directly onto the plates. Filters were at all times handled using Millipore forceps sterilized between each filter handling by flaming the tips after these had been dipped in alcohol.

Twenty-five ml aliquots of the dilutions were filtered for culture in each instance, the aliquot obtained using a sterile syringe. In each particular dilution series, the highest dilutions were filtered first, sequentially until the lowest dilution was finally filtered. By this means one syringe was used during a particular dilution series, the syringe merely being flushed with liquid from a lower dilution before drawing that aliquot. Similarly, the filter-holder was neither rinsed nor re-sterilized during one series. This procedure saved both time and equipment and was rationalised by the argument that contamination between filtrations was negligible as at least ten times lower dilutions were filtered sequentially and this would negate the contamination from a higher dilution.

For a different dilution series, either a sterile 50 ml filter apparatus was used or the 100 ml filter-holder was re-used after sterilising it by coating it internally with alcohol and then flaming it, followed by

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subsequent cooling of the filter-holder by flushing it internally with sterile, distilled water.

Dilutions were filtered which from experience were known to result in 20-200 colonies growing on the filter after incubation. Generally it was sought to have 20-80 colonies per filter. Duplicates of the dilutions filtered were obtained. Apart from the aliquot dispensing, usual aseptic precautions were adopted.

6.3.1 Coliform Culture

The dilutions used in coliform culture, the methods of identification and the media used were largely the same as those of the Natal Regional Laboratory of the NIWR of the CSIR.

6.3.1.1 Total Coliform Counts

Total coliform counts were obtained by filtering a sample as described in 6.3. The bacteria retained on the filter were cultured by initially placing the filter on a sterile Millipore absorbent pad contained in a sterile, glass petri dish and saturated with sterile Oxoid Resuscitation Membrane Broth which had been dispensed using a sterile pipette. The filters were incubated in an incubator for 1 hour at 37 C. The absorbent pads were saturated with the Resuscitation Membrane Broth prior to the samples being drawn. Thereafter the filters were transferred to sterile

nutrient / .....

nutrient pads impregnated with sterile Oxoid MacConkey Membrane Broth and re-incubated for a further 17-19 hours at 37 C. After incubation the filters were examined using a stereo dissection microscope (10x magnification) and all yellow colonies were recorded as total coliforms. The total coliform count was adjusted to a count per 100 ml of the sea-water sample, allowing for the dilution factor.

6.3.1.2 Presumptive E.coli Counts

The procedure to obtain presumptive E.coli counts is similar to that of the total coliform count. In this instance, however, the filters were first placed on Membrane Resuscitation Broth impregnated pads for 2 hours before being transferred to MacConkey Membrane Broth pads. These petri dishes were then placed in weighted, heavy duty, polyethylene bags, the air expelled from the bags, the bags sealed and placed in a water-bath sufficiently deep to cover the bags and incubated for a further 16-18 hours at 44 C. At the end of the incubation period, the filters were examined (10x magnification) and all yellow colonies were recorded as presumptive E.coli bacteria per 100 ml of sea-water sample after the necessary adjustments had been made.

6.3.1.3 Confirmatory E.coli Type I tests

Although serotyping is a useful means of coliform identification, it is expensive and the requisite sera

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are not always readily available in South Africa. For this reason and the fact that the confirmatory tests necessary in this instance were easily executed, established techniques were used.

Randomly selected yellow colonies growing on MacConkey Membrane Broth at 44 C were sub-cultured to Difco 1% Bacto-Tryptone Broth to ascertain indole production using Kovacs test reagent and for growth in Difco Koser's Citrate Medium (Society of American Bacteriologists, 1957).

The bacteria were differentiated by these tests as follows :

Indole Production	Growth in Koser's Citrate Medium	
+	-	E.coli Type I, faecal origin
-	-	E.coli Irregular Type II, origin unknown
-	+	E.coli Irregular Type VI, and paper origin

The typed E.coli bacteria were compared to the original presumptive E.coli count to obtain the relative counts of each type. A Gram stain reaction (Society of American Bacteriologists, 1957) was the only other confirmatory test conducted. It was found that almost all the presumptive E.coli bacteria sub-cultured were categorised as E.coli Type I bacteria. These confirmatory tests were conducted on three occasions during

the / .....

the study.

6.3.2 Culture of Aerobic, Heterotrophic Bacteria

Samples for what is termed the total bacterial count were diluted and filtered as described in 6.2 and 6.3. The term 'total bacterial count' is a misnomer as the bacteria cultured on the medium used represent only a portion of the non-sporeforming, aerobic heterotrophs and exclude for example anaerobes and specific bacteria such as the sulphur bacteria.

The total bacterial count was obtained by counting all the colonies growing on a filter placed on ZoBell's 2216 Medium (ZoBell, 1946). This medium was later replaced by Difco 2216 Marine Agar. The filters were not placed on nutrient pads, but on sterilised agar medium in pre-sterilized, disposable petri dishes (BTL plastic pre-sterilized, disposable, petri dishes, 100 mm diameter). These agar plates were prepared before each sampling cycle. Incubation temperature for the samples was initially 15 C, but due to circumstances this was changed to room temperature (20 C-23 C). This temperature was within the range of ZoBell's (1946) stated optimum incubation temperature for maximum counts.

Total counts were usually made after 5-7 days incubation. In some instances, however, incubation periods were reduced as certain bacteria swarmed across the plates

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and masked any other growth. Incubation periods longer than 7 days had little effect on the total count.

The vibriostat compound O/129 used in the antibiotic tests was a pteridine compound, 6:7 di-isopropylpteridine, described by Collier et al (1950).

The O/129 sensitivity discs were made by aseptically dissolving the vibriostat in acetone to make a 1% solution and by applying 0,2 ml of this solution to each disc.

RESULTS

7.1 VERTICAL BACTERIAL DISTRIBUTION

Vertical distribution of the bacteria in the water column at Hottentotshuisie was determined in March, April and May 1975 (by total counts of the bacteria on ZoBell's nutrient agar). The results are given in Table III below:

TABLE III

Vertical Distribution of Bacteria at Hottentotshuisie

Sampling Period 1975	Total Bacterial Count		Temperature, C	
	Surface	Bottom	Surface	Bottom
March	226	220	13,7	11,0
April	540	472	11,0	9,8
May	1960	1280	13,0	13,0

The samples taken in March and April were drawn at a water depth of 12 m and those in May at a water depth of 4-7 m. Despite the consistently higher counts obtained in the surface samples, it was decided that the variation was sufficiently small that the influence of vertical distribution on the total bacterial count could be discounted. It is interesting to note that the presence of a thermocline in March and April did not affect the vertical distribution of the bacteria. The surface samples taken in May showed coliform counts of 600 coliforms/100 ml, but they were absent in the bottom sample counts.

7.2 COMPARATIVE / ....

7.2 COMPARATIVE SAMPLING AT A, B and C

Samples for bacterial analysis were drawn at A, B and C on five occasions, namely, 17/10/75, 4/11/75, 16/11/75, 20/1/76 and 28/1/76. Apart from the bacterial sampling, DO, temperature and pH were measured in situ or on-shore on each sampling occasion at each site using a YSI Model 57 DO meter, thermometer and/or a YSI DO meter and a Fischer Accumet Model 150 portable pH meter.

7.2.1 Non-biological Measurements

The results of the DO, temperature and pH measurements are given in Table IV below.

TABLE IV

DO, Temperature and pH at A, B and C

(a) : DO (in ppm) at A, B and C

Date	A	B	C
17/10/75			
4/11/75	10,4	10,4	10,5
16/11/75	10,4	10,4	10,4
20/ 1/76	10,0	10,4	10,4
28/ 1/76	9,6	10,0	10,0

(b) : Temperature / ...

(b) : Temperature (in C) at A, B and C

Date	A	B	C
17/10/75	14,0	13,0	15,0
4/11/75	11,0	11,0	11,0
16/11/75	13,5	13,0	13,5
20/ 1/76	13,5	13,5	14,0
28/ 1/76	14,0	13,5	14,5

(c) : pH at A, B and C

Date	A	B	C
17/10/75	7,6	7,3	7,3
4/11/75	7,4	7,4	7,4
16/11/75	7,4	7,3	7,4
20/ 1/76	7,5	7,4	7,4
28/ 1/76	7,3	7,2	7,4

Apart from a DO at A on 28/1/76 of 9,6 ppm, all the other measurements are within accepted limits of a good quality water. A DO of less than 10 ppm is generally considered suspect, but 9,6 ppm is not so low as to cause concern.

7.2.2 Bacterial Counts

The bacterial counts conducted were coliform, presumptive E.coli and total counts as previously described in Section 6.

The results obtained are given in Tables V, VI and VII. Confirmatory E.coli type I tests were not routinely

carried / .....

carried out, but when these tests as described in Section 6.3.1.2.1 were applied to organisms isolated at sites A and B, almost all the isolates were confirmed as E.coli type I bacteria.

TABLE V

Coliform Bacteria / 100 ml Sea-water

Date	A	B	C
17/10/75	280	5 000	0
4/11/75	2 120	1 360	160
16/11/75	400	400	0
20/ 1/76	1 910	1 200	40
28/ 1/76	80	250	0

TABLE VI

Presumptive E.coli Bacteria / 100 ml Sea-water

Date	A	B	C
17/10/75	72	1 080	0
4/11/75	992	476	40
16/11/75			
20/ 1/76	440	232	12
28/ 1/76	20	66	0

TABLE VII / ....

TABLE VII

Total Bacterial Count / ml Sea-water

Date	A	B	C
17/10/75	240	320	140
4/11/75	2 800	2 300	280
16/11/75	3 800	2 200	580
20/ 1/76	2 760	2 140	706
28/ 1/76	1 280	2 310	160

These results show a wide variation in the counts between the polluted and reference stations. The variation in the counts between A and B is most likely explained by the localised current patterns which can occur on any particular day. The counts at A and B in most cases are, however, very similar, and are markedly higher than those at C.

That the coliform counts and particularly the presumptive E.coli counts at A and B are so similar appears to be in conflict with previous work (Breyer-Menke, unpublished) conducted in Table Bay which showed a rapid die-off or dilution of these organisms at distances of 100 m and 200 m from the mouth of the coliform source. However, the residence time of the coliform bacteria in the marine environment at different sites is unknown. Also the coliforms in Table Bay would have been exposed to sunlight for some time before arriving in Table Bay. Finally, nutrient enrichment of the sea

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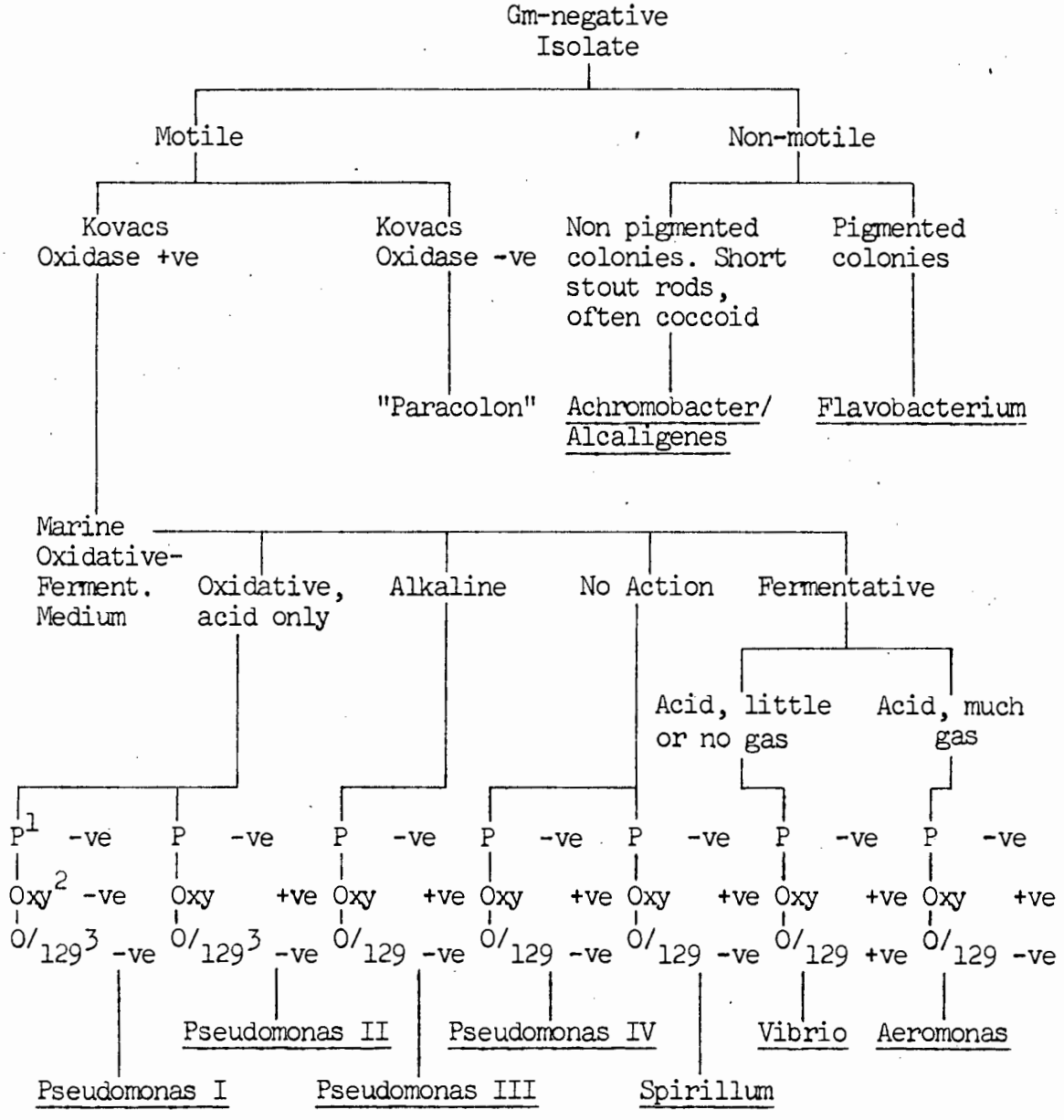
at the Table Bay station would be insufficient to benefit the bacteria.

Randomly chosen colonies from the total count plates were picked off the plates and streaked onto ZoBell's Nutrient Agar or Difco 2216 Marine Agar. Isolates were sub-cultured at least three times to obtain a pure culture. Amongst the flavobacteria in particular a peculiar phenomenon was a persistent association with cocci. These extremely small cocci could not always be satisfactorily stained and it was very difficult to eradicate them from the Flavobacterium colonies. It is possible that these contaminants persisted in certain instances of 'pure' flavobacteria cultures. Subsequently, staining showed these cocci to be Gram-positive bacteria, and they were thought to belong to the genus Micrococcus.

Having obtained randomly selected pure cultures from the total count plates, the cultures were classified according to the hierarchical classification scheme proposed by Shewan et al (1960) for the identification of certain Gram-negative bacteria, especially the Pseudomonadaceae.

7.3 IDENTIFICATION OF TOTAL COUNT ISOLATES

The scheme of Shewan et al (1960) was largely applied as follows :-



1 Penicillin

2 Oxytetracycline

3 Vibriostat O/129

In their tests Shewan et al (1960) used Penicillin with an activity of 2,5 I.U., Terramycin of 10 µg per disc

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and Shewan et al (1954) Streptomycin at 80 µg per disc and Chloramphenicol at 100 µg per disc. Colwell and Wiebe (1970) recommend the use of penicillin of 10 I.U., Tetracycline at 30 µg per disc and Dihydrostreptomycin at 10 µg per disc. In this study, apart from the vibriostatic agent, compound 0/129, the following antibiotic discs available from Mast Laboratories were used :

Penicillin G	4 I.U. per disc
Oxytetracycline	25 µg per disc
Streptomycin	25 µg per disc
Chloramphenicol	10 µg per disc

The scheme as applied in this programme varies from that described by Shewan as follows :

1) The presence and position of flagella was not examined. The flagella staining technique is time consuming and flagella stains are often misinterpreted (Hodgkiss, 1960). The electron microscope is not suitable for routine use.

2) Terramycin as used by Shewan et al (1960) was not available and was replaced by Oxytetracycline. Although Oxytetracycline has the same chemical formulation as Terramycin, dissimilar results were obtained. For this reason, the Oxytetracycline test has been ignored, and Pseudomonas I and Pseudomonas II grouped together.

3) Chloramphenicol / ....

3) Chloramphenicol and Streptomycin were included in the antibiotic tests as described by Shewan et al (1954) and Colwell and Wiebe (1970). These antibiotics proved of little use in the classification scheme, but had been included because the number of Gram-positive and other Bacteriaceae that would be isolated was unknown, and also to ascertain whether any of the isolates, 'Paracolons' in particular, showed multiple drug resistance.

Multiple drug resistance was recorded in three isolates, namely a Flavobacterium and two Pseudomonas I or II organisms. It is thought likely that the seeding of the plates in these instances were unsuccessful and that these results are erroneous. A few other less marked but unexpected antibiotic test results were found probably because great sensitivity of certain isolates to one or more antibiotics masked their reactions to other antibiotics. This may also have been true for some of the Oxytetracycline reactions.

The organisms classified according to the hierarchical classification scheme on each sampling occasion are listed in Appendix 3. Table VIII gives the number of bacteria in each genus or group as a proportion of the total count.

TABLE VIII / .....

TABLE VIII

Genera as Proportions of the Total Count

(a) Bacteria at A

Bacteria	Bacteria / ml 17/10/75	4/11/75	16/11/75	20/1/76	28/1/76
1. Flavobacterium			1003(25%)	690(26%)	256(20%)
2. Achromobacter/ Alcaligenes		431(15%)		230(8%)	
3. 'Paracolon'	80(33%)	215(9%)	1432(38%)		597(47%)
4. Pseudomonas I or II	53(22%)		1003(25%)	1150(42%)	171(13%)
5. Pseudomonas III	27(12%)				
6. Pseudomonas IV		431(15%)	281(6%)		
7. Vibrio	80(33%)	646(23%)	281(6%)		
8. Aeromonas		646(23%)			
9. Spirillum		431(15%)		230(8%)	
10. Gram-positive				460(16%)	256(20%)
TOTAL COUNT	240	2800	3800	2760	1280

(b) Bacteria / ....

(b) Bacteria at B

Bacteria	Bacteria / ml 17/10/75	4/11/75	16/11/75	20/1/76	28/1/76
1. Flavobacterium	16(5%)		560(25%)	458(21%)	462(20%)
2. Achromobacter/ Alcaligenes	46(14%)			186(9%)	308(13%)
3. 'Paracolon'	46(14%)		210(10%)		
4. Pseudomonas I or II	120(38%)	256(11%)	1220(55%)	1310(61%)	1386(60%)
5. Pseudomonas III					
6. Pseudomonas IV	16(5%)	767(34%)		186(9%)	
7. Vibrio	76(24%)	511(22%)			
8. Aeromonas		511(22%)			
9. Spirillum		255(11%)	210(10%)		154(7%)
10. Gram-positive					
TOTAL COUNT	320	2300	2200	2140	2310

(c) Bacteria at C

Bacteria	Bacteria / ml 17/10/75	4/11/75	16/11/75	20/1/76	28/1/76
1. Flavobacterium			49(8%)	152(22%)	62(39%)
2. Achromobacter/ Alcaligenes	18(13%)	17(6%)	49(8%)	50(7%)	24(15%)
3. 'Paracolon'		70(25%)			
4. Pseudomonas I or II	88(63%)	140(51%)	193(33%)	454(64%)	62(39%)
5. Pseudomonas III		18(6%)	48(9%)		
6. Pseudomonas IV		18(6%)	97(17%)	50(7%)	12(7%)
7. Vibrio	17(12%)		96(17%)		
8. Aeromonas		17(6%)			
9. Spirillum	17(12%)		48(8%)		
10. Gram-positive					
TOTAL COUNT	140	280	580	706	160

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The data in TAble VIII is summarized in Fig. 5. The groups in Fig. 5 are listed in the following sequence in each time period :

Column

1	Flavobacterium
2	Achromobacter/Alcaligenes
3	'Paracolon'
4	Pseudomonas I or II
5	Pseudomonas III
6	Pseudomonas IV
7	Vibrio
8	Aeromonas
9	Spirillum
10	Gram-positive bacterium

Although this form of presentation is initially confusing it has the following merits :

(i) the data is presented concisely

(ii) this presentation allows comparison of the complete community structure at each sampling station at each time period (this point is stressed in the discussion on p. 57)

(iii) the graph indicates the relative abundance of the organisms at each sampling station and one can observe the relationship between abundance and diversity (this is further emphasized in Fig. 6 and the significance is discussed on p.53-55).

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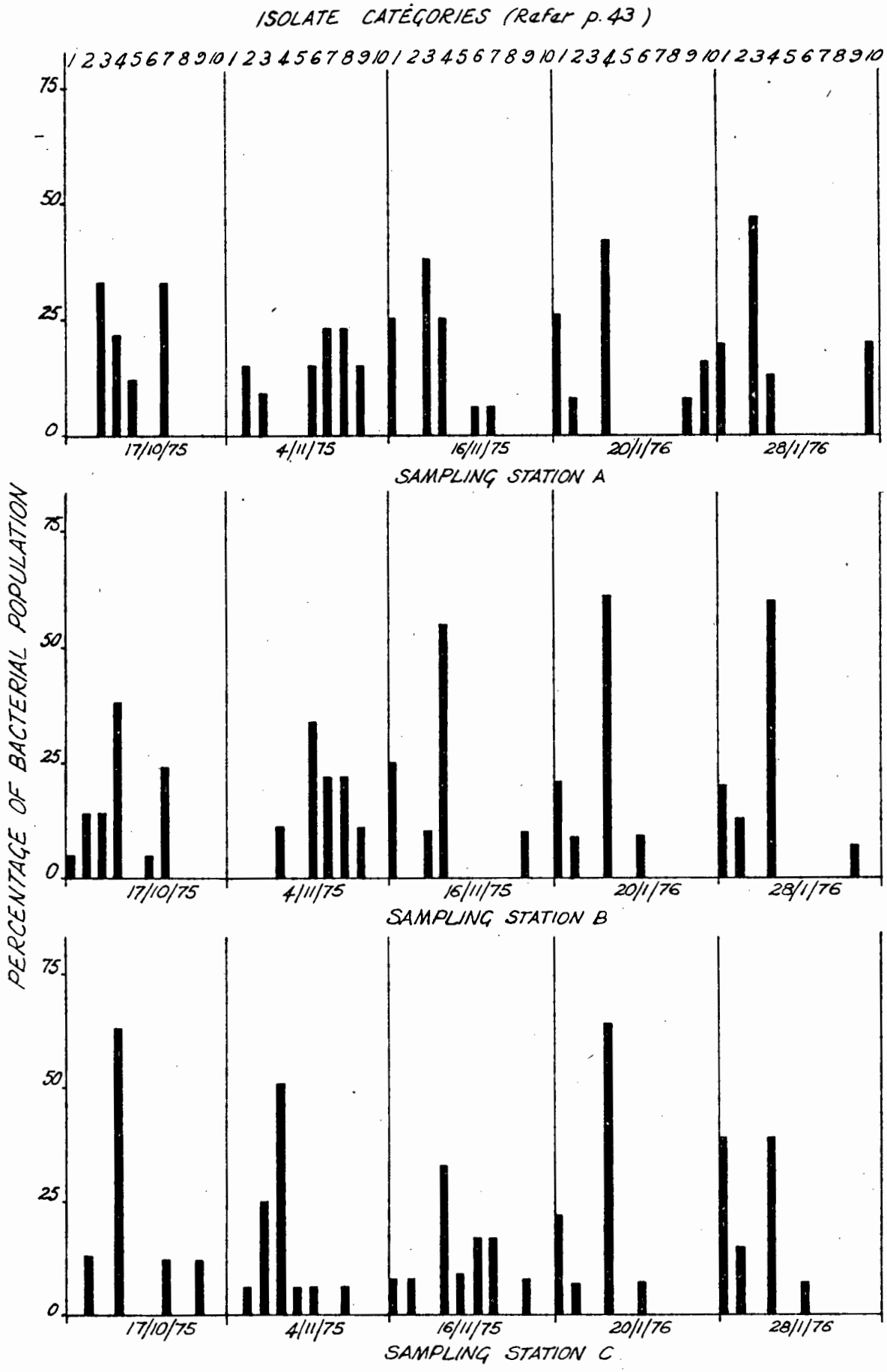

FIG. 5 : THE GENERA AT A, B AND C AS A PERCENTAGE OF THE TOTAL COUNT.

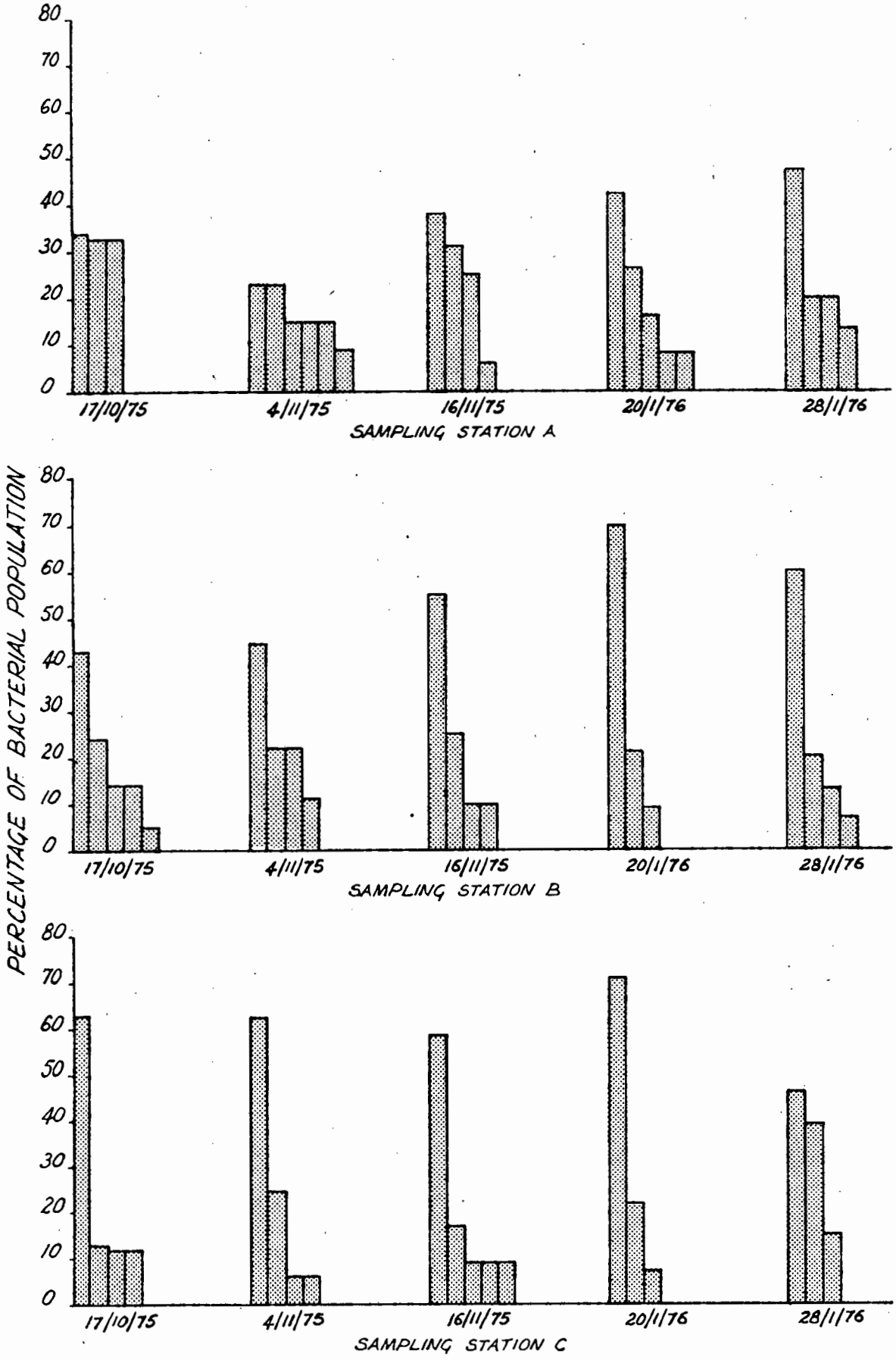

FIG. 6 : BARCHART OF BACTERIAL POPULATION AND DISTRIBUTION.

The disadvantage of this presentation is that it is not easy to see fluctuations of the organisms or groups with time. Changes taking place with time are shown in Fig. 7 where each genus or group is presented separately. By including results from A, B and C together in one sequence, changes in time which tend to be obscured if the stations are presented separately, are apparent.

Flavobacteria were present on the last three sampling occasions and absent or rare on the first two sampling dates. Gram-positive organisms were also recorded on the last two sampling dates. In contrast, Vibrio were identified on the first three and Aeromonas on the second sampling dates. Other organisms or groups either did not occur regularly or did not show clear trends. The existence of these trends in community structure with time emphasize the need for comparison between a polluted and a reference station at the same time to avoid confusion with seasonal or other time changes.

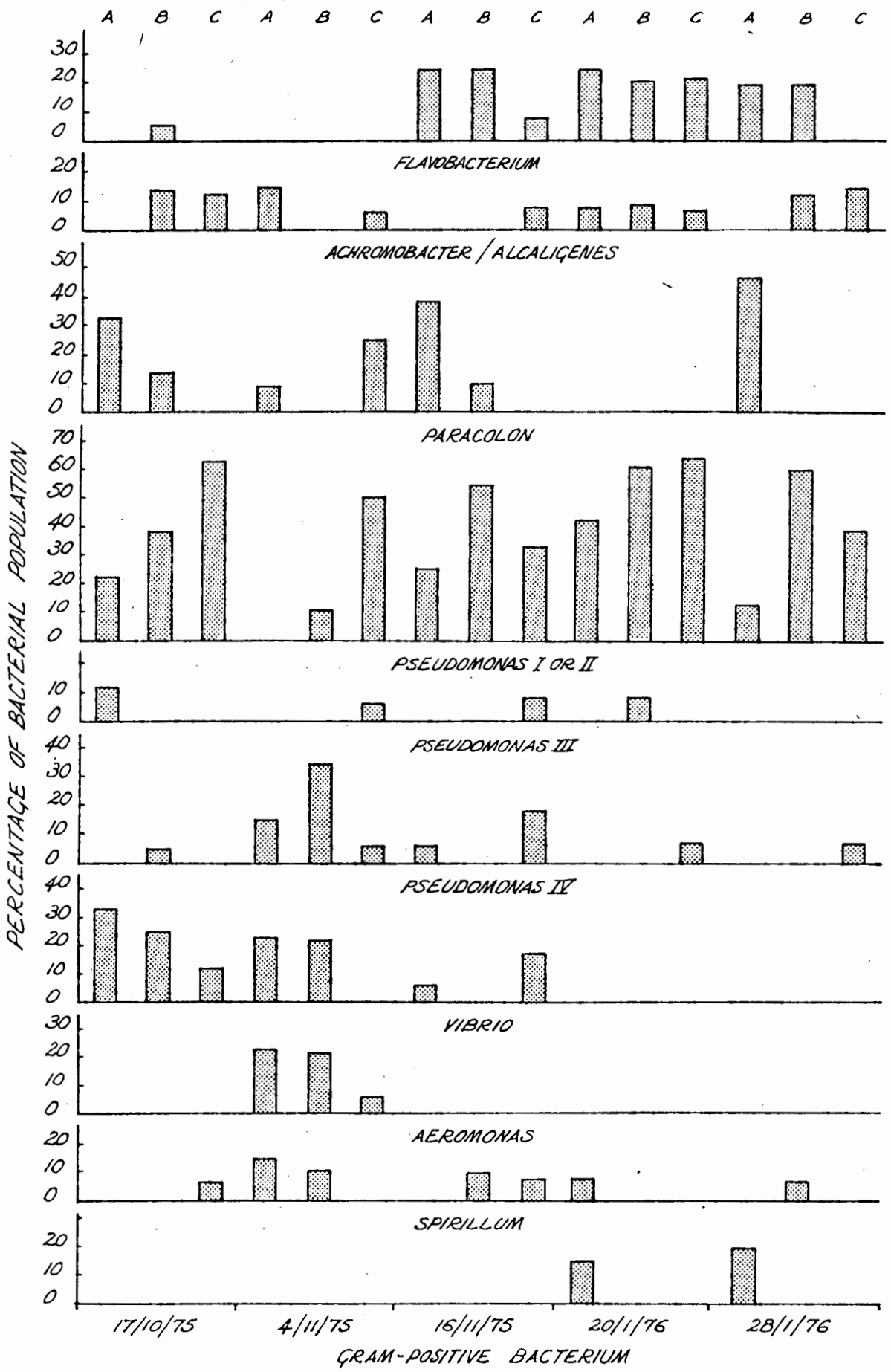

FIG. 7 : THE GENERA AT A, B AND C AS A PERCENTAGE OF THE TOTAL COUNT.

DISCUSSION

8.1 THE MEMBRANE FILTER TECHNIQUE FOR THE ENUMERATION OF MARINE BACTERIA

A number of techniques have been used for the enumeration of bacteria in sea-water. These are principally of two types, direct microscopic cell counts and colony culture methods.

Direct microscopic cell counts may be done by means of a Petroff-Hauser counting chamber, but unfortunately these are no longer available in South Africa. Another technique (Shanahan et al, 1955 and Jannasch and Jones, 1959) involves concentrating the bacteria on a membrane filter, staining this with a suitable dye and direct microscopic observation of the filter which is mounted on a slide using an oil with a suitable refractive index. In this study it was felt that confusion of bacteria with particulate material could occur, especially as bacteria tend to adhere to particulates. The great amount of particulate material found at the polluted sites could lead to large inaccuracies. Furthermore, this technique does not differentiate between living and dead bacteria. In recent years, however, vital stains have successfully been used in staining bacteria from a marine environment. Hobbie et al (1972), Francisco et al (1973), Jones (1974) and Daley and Hobbie (1975) have stained formalin-fixed slides with acridine orange

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which causes living cells to fluoresce when seen in ultra-violet light.

Culture counting techniques indicate only those bacteria capable of growing on the enumeration medium used. The most common bacterial enumeration methods which rely on cell multiplication are the most-probable-number method (Gunkel and Rheinheimer, 1972), the pour plate, the spread plate (Buck and Cleverdon, 1960) and the membrane filter technique (Oppenheimer, 1952). Of these, the membrane filter technique has been widely used for the enumeration of coliforms in aquatic systems by Taylor et al (1972), Dufoux and Cabelli (1974) and Presnell and Andrews (1976). This technique of concentrating the bacteria onto a filter is equally applicable to total counts and was the favoured enumeration method throughout the programme. Seki (1973) described a solid medium incorporating silica gel instead of agar to prevent agar-decomposing bacteria from liquefying the plates. In this study a number of flavobacteria were found to be agar digesters. This did not, however, interfere with the laboratory work, and agar was used as the solidifying medium.

Schaeffer et al (1974) found that higher total coliform recoveries were obtained using Gelman rather than Millipore membrane filters although no differences between the filters were found in faecal coliform enumeration. The reasons for these differences are as yet unclear, and in this study Millipore filters were used.

8.2 THE / ....

8.2 THE EFFECT OF DO, TEMPERATURE AND PH ON THE BACTERIAL COUNTS

DO, temperature and pH data recorded in Table IV show that these parameters vary little between the sites, and it is unlikely that they would have affected the bacterial distribution or the total counts at the three sites. The DO of 9,6 ppm recorded at A on 28/1/76 is, however, slightly low and could possibly explain the reduced counts at A on 28/1/77, although further investigation would be needed to verify this.

It is interesting that the DO, temperature and pH results at the three sites are so similar. The loading at A appears to be sufficiently small that the dilution affect of the sea negates DO, temperature and pH variances that might be expected between A, B and C. This despite the fact that the sampling site A was close to the sewage outfall. The relatively heavy wave action at A would, however, greatly assist the mixing of the sewage effluent into the sea-water.

In this study, ZoBell (1957) found that temperature seems to have only an indirect effect on the bacteria in the sea, although Sieburth (1967) found that the growth range of natural bacterial populations in an estuary varied with water temperature. However, there was no apparent suppression or enhancement of any taxonomic group due to temperature.

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A physical parameter that is believed to profoundly affect the bacterial counts is wave action. This premise is supported by Mazure (1976). The turbulence caused by strong wave action would serve to mobilise sediment particles into the water column and towards the water surface. This would increase the bacterial load in the water as bacteria tend to adhere to particulate matter and would likely increase bacterial counts even via culture techniques.

Visual observation of the sea on the sampling days and experiences during actual sampling periods give rise to this opinion. The sea was very still on 17/10/75, whereas a heavy sea was running on 4/11/75 and a strong surge was felt on the sea bottom. The appearance of the sea on 16/11/75 would seem to belie the supposition that increased wave action increases the bacterial count, because although high bacterial counts were obtained the sea was very calm. However, evidence of turbulent sea conditions a short time before sampling on 16/11/75 was indicated by the white foam on the sea, the poor visibility in the water and the large number of broken kelp fronds suspended in the water column. Similarly, a strong swell was running on 20/1/76, but sea conditions were quiet on 28/1/76.

8.3 COLIFORM COUNTS AT A, B AND C

Gameson et al (1973) found that the rate of disappearance of sewage coliform bacteria in the sea was generally

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much faster than could be accounted for either by the effects of physical dilution or the mortality that was found to occur in sea-water samples incubated in the dark. These workers concluded that bacteria were inactivated by ultra-violet radiation. The effect of this radiation on the bacteria was ameliorated depending on the degree of the turbidity of the water. There are, however, many variables in the natural aquatic environment, and McFeters et al (1974) caution that water quality, phage and competing or predatory organisms may also influence bacterial survival. Vind et al (1975) conducted in situ E.coli survival studies in the sea and concluded from their results that E.coli are very sensitive to sunlight. Unless protected inside a grease ball or faecal mass, E.coli discharged near the open surface would perish within a few days. To add to the multiple factors affecting coliform survival, Bissonette et al (1974) conclude that E.coli survival rates from streams would be greater if the isolates were cultured on nutritionally rich, non-selective media and that selective media inhibited multiplication of E.coli cells injured during exposure in natural waters.

It is therefore apparent that a number of unmeasured influences could affect the counts obtained at A, B and C. From the foregoing, it would appear that coliforms isolated at A and to a lesser extent at B would be better protected against injury or inactivation than those coliforms isolated at C. Particularly at A the effects

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of sunlight would be minimised due to the turbidity of the water and bacterial cells might persist through incorporation in faecal material. Also the nutrient enriched water at A would encourage cell multiplication.

As expected, the coliform counts and presumptive E.coli counts were generally greater at A than at B and both these sites gave higher counts than C. The coliforms and presumptive E.coli isolated at C probably resulted from faecal contamination from a nearby construction camp rather than being transported from A. That greater coliform and presumptive E.coli counts were obtained at B rather than A on 17/10/75 and 28/1/76 could possibly be ascribed to a local current regime which could transport the sewage in a circular pattern around Bakoven Bay and deliver it first to B before reaching A.

The total coliform and presumptive E.coli counts obtained at A, B and C confirm that coliform analysis remains an eminently suitable means of identifying faecal contamination of sea-water.

8.4 AEROBIC, HETEROTROPHIC BACTERIA AT A, B AND C (Refer Fig. 5, 6, 7 and 8)

This study showed a high number of Gram-negative, rod-shaped bacteria in relation to the other isolates in plate counts on marine nutrient media. This is in accordance with the findings of ZoBell (1946), Ingram

and / .....

and Shewan (1960) and Shewan et al (1960) although in the last instance isolates were obtained from fresh and spoiling white fish. MacLeod (1965) found that most of these Gram-negative bacteria were motile as was the case in the present study. Murchelano and Brown (1970) in a study of the heterotrophic bacteria in Long Island Sound found in contrast that non-motile bacteria were more abundant than motile forms unless examined using phase contrast microscopy as was done in the present study. Murchelano and Brown (1970) found that over 90% of population comprised the genera Pseudomonas, Flavobacterium and Achromobacter. In the present study although such high percentage representation by these genera did occur in the total count, the average was not as high as 90%. The average percentage representation of the three genera in the total heterotrophic population in this study was nevertheless of interest. At A it was 48%, 76% at B and 84% at C. The effect of this can be seen in the bar chart (Fig. 6). In this bar chart because of the usually overwhelming preponderance of Pseudomonas I or II group over the other pseudomonads, they have all been placed into a single Pseudomonas group. In this bar chart, the relative numbers of each group as a percentage of the total population at each site are given. Unlike in Fig. 5, in this case the group with largest percentage population representation has been placed in the first column, the second largest group adjacent to it and so on.

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As this bar chart is a function of both the number of genera or groups at each site and the number of individuals per genus, it is more complex than the bar charts of both Crossman et al (1974) and those in a study directed by Cairns and Dickson (1973) which compare either the number of taxa or the density of organisms at any site. The bar chart in this study because of the lesser number of groups in the population is not as elaborate as the histograms designed by Patrick and described by Cairns et al (1973). Patrick's histograms uitilise a diatom community structure to describe stream pollution level.

A number of interesting points emerge from this bar chart. Bar graphs described by Cairns (1967) are used to characterize different degrees of stream pollution by means of the community structure of the aquatic stations. Relating the bar charts of Cairns (1967) to those in Fig. 6, the horizontal width of the bacterial bar charts are a function of the number of bacterial genera or groups and give an indication of the diversity of the population. The greater the horizontal spread, the greater the diversity. The bacteria in this study substitute for the wide range of aquatic organisms studied by Cairns which he placed into classes and represented in his bar charts. Applying Cairns' interpretation of his own bar graphs to those in Fig. 6, A would be considered a healthy to semi-healthy station and C, which in comparison to A has fewer genera and a

greater / .....

greater variation in the number of individuals in each genus, a polluted site. This result is based on the argument that most forms of stress (or pollution) cause a reduction in the complexity of the system, a simplification. The analysis of the bacterial population in Fig. 6 shows that A is a more complex and therefor apparently healthier site than C. The differences between A and C are, however, small and it must be noted that the total counts were far higher at A and significantly higher at B than at C. There is therefore an increase in diversity from A to B to C. This is demonstrated in Table IX. This table uses the diversity index of Menhinick (1964), $d = \frac{S}{N}$, where d is the diversity index, S the number of groups isolated and N the total count.

TABLE IX

Bacterial Diversity Indices at A, B and C

	Diversity Index					
Station	17/10/75	4/11/75	16/11/75	20/1/76	28/1/76	Mean
A	0,26	0,11	0,08	0,10	0,11	0,13
B	0,34	0,10	0,09	0,09	0,08	0,14
C	0,34	0,36	0,29	0,15	0,32	0,29

A number of workers have indicated the effects of nutrient limitation on bacterial numbers. Sykes (1973) has described the effect of nutrient limitation on the specific growth rate. Organic nutrients have been

described / .....

described as being important by Gundersen et al (1972) to the distribution of bacteria and by Anson and Ware (1974) to the total number of viable aerobic bacteria. In a bibliography, ZoBell (1957) summarizes the work of Reuszer and notes that the organic content of sea-water and mud appears to be the most important factor affecting the abundance of bacteria. The total counts obtained at A, B and C support this last statement, and the presumptive evidence is that the organic enrichment at A and also B could be responsible for the differences in the structure of the bacterial populations at A, B and C.

Attention should also be drawn to the striking decrease in evenness in community structure appearing in the data from B and C (Fig. 6). The true diversity is probably greater than that appearing in the present results because the number of organisms that will grow on any particular medium is limited. As described earlier most workers have found an abundance of Gram-negative, rod-shaped, motile bacteria in their studies and it is generally accepted (Ferguson-Wood, 1965) that these bacteria form the largest percentage of the heterotrophic bacterial population (about 80%) in the ocean. In his studies ZoBell (1946) found that the medium used in this study gave the highest total count and the greatest diversity of species. However, certain bacteria such as the slow-growing alginolytic organisms and bacteria with specific nutrient requirements such as the

sulphur / .....

sulphur bacteria amongst a number of others would either be overgrown or would not grow at all on this medium.

Aprart from the result on 28/1/76, the total count at C rose markedly and steadily during the sampling period. The increase in the total count at C was also noticeable in relation to the fairly static counts at A and B, apart from the increase in the counts at these sites between 17/10/75 and 4/11/75. A possible explanation for this could be the result of increased availability of nutrients at C as a result of upwelling, a condition known to occur at C. Sorokin (1971) says that dissolved organic material made available by upwelling is the main source of external energy for additional bacterial production in tropical waters. Grindley and Taylor (1968) described the positive influence of upwelling on the occurrence of plankton blooms in False Bay.

From Fig. 5 and in particular Fig. 6, it is apparent that although there exists a continuous observable difference between the sites, particularly between A and C, the sites in terms of vertical and horizontal spread in Fig. 6 can only successfully be compared on a day to day basis. This means that each time a suspected pollution site is sampled, a sample must be drawn for the relevant reference site and similarly analysed before comparisons between the sites can be made and conclusions drawn.

For / .....

For the purposes of comparing the degree of pollution at different sites, the procedure followed in Fig. 6 of incorporating all the pseudomonads in a single group seems to be satisfactory. This would obviate the need for including the response of any bacterium to the antibiotics penicillin and terramycin and retain only the vibriostat 0/129 in the classification scheme. Also only the reaction of an isolate in the anaerobic marine oxidative-fermentative medium would have to be monitored. This simplified system would remove the subjectivity which is at times present in applying Shewan's classification scheme as can be seen in Appendix 3. The difficulties in classifying Gram-negative, rod-shaped bacteria are apparent in a paper by De Ley (1964) concerning Pseudomonas and related genera wherein he states that differences between strains are inconspicuous and numerous tests are required to reveal them, concluding that apart from other properties which should be known a definitive division of this group into genera requires the knowledge of the bacterium's DNA base composition.

One method of obviating the subjectivity which can enter into an hierarchical classification scheme is to employ numerical taxonomy based on Adansonian principles. Sneath (1962) described numerical taxonomy as having the advantages of yielding stable, precise taxonomies based on objective criteria. The tenets of Adansonian classification are that all possible

characters / .....

characters are tested for each strain, that each feature carries equal weight and that taxa are based on similarity (Sneath, 1957).

Colwell et al (1974) used numerical taxonomy for the classification of the Eubacteriaceae and Colwell and Wiebe (1970) developed a numerical taxonomy system for classifying aerobic, heterotrophic bacteria.

The advantages of numerical taxonomy are many, but its consideration for use in a rapid, routine monitoring procedure is less attractive. In order that a successful and stable division into taxa be achieved, numerical taxonomy demands that a large number of characters be measured. To do this is time-consuming and conflicts with the requirements of a rapid, routine monitoring procedure. Because of this, it was decided to maintain a hierarchical classification scheme in this study, and investigate the possibility of simplifying the Shewan scheme without influencing the bacteriological integrity required in this study.

The Pseudomonas group was the most numerically powerful group throughout the study and it was decided to review this numerical superiority to establish whether it was consistent. Fig. 8 shows graphs of the relative number of pseudomonads as a percentage of the total aerobic, heterotrophic count at A, B and C on each of the sampling occasions. As was apparent in Fig. 5, day by

day / .....

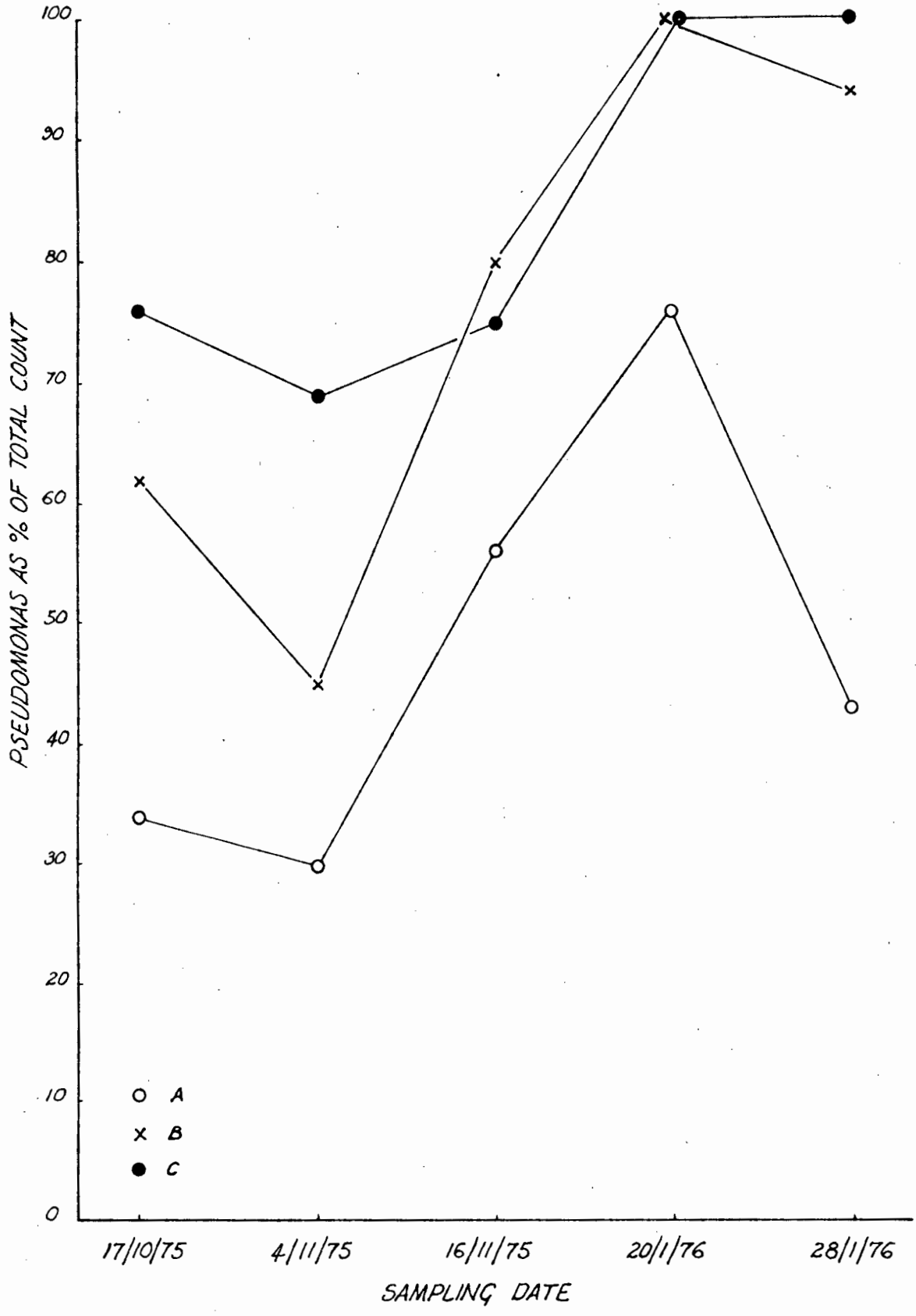


FIG. 8 : PSEUDOMONAS AS A PERCENTAGE OF THE TOTAL HETEROTROPHIC BACTERIAL POPULATION AT SAMPLING STATIONS A, B AND C

day comparisons between the reference station C and the polluted sites A and to a lesser extent B show distinct differences. This result suggests that in the case of sewage pollution in a marine environment, analysis of the pseudomonad population in relation to the total count between a suspected polluted site and a comparable but unpolluted reference site might indicate whether or not the suspected site is polluted.

The benefit that this would have on the laboratory manipulations would be very great. The only bacterial examinations that would have to be undertaken would be the Gram stain, motility test, the Kovacs oxidase test and the reaction in a marine oxidative-fermentative medium. It would also allow for a greater number of isolated cultures to be examined, thereby minimising the influence one incorrect classification could have on the number of bacteria in one group in relation to the population as a whole.

8.5 A COMPARISON OF THE COLIFORM AND HETEROTROPHIC BACTERIA AT A, B AND C

The total coliform count, the presumptive E.coli count and the total count were always higher at A and B than at C. This in itself shows the bacteriological difference between the water at Bakoven Bay and at Hottentotshuisie.

Uncertainty / .....

Uncertainty about the success of using coliform bacteria as indicators of faecal pollution persist. Nusbaum and Gaver (1955) in their study of coliform organisms in Pacific Ocean Coastal waters commented that the survival of enteric bacteria in sea-water is highly variable. Boyd et al (1972) conducted in situ studies of pure cultures of bacteria in a polar lagoon at a temperature range of 9,4 C. Boyd's study showed that indicator organisms died off rapidly, while as might be expected indigenous Pseudomonas species showed greater stability.

Smith et al (1973) quoted earlier work which showed that Salmonella densities could vary while faecal coliform counts were high. These workers nevertheless concluded that the faecal coliform concentration remains a useful method for making a qualified judgment of water quality. Womack et al (1973) undertook a comprehensive study of the survival of enteric pathogens and indicator organisms in natural waters, and their research showed the validity of using faecal coliforms as indicator organisms. An interesting statement by Grabow et al (1974) called for a reappraisal of water quality criteria. These workers maintained that due to the possibility of plasmid combination conferring drug resistance to coliforms, these bacteria could no longer be regarded as only indicator organisms, as the drug resistant R factors are transmissible among Gram-negative bacteria such as the enterobacteria.

In / .....

In this study the results have confirmed that either the total coliform or the presumptive E.coli count can be used as indicators of faecal pollution in sea-water. Similarly (Fig. 8), it has been shown that the percentage of Pseudomonas to the total count could equally be used as an indication of pollution. In cases of suspected sewage pollution, it is suggested that both indicator organisms and the Pseudomonas: total count ratio be monitored. It could be postulated that in cases of organic enrichment from sources other than sewage, a comparison of the Pseudomonas:total count ratio between the stressed site and a reference station might show meaningful differences. It would also be worthwhile to investigate the use of the Pseudomonas: total count ratio in stressed conditions resulting from causes other than nutrient enrichment. In a recent report, Cabelli et al (1976) when comparing Pseudomonas aeruginosa - faecal coliform relationships in estuarine and fresh recreational waters found that high Pseudomonas aeruginosa densities could be expected wherever recreational waters are subject to a combined nutrient discharge resulting from a textile finishing plant, private wastewater lines and several marinas. The data of Cabelli et al (1976) showed that Pseudomonas aeruginosa alone was a poor indicator of faecal pollution and therefore of itself could not be used as the basis of water standards for the prevention of enteric disease during the recreational use of surface waters. They suggest that although no

conclusive / .....

conclusive evidence exists of a correlation between coliform levels in recreational waters and the incidence of illness in populations bathing therein, the assay of faecal coliforms or some other indicator organism in conjunction with Psedomonas aeruginosa determinations could be of considerable value in the development of criteria for recreational waters.

CONCLUSION

The results of this study have shown that despite reservations concerning the coliform indicators of faecal pollution, the use of total coliform or presumptive E.coli counts as indicators of sewage pollution in a coastal marine environment has been validated. Therefore in instances of suspected marine contamination by sewage, these bacterial analyses could be employed.

It has also been shown that a wider bacterial population analysis, in this case using aerobic heterotrophic bacteria, is a possible means of analysing faecal pollution in the marine environment. In fact, in the case of marine faecal pollution, the Pseudomonas:total count ratio seems to be a satisfactory means of analysis and would be particularly suited to a routine monitoring programme. High numbers of Pseudomonas in relation to the total count appear to indicate unpolluted conditions. On the basis of the findings in this study, it is necessary that a site chosen for bacterial analysis be assessed in relation to a reference station.

Although the suitability of coliform indicator organisms for marine faecal contamination has been indicated the inclusion of an easily executed heterotrophic bacterial analysis when sewage pollution is suspected would be an advantage. The coliform tests would immediately confirm sewage pollution if this was the cause of the stressed environment. However, a stressed coastal marine environment might be identified using a

method / .....

method of heterotrophic bacterial analysis the pollution was due to some other cause, or at least if it resulted from organic enrichment other than from a sewage source. The results also seem to indicate that sewage pollution causes a reduction in diversity in the marine bacterial community.

REFERENCES

(Journal abbreviations are according to ACCESS and CASSI published by the American Chemical Society).

APHA, 1971. Standard methods for the enumeration water and wastewater. 13th ed. American Public Health Association, Inc., New York. 874p.

ANSON, Avril and Ware, G.C. 1974. Survey of distribution of bacterial pollution in the Bristol Channel. J. Appl. Bacteriol., 37, 657-661.

ATLAS, R.M. and Bartha, R. 1973a. Fate and effects of polluting petroleum in the marine environment. Residue Rev., 49, 49-85.

ATLAS, R.M. and Bartha, R. 1973b. Abundance, distribution and oil biodegradation potential of microorganisms in Raritan Bay. Environ. Pollut., 4, 291-300.

BISSONETTE, G.K., Jezeski, J.J., McFeters, G.A. and Stuart, D.G. 1975. Influence of environmental stress on the enumeration of indicator bacteria from natural waters. Appl. Microbiol., 29, 186-194.

BOYD, W.L., Klubek, B.P. and Boyd, Josephine, W. 1972. Clean water ecology in the Polar regions. Naval Research Rev., November, 1972, 17-24.

BRUMMAGE, K.G. 1973. What is marine pollution? The Royal Institution of Naval Architects' Symposium of Marine Pollution, 27-28 February, 1973, 6p.

BRYAN, G.W. and Hummerstone, L.G. 1973. Brown seaweed as an indicator of heavy metals in estuaries in South-West England. J. Mar. Biol Ass. U.K., 53, 705-720.

BUCK, J.D. and Cleverdon, R.C. 1960. The spread plate as a method for the enumeration of marine bacteria. Limnol. Oceanogr., 5, 78-80.

BUIKEMA, A.L., Cairns, J. and Sullivan, Gail W. 1974. Rotifers as monitors of heavy metal pollution in water. Virginia Polytechnic Institute Water Resources Research Centre, Bulletin No. 71. 74p.

CABELLI, V.J. and McCabe, L.J. 1974. Recreational water quality criteria. EPA (Cincinnatti) Survey Report, 11 November, 1974. 4p.

CABELLI, V.J., Kennedy, Harriet and Levin, M.A. 1976. Pseudomonas aeruginosa - faecal coliform relationships in estuarine and recreational waters. J. Water Pollut. Contr. Fed., 48, 367-376.

CAIRNS, J. 1967. The use of quality control techniques in the management of aquatic ecosystems. Water resour. Bull., 3, 47-53

CAIRNS, J. and Sparks, R.E. 1971. The use of bluegills to detect zinc. EPA Water Pollution Control Research Series No. 18050. 45p.

CAIRNS, J., Dickson, K.L., Lanza, G.R., Almeida, S.P. and Balzo, D.D. 1972. Coherent optical spatial filtering of diatoms in water pollution monitoring. Arch. Midrobiol., 83, 141-146.

CAIRNS / .....

CAIRNS, J. and Dickson, K.L. 1973. The effects of waste discharges from Radford Army Ammunition Plant on the biota of the New River, Virginia. Virginia Polytechnic Institute Water Resources Research Centre, Bulletin No. 57. 57p.

CAIRNS, J., Dickson, K.L. and Lanza, G. 1973. Rapid biological monitoring systems for determining aquatic community structure in receiving systems. Biological Methods for the Assessment of Water Quality. American Society for Testing and Materials. p.148-163.

CAIRNS, J. and Messenger, D. 1974. An interim report on the effects of prior exposure to sublethal concentrations of toxicants upon the tolerance of snails to thermal shock. Arch. Hydrobiol., 74, 441-447.

CHERMISINOFF, P.N. 1974. Water pollution sampling, monitoring and analysis instrumentation. Pollut. Eng., 6, 36-47.

CLASBY, R.C., Reeburgh, W.S. and Alexander, Vera. 1972. A close-interval syringe sampler. Limnol. Oceanogr., 17, 632-633.

COLLIER, H.O.J., Campbell, N.R. and Fitzgerald, M.E.H. 1950. Vibriostatic activity in certain series of pteridines. Nature, 165, 1004-1005

COLWELL, Rita R. and Wiebe, W.J. 1970. 'Core' characteristics for use in classifying aerobic, heterotrophic bacteria by numerical taxonomy. Bull. G. Acad. Sci., 28, 165-185.

COLWELL/ .....

COLWELL, Rita, R., Johnson, R., Wan, L., Lovelace, T.E. and Brenner, D.J. 1974. Numerical taxonomy and deoxyribonucleic acid reassociation in the taxonomy of some Gram-negative, fermentative bacteria. International Journal of Systematic Bacteriology, 24, 422-433.

DALEY, R.J. and Hobbie, J.E. 1975. Direct counts of aquatic bacteria by a modified epifluorescence technique. Limnol. Oceanogr., 20, 875-882.

DE LEY, J. 1964. Pseudomonas and related genera. Annu. Rev. Microbiol., 18, 17-46.

DUFOUX, A.P. and Cabelli, V.J. 1975. Membrane filter procedure for enumerating the component genera of the coliform group in sea-water. Appl. Microbiol., 29, 826-833.

FERGUSON-WOOD, E.J. 1965. Marine Microbiol. Ecology. Chapman and Hall Ltd., London. 243p.

FRANCISCO, D.E., Mah, R.A. and Rabin, A.C. 1973. Acridine orange-epifluorescence technique for counting bacteria in natural waters. Trans. Amer. Micros. Soc., 92, 416-421.

GAMESON, L.H., Pike, E.B. and Davey, K.W. 1973. Mortality of coliform bacteria in sea-water samples in daylight. Atti Del 5° Colloquio Internazionale Di Oceanografia Medica. Editrice Libraria Bonanzinga, Messina. p 353-362.

GRABOW, W.O.K., Prozesky, O.W. and Smith, L.S. 1975. Drug resistant coliforms call for re-evaluation of water quality standards. Water Pollut. Contr., 74, 217-224.

GRINDLEY / .....

GRINDLEY, J.R. and Taylor, F.J.R. 1968. Factors affecting plankton blooms in False Bay. Trans. Roy. Soc. S. Afr., 41, 201-210.

GRINDLEY, J.R. 1972. Pollution of the sea. S. Afr. J. Sci., 68, 162-170.

GUNDERSON, C.W., Mountain, Diane T., Ohye, R. and Shea, J. 1972. Some Chemical and micro-biological observations in the Pacific Ocean off the Hawaiian Islands. Limnol. Oceanogr., 17, 524-531.

GUNKEL, W. and Rheinheimer, G. 1972. Bacteria. Research Methods in Marine Biology. Sidgwick and Jackson, London. 356p.

HOBBIE, J.E., Holm-Hansen, O., Packard, T.T., Pomeroy, L.R., Sheldon, R.W., Thomas, J.P. and Wiebe, W.J. 1972. A study of the distribution and activity of micro-organisms in ocean water. Limnol. Oceanogr., 17, 544-555.

HODGKISS, W. 1960. The interpretation of flagella stains. J. Appl. Bacteriol., 23, 389-399.

INGRAM, M. and Shewan, J.M. 1960. Introductory reflections on the Pseudomonas - Achromobacter group. J. Appl. Bacteriol., 23, 373-378.

INMAN, D.L. and Brush, B.M. 1973. The coastal challenge. Science, 181, 20-32.

JANNASCH, H.W. and Jones, G.E. 1959. Bacterial populations in sea-water as determined by different methods of enumeration. Limnol. Oceanogr., 4, 128-139.

JONES / .....

JONES, J.G. 1974. Some observations on direct counts of freshwater bacteria obtained with a fluorescence microscope. Limnol. Oceanogr., 19, 540-543.

KRISS, E.A. 1963. Marine microbiology. Oliver and Boyd, London. 563p.

LEE, T.N. and Rooth, C. 1973. Water movements in shallow coastal bays and estuaries. University of Miami Coastal Zone Management Series No. 3. 19p.

LIVINGSTONE, D.J., De Goede, J.W. and Warren-Hansen, Barbara A. 1969. The distribution and occurrence of coliform and pathogenic indicators of pollution within the surf zone and near-shore waters along a section of the Natal Coast. CSIR Research Report No. 278. 100p.

LIVINGSTONE, D.J. 1969. An appraisal of sewage pollution along a section of the Natal Coast. J. Hyg., 67, 209-223.

MacLEOD, R.A. 1965. The question of the existence of specific marine bacteria. Bacteriol. Rev., 29, 9-23.

MAZURE, H.G.F. 1976. Bacterial biomass in coastal waters off the Cape Peninsula. 1st Interdisciplinary Conference on Marine and Freshwater Research in South Africa, 5-10 JUly, 1976. 12p.

McFETERS, G.A., Bissonnette, G.K., Jezeski, J.J., Thomson, Carole A. and Stuart, D.G. 1974. Comparative survival of indicator bacteria and enteric pathogens in well water. Appl. Microbiol., 27, 823-829.

MENHINICK / .....

MENHINICK, E.F. 1964. A comparison of some species diversity indices applied to samples of field insects. Ecology, 45, 859-861.

MIRONOV, O.G. 1970. Role of micro-organisms growing on oil in the self-purification and indication of oil pollution in the sea. Oceanology, 11, 650-656.

MURCHELANO, R.A. and B rown, C. 1970. Heterotrophic bacteria in Long Island Sound. Mar. Biol., 7, 1-6.

NUSBAUM, I. and Garner, R.M. 1955. Survival of coliform organisms in Pacific Ocean coastal waters. Sewage Ind. Wastes, 27, 1383-1390.

OLIFF, W.D. 1976. The sea and pollution. 1st Interdisciplinary Conference on Marine and Freshwater Research in South Africa, 5-10 July, 1976. 17p.

OPPENHEIMER, C. 1952. The membrane filter in marine microbiology. J. Bacteriol., 64, 783-786.

PRESNELL, M.W. and Andrews, W.H. 1976. Use of the membrane filter and a filter aid for concentrating and enumerating indicator bacteria and Salmonella from estuarine waters. Water Res., 10, 549-554.

RIDDLE, A.M. 1973. Residual current flows off North East England. Mar. Pollut. Bull., 7, 128-130.

RUPERT, E.E. 1972. An efficient, quantitative method for sampling the meiobenthos. Limnol. Oceanogr. 17, 629-631.

SABS, 1971. Specification for domestic water supplies. Council of the South African Bureau of Standards, Pretoria. 13p.

SCHAEFFER, D.J., Long, M.C. and Janardau, K.G. 1974. Statistical analysis of the recovery of coliform organisms on Gelman and Millipore membrane filters. Appl. Microbiol., 28, 605-607.

SEKI, H. 1973. Silica Gel medium for the enumeration of petroleumlytic micro-organisms in the marine environment. Appl. Microbiol., 26, 318-320.

SEKI, H., Nakai, T. and Otobe, H. 1974a. Petroleumlytic bacteria in different water masses of the Pacific Ocean in January 1973. La Mer, 12, 16-19.

SEKI, H., Otobe, H. and Nakai, T. 1974b. SON microbiological sampler for hydrobiological investigation. J. Oceanogr. Soc. of Japan, 30, 203-204.

SHANAHAN, A.J., Fornwald, R.E. Chapman, D.M. and Beachley, A.N. 1956. Visualizing bacterial cells for direct microscopic counting on the Millipore filter, Hydrosol type. J. Bacteriol., 71, 499-500.

SHEWAN, J.M., Hodgkiss, W. and Liston, J. 1954. A method for the rapid differentition of certain non-pathogenic, asporogenous bacilli. Nature, 173, 173-209.

SHEWAN / .....

SHEWAN, J.M., Hobbs, G. and Hodgkiss, W. 1960a. A determinative scheme for the identification of certain genera of Gram-negative bacteria, with special reference to the Pseudomonodaceae. J. Appl. Bacteriol., 23, 379-390.

SHEWAN, J.M., Hobbs, G. and Hodgkiss, W. 1960b. The Pseudomonas and Achromobacter groups of bacteria in the spoilage of marine white fish. J. Appl. Bacteriol., 23, 463-468.

SIEBURTH, J. McN., Frey, J.A. and Conover, J.T. 1963. Microbiological sampling with a piggy-back device during routine Nansen bottle casts. Deep Sea Res., 10, 757-758.

SIEBURTH, J. McN. 1963. A simple form of the ZoBell bacteriological sampler for shallow water. Limnol. Oceanogr., 8, 489-492.

SIEBURTH, J. McN. 1965. Bacteriological samplers for air-water and water-sediment interfaces. Transactions of the Joint Conference, Marine Technology Society and American Society of Limnology and Oceanography. June, 1965.

SIEBURTH, J. McN. 1967. Seasonal selection of estuarine bacteria by water temperature. J. Exp.Mar. Biol. Ecol., 1, 98-121.

SMITH, R.J., Tweedt, R.M. and Flanigan, Linda K. 1973. Relationships of indicator and pathogenic bacteria in stream waters. J. Water Pollut. Contr. Fed., 45, 1736-1745.

SNEATH, P.H.A. 1957. The application of computers to taxonomy. J. Gen. Microbiol., 17, 201-226.

SNEATH / .....

SNEATH, P.H.A. 1962. The construction of taxonomic groups. Microbial classification. Cambridge University Press, Cambridge. 483p.

SOCIETY of American Bacteriologists, 1957. Manual of microbiological methods. McGraw-Hill Co., New York. 357p.

SOROKIN, Y.I. 1971. Bacterial populations as components of oceanic ecosystems. Mar. Biol., 11, 401-405

SPARKS, R.E., Cairns, J., McNabb, R.A. and Suter, G. 1972. Monitoring zinc concentrations in water using the respiratory response of Bluegills (Lepomis macrochirus rafinesque). Hydrobiol., 40, 361-369.

STAUFFER, J.R., Dickson, K.L. and Cairns, J. 1974. A field evaluation of the effects of heated discharges on fish distribution. Water Resour. Bull. AWRA, 10, 860-876.

SYKES, R.M. 1973. Identification of the limiting nutrient and specific growth rate. J. Water Pollut. Contr. Fed., 45, 888-895.

TAYLOR, R.H., Bordner, R.H., and Scarpino, P.V. 1973. Delayed-incubation membrane-filter test for fecal coliforms. Appl. Microbiol., 25, 363-368.

VIND, H.P., Muraoka, J.S. and Mathews, C.W. 1975. The survival of sewage bacteria at various ocean depths. Technical Note No. 1396, Civil Engineering Laboratory, Naval Construction Battalion Center (California). 19p.

WALDICHUK, M. 1973. International approach to the marine pollution problem. Ocean Management, 1, 211 - 261.

WILLIAMS, E.D.F. 1969. A submerged membrane filter apparatus for microbiological sampling. Mar. Biol., 3, 78-80.

WOMACK, T.D., O'Connor, P.B. and Williams, P. 1973. Survival of enteric pathogens and indicator organisms in natural waters. Water Resources Research Center Research Report, University of Tennessee. 46p.

ZOBELL, C.E. 1946. Marine microbiology. A monograph on hydrobiology. Chonica Botanica Co., Waltham. 240p.

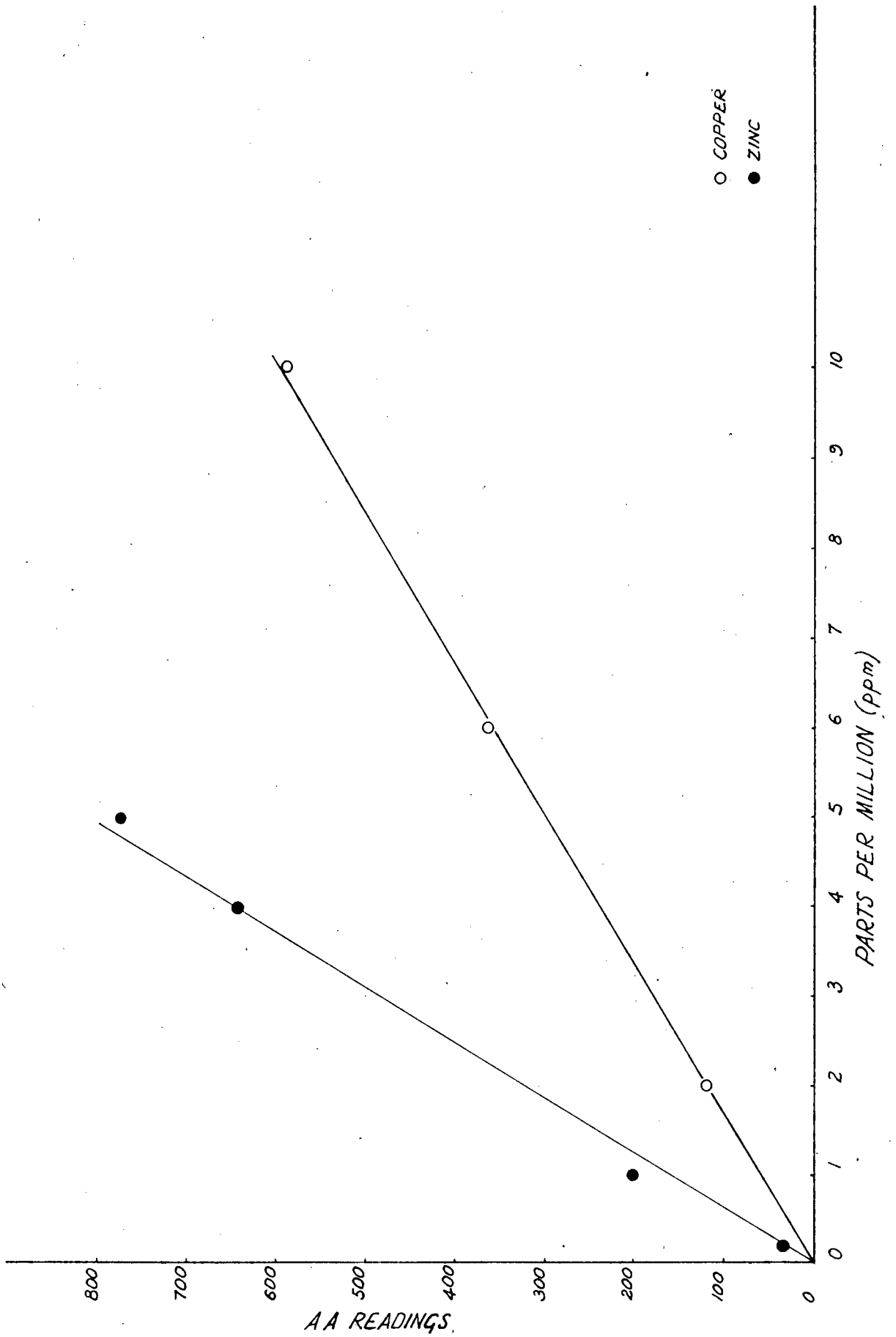
ZOBELL, C.E. 1957. Marine bacteria. Geol. Soc. America, 1, 1035-1040.

ZOBELL, C.E. 1969. Microbiological modification of crude oil in the sea. Proceedings of the Joint Conference on Prevention and Control of Oil Spills. American Petroleum Institute, New York. p. 317-326.

APPENDICES

The appendices are listed on p. 79 - 86.

APPENDIX 1: CALIBRATION CURVE FOR ATOMIC ABSORPTION ANALYSER



APPENDIX 2: ATOMIC ABSORPTION ANALYSER

Readings for Copper and Zinc Levels in Sea-water contained in Brass and Glass Bacteriological Samplers

(a) Copper

Sampler	Time 0h	2h	3h
Brass Sampler 1	0,024	0,319	0,438
Brass Sampler 2	0,083	0,486	0,559
Brass Sampler 3	0,059	0,350	0,436
Brass Sampler 4	0,065	0,408	0,432
Glass Sampler	0,003	0,007	0,008

(b) Zinc

Sampler	Time 0h	2h	3h
Brass Sampler 1	0,111	0,520	0,609
Brass Sampler 2	0,177	0,612	0,654
Brass Sampler 3	0,123	0,321	0,436
Brass Sampler 4	0,150	0,331	0,502
Glass Sampler	0,015	0,015	0,012

APPENDIX 3: HIERARCHICAL CLASSIFICATION OF BACTERIA ISOLATED FROM THE TOTAL COUNT MEDIUM

The classification tables are given on pages p. 82 - 86.

The isolate number is constructed as follows :

1. The first letter refers to the sampling station where the sample was drawn.

2. The second letter refers to one or other of the duplicate samples.

3. The first number refers to the sample dilution.

4. The second number refers to the number of the isolate from a particular dilution.

The following symbols are used to describe the bacterial growth in the marine oxidate-fermentative medium :

O - Oxidative

F - Fermentative

G - Gas producing

SAMPLING DATE : 17th OCTOBER, 1975.

ISOLATE NUMBER	GRAM REACTION	MOTILITY	COLONY COLOUR	MORPHOLOGY	KOVACS OXIDASE	MARINE OXID.-FERM. MEDIUM	GROWTH AT 37 C	ANTIBIOTICS					GENUS
								PENICILLIN	OXYTETRACYCLINE	VIBRIOSTAT 0/129	CHLORAMPHENICOL	STREPTOMYCIN	
AA 31	−	+	ORANGE	STR. RODS, SINGLE	+	O	+	−	−	−	+	+	PSEUDOMONAS I or II
32	−	−	CREAM	SHORT RODS TO COCCI, SINGLE	+	O	−	−	−	−	+	+	ACHROMOBACTER / ALCALIGENES
33	−	+	COLOURLESS	CURVED RODS, SINGLE	+	NO ACTION	−	+	−	−	+	+	SPIRILLUM
41	−	−	YELLOW	COCCI, SINGLE	−	NO ACTION	−	−	−	+	+	+	?
AB 31	−	+	CREAM	STR. RODS, SINGLE	+	F	−	+	−	+	+	+	VIBRIO
32	−	+	COLOURLESS	STR. RODS, SINGLE	+	O	+	−	−	−	+	+	PSEUDOMONAS I or II
34	−	+	YELLOW/ ORANGE	SLENDER RODS, SINGLE	+	O	+	−	−	−	+	+	PSEUDOMONAS I or II
35	−	+	COLOURLESS	SHORT, STR. RODS, SINGLE	+	O	+	−	−	−	+	+	PSEUDOMONAS I or II
41	−	+	CREAM	STR. RODS, SINGLE	+	O	−	+	−	−	+	+	PSEUDOMONAS I or II
BA 31	−	+	CREAM	SLENDER, STR. RODS, SINGLE	+	O	−	−	−	−	+	+	PSEUDOMONAS I or II
32	−	+	CREAM	SLENDER, STR. RODS SINGLE	+	O	−	−	−	−	+	+	PSEUDOMONAS I or II
33	−	+	CREAM	SLENDER, STR. RODS SINGLE	+	O	−	−	−	−	+	+	PSEUDOMONAS I or II
34	−	+	CREAM	STR. RODS, SINGLE	+	F	−	−	−	+	+	+	VIBRIO
35	−	+	ORANGE	STR. RODS, SINGLE	−	F+G	+	+	−	−	+	+	PARACOLON
36	−	−	COLOURLESS	STR. RODS, SINGLE	+	O	+	−	−	−	+	+	ACHROMOBACTER / ALCALIGENES
37	−	+	WHITE	STR. RODS, SINGLE	−	F+G	+	+	−	−	+	+	PARACOLON
41	−	+	GREY/ BLACK	STR. RODS, SINGLE	+	F	−	−	−	+	+	+	VIBRIO
42	−	+	CREAM	STR. AND CURVED RODS, SINGLE	+	O	−	−	−	−	+	+	PSEUDOMONAS I or II
43	−	+	CREAM	STR. RODS, SINGLE	+	O	−	−	−	−	+	+	PSEUDOMONAS I or II
44	−	+	COLOURLESS	SHORT RODS, SINGLE	+	F	−	−	−	+	+	+	VIBRIO
51	−	+	COLOURLESS	SLENDER RODS, SINGLE	+	NO ACTION	−	−	−	+	+	−	PSEUDOMONAS IV
BB 33	−	+	WHITE	CURVED RODS, SINGLE	+	F	−	+	−	+	+	+	VIBRIO
34	−	−	CREAM	STR. RODS, SINGLE	+	O	−	−	−	−	+	+	ACHROMOBACTER / ALCALIGENES
35	−	−	WHITE	SHORT, STOUT RODS TO COCCI	+	O	−	−	−	−	+	+	ACHROMOBACTER / ALCALIGENES
36	−	+	CREAM	STR. RODS, SINGLE	+	O	+	−	−	−	+	+	PSEUDOMONAS I or II
37	−	−	YELLOW	SLENDER RODS, SINGLE	+	NO ACTION	−	−	−	−	+	+	FLAVOBACTERIUM
38	−	+	WHITE	STR. RODS, SINGLE	+	O	−	−	−	−	+	+	PSEUDOMONAS I or II
39	−	+	COLOURLESS	STR. RODS, SINGLE	−	F+G	−	+	−	−	+	+	PARACOLON
41	−	+	CREAM	STR. RODS, SINGLE	+	O ?	−	+	−	+	+	+	VIBRIO
42	−	+	CREAM	STR. RODS, SINGLE	+	O	−	−	−	−	+	+	PSEUDOMONAS I or II
51	−	−	COLOURLESS	SLENDER, STR. RODS, SINGLE	+	NO ACTION	+	+	+	−	+	+	?
CA 33	−	+	ORANGE	LONG, SLENDER RODS, SINGLE	+	O	−	−	+	+	+	+	PSEUDOMONAS I or II
34	−	+	CREAM	LONG, SLENDER RODS, SINGLE	+	O	+	+	−	+	+	+	PSEUDOMONAS I or II
36	−	+	CREAM	STR. RODS, SINGLE	−	F	+	+	−	−	+	+	PARACOLON
41	−	+	ORANGE	STR. RODS, SINGLE	+	F	−	+	−	+	+	+	VIBRIO
CB 31	−	+	GREY/ BLACK	STR. RODS, SINGLE	−	F+G	−	−	−	−	+	+	PARACOLON
34	−	+	ORANGE	STR. RODS, SINGLE	+	F	−	+	−	+	+	+	VIBRIO
35	−	+	WHITE	STR. RODS, SINGLE	+	F	−	+	−	+	+	+	VIBRIO
36	−	+	ORANGE	STR. RODS, SINGLE	−	F	−	−	−	−	+	+	PARACOLON
41	−	+	ORANGE	STR. RODS, SINGLE	+	NO ACTION	+	−	−	−	+	+	PSEUDOMONAS IV

SAMPLING DATE : 4 th NOVEMBER, 1975.

ISOLATE NUMBER	GRAM REACTION	MOTILITY	COLONY COLOUR	MORPHOLOGY	KOVACS OXIDASE	MARINE OXID.-FERM. MEDIUM	GROWTH AT 37 C	ANTIBIOTICS: PENICILLIN	OXYTETRACYCLINE	VIBRIOSTAT 0/129	CHLORAMPHENICOL	STREPTOMYCIN	GENUS
AA 31	—	+	CREAM	LONG STR. RODS, SINGLE	+	O	—	—	—	—	+	—	PSEUDOMONAS I OR II
32	—	+	CREAM	LONG STR. RODS, SINGLE	+	O	—	—	—	—	+	—	PSEUDOMONAS I OR II
33	—	+	YELLOW	STR. RODS, SINGLE	+	O	—	—	—	—	+	—	PSEUDOMONAS I OR II
34	—	+	CREAM	SHORT RODS, SINGLE AND PAIRS	+	O	—	—	—	—	+	+	PSEUDOMONAS I OR II
35	—	+	CREAM	SHORT RODS, SINGLE AND PAIRS	+	O	—	—	—	—	+	—	PSEUDOMONAS I OR II
36	—	+	YELLOW	LONG RODS, CURVED OR STR., SINGLE	+	O	+	—	—	—	+	+	PSEUDOMONAS I OR II
41	—	+	CREAM	SHORT RODS, SINGLE	+	O	—	—	—	—	+	+	PSEUDOMONAS I OR II
AB 31	—	+	ORANGE/ RED	LONG STR. RODS, SINGLE	—	F	—	—	—	—	+	+	PARACOLON
32	—	—	CREAM	STR. RODS, SINGLE AND PAIRS	+	O	—	+	+	—	+	+	ACHROMOBACTER / ALCALIGENES
33	—	+	CREAM	LONG STR. AND CURVED RODS, SINGLE	+	NO ACTION	+	—	—	—	+	+	PSEUDOMONAS IV
34	—	+	CREAM	SHORT, STOUT RODS, SINGLE OR PAIRS	—	F	+	—	—	—	+	+	PARACOLON
35	—	+	CREAM	LONG, STR. RODS, SINGLE OR PAIRS	+	NO ACTION	+	+	—	—	+	+	PSEUDOMONAS IV
36	—	+	ORANGE/ RED	LONG. STR. RODS, SINGLE OR PAIRS	+	O	—	—	—	—	+	—	PSEUDOMONAS I OR II
37	—	+	YELLOW	LONG. STR. RODS, SINGLE OR PAIRS	+	F+G	+	—	—	—	+	—	AEROMONAS
38	—	+	ORANGE/ RED	LONG, STR. RODS SINGLE OR PAIRS	—	NO GROWTH	—	—	—	—	+	+	PARACOLON
39	—	+	CREAM	PLEOMORPHIC	—	F	—	+	—	—	+	+	PARACOLON
BA 41	—	+	ORANGE	LONG, STR. RODS, SINGLE	+	NO GROWTH	+	—	—	—	+	+	PSEUDOMONAS IV
42	—	+	YELLOW	SHORT, STR. AND CURVED RODS, SINGLE	+	F	—	+	—	+	+	+	VIBRIO
43	—	+	ORANGE	LONG (NEEDLE-LIKE) RODS, STR, SINGLE OR CHAIN	+	NO ACTION	—	—	—	—	+	—	PSEUDOMONAS IV
44	—	S	CREAM	SHORT, STR. TO CURVED RODS, SINGLE	+	NO ACTION	+	—	—	—	+	—	SPIRILLUM
45	—	+	CREAM	STR. RODS, SINGLE	+	O, F+G	+	—	—	—	+	—	AEROMONAS
46	—	+	ORANGE	SHORT, STR. TO CURVED RODS, SINGLE	+	O	+	—	—	—	+	—	PSEUDOMONAS I OR II
47	—	+	ORANGE	SHORT, STR. AND CURVED RODS, SINGLE	+	O, F+G	+	—	—	—	+	—	AEROMONAS
BB 41	—	+	CREAM	SHORT, VERY STOUT RODS, SINGLE	+	F	—	+	—	+	+	+	VIBRIO
42	—	—											CULTURE LOST
43	—	+	CREAM	STR. RODS, SINGLE AND PAIRS	+	NO ACTION	—	—	+	—	+	—	PSEUDOMONAS IV
CA 41	—	+	CREAM	STR. RODS, SINGLE	+	NO ACTION	—	—	—	—	+	+	PSEUDOMONAS IV
42	—	S	CREAM	STR. RODS, SINGLE	+	NO ACTION	—	—	—	—	+	+	SPIRILLUM
43	—	+	WHITE	SHORT, STR. RODS, SINGLE	+	O, F+G	+	—	—	—	+	—	AEROMONAS
44	—	+	COLOURLESS	SHORT, STR. RODS, SINGLE	+	F	—	—	+	+	+	+	VIBRIO
45	—	—	WHITE	SHORT, STR. RODS, SINGLE	+	AEROBIC GROWTH	—	—	—	—	+	+	ACHROMOBACTER ALCALIGENES
46	—	+	COLOURLESS	STR. RODS, SINGLE	+	NO ACTION	—	—	—	—	+	+	PSEUDOMONAS IV
47	—	+	CREAM	STR. RODS, SINGLE	+	F+G	+	—	—	—	+	+	AEROMONAS
48	—	+	CREAM	SHORT RODS, SINGLE	+	F	+	—	—	—	+	+	? AEROMONAS
CB 41	—	—	CREAM	SHORT, STR. RODS TO COCCI, SINGLE	—	O	—	—	—	—	+	+	ACHROMOBACTER ALCALIGENES
42	—	+	CREAM	SHORT RODS, SINGLE	+	F	—	+	+	+	+	+	VIBRIO
43	—	S	CREAM	SHORT RODS, STR. TO CURVED, SINGLE	+	NO ACTION	—	—	—	—	+	+	SPIRILLUM
44	—	+	CREAM	SHORT RODS, SINGLE	—	F+G	—	—	—	—	+	+	PARACOLON
45	—	+	ORANGE	SHORT RODS, CURVED, SINGLE	+	F+G	—	—	+	+	+	+	VIBRIO

SAMPLING DATE : 16th NOVEMBER, 1975.

ISOLATE NUMBER	GRAM REACTION	MOTILITY	COLONY COLOUR	MORPHOLOGY	KOVACS OXIDASE	MARINE OXID.-FERM. MEDIUM	GROWTH AT 37 C	ANTIBIOTICS					GENUS
								PENICILLIN	OXYTETRA-CYCLINE	VIBRIOSTAT 0/129	CHLORAM-PHENICOL	STREPTO-MYCIN	
AA 31	—	+	ORANGE	STR. AND CURVED RODS, SINGLE	+	O	+	—	—	—	+	+	PSEUDOMONAS I OR II
32	—	+	YELLOW	RODS, SINGLE AND PAIRS	+	O+F	—	+	—	—	+	+	FLAVOBACTERIUM
33	—	S	ORANGE	RODS, SPIRAL, SINGLE	+	NO GROWTH	+	+	+	—	+	+	SPIRILLUM
34	—	+	YELLOW	SHORT RODS, SINGLE, PAIRS AND CHAINS	+	O	+	—	—	—	+	+	PSEUDOMONAS I OR II
35	—	—	GREY	SHORT STOUT RODS, SINGLE	+	ANAEROBIC GROWTH	—	—	—	—	+	+	ACHROMOBACTER/ ALCALIGENES
36	—	+	CREAM	RODS, PLEOMORPHS	+	NO ACTION	—	—	—	—	+	+	PSEUDOMONAS IV
41	—	+	CREAM	OVAL BACTERIA, SINGLE AND PAIRS	+	F	—	+	+	+	+	+	VIBRIO
42	—	+	RED MICRO-COLONY	COCCI									CULTURE LOST
AB 41	—	+	ORANGE	STR. RODS, SINGLE	+	O	+	+	—	—	+	+	PSEUDOMONAS I OR II
42	+	+	DARK BROWN										CULTURE LOST
43	—	+	YELLOW	STR. RODS, SINGLE	+	O	+	+	—	—	+	+	PSEUDOMONAS I OR II
44	—	+	YELLOW	LONG STR. RODS, SINGLE	+	ALKALINE	+	+	—	—	+	+	PSEUDOMONAS III
45	—	+	WHITE	SHORT STOUT RODS, SINGLE	+	F	—	+	+	+	+	+	VIBRIO
46	—	+	ORANGE	LONG, STR. RODS, SINGLE	+	NO ACTION	+	+	—	+	+	+	PSEUDOMONAS IV
BA 41	—	+	ORANGE	STR. AND CURVED RODS, SINGLE	+	O	—	—	—	—	+	+	PSEUDOMONAS I OR II
42	—	+	CREAM	STR. AND CURVED RODS, SINGLE	+	GROWTH	—	+	+	—	+	+	SPIRILLUM
43	—	+	CREAM	STR. AND CURVED RODS, SINGLE	+	O	+	—	—	—	+	+	PSEUDOMONAS I OR II
44	—	+	CREAM	STR. AND CURVED RODS, SINGLE	+	O	+	—	—	—	+	—	PSEUDOMONAS I OR II
45	—	+	CREAM	SHORT, STOUT RODS, SINGLE	—	F+G	+	—	—	—	+	+	PARACOLON
BB 41	—	+	YELLOW	STR. RODS, SINGLE	+	O+F	—	+	—	—	+	+	FLAVOBACTERIUM
42	—	+	YELLOW	STR. RODS, SINGLE	+	O+F	—	+	+	+	+	+	FLAVOBACTERIUM
43	—	+	YELLOW	STR. RODS, SINGLE	+	O	+	+	+	+	+	+	PSEUDOMONAS I OR II
44	—	+	ORANGE	STR. RODS, SINGLE	+	O	—	—	—	—	+	+	PSEUDOMONAS I OR II
45	—	+	CREAM	STR. RODS, SINGLE	+	O	—	—	—	—	+	+	PSEUDOMONAS I OR II
46	—	+	ORANGE	STR. RODS, SINGLE	+	O+F	—	+	+	+	+	+	FLAVOBACTERIUM
CA 41	—	+	BROWN	LONG RODS, SINGLE AND PAIRS	+	O	—	—	—	—	+	+	PSEUDOMONAS I OR II
42	—	+	CREAM	STR. RODS, SINGLE	+	O	+	—	—	—	+	—	PSEUDOMONAS I OR II
43	—	+	CREAM	LONG SLENDER RODS, SINGLE	+	ALKALINE	+	+	+	+	+	+	PSEUDOMONAS III
44	—	+	WHITE	SMALL, SINGLE BACTERIA	—	F+G	+	+	—	—	+	+	PARACOLON
45	—	+	BROWN	SHORT, STOUT RODS, SINGLE	+	O+F	+	+	+	+	+	+	FLAVOBACTERIUM
46	—	+	ORANGE	SHORT, STOUT RODS, SINGLE	+	O+F	+	+	—	—	+	+	FLAVOBACTERIUM
47	—	+	CREAM	STR. RODS, SINGLE	+	F	—	+	+	+	+	+	VIBRIO
CB 41	—	+	BROWN	STR. RODS TO OVAL SINGLE	+	O+F	+	—	—	—	+	—	FLAVOBACTERIUM
42	—	+	BROWN	STR. RODS, SINGLE	—	O	+	+	—	—	+	—	PARACOLON
43	—	+	WHITE	RODS TO COCCOID FORMS, SINGLE	—	AEROBIC GROWTH	+	—	—	—	+	—	PARACOLON
44	—	+	CREAM	STR. RODS, SINGLE	+	O	+	+	+	+	+	+	PSEUDOMONAS I OR II
45	—	+	BROWN	STR. AND CURVED RODS, SINGLE AND PAIRS	—	O+F	+	+	—	—	+	—	PARACOLON
46	—	+	CREAM	STR. RODS, SINGLE AND PAIRS	+	O	+	—	—	—	+	—	PSEUDOMONAS I OR II
47	—	+	BROWN	STR. RODS, SINGLE	+	O+F	+	+	—	—	+	—	FLAVOBACTERIUM
48	—	+	BROWN	STR. AND CURVED RODS, SINGLE	—	O+F	+	+	—	+	+	+	PARACOLON

SAMPLING DATE : 20th JANUARY, 1976

ISOLATE NUMBER	GRAM REACTION	MOTILITY	COLONY COLOUR	MORPHOLOGY	KOVACS OXIDASE	MARINE OXID.-FERM. MEDIUM	GROWTH AT 37 C	ANTIBIOTICS					GENUS
								PENICILLIN	OXYTETRA-CYCLINE	VIBRIOSTAT 0/129	CHLORAM-PHENICOL	STREPTO-MYCIN	
AA 41	—	+	YELLOW	STR. RODS, SINGLE	+	O	+	—	—	--	+	+	PSEUDOMONAS I OR II
42	—	+	YELLOW	SHORT RODS, SINGLE	+	O	+	—	—	—	+	+	PSEUDOMONAS I OR II
43	—	+	YELLOW	STR. RODS, SINGLE	+	O	+	—	—	—	+	+	PSEUDOMONAS I OR II
44	—	+	ORANGE	STR. RODS, SINGLE	+	O	+	—	—	—	+	+	PSEUDOMONAS I OR II
45	—	+	ORANGE	STR. RODS, SINGLE	+	O	+	—	—	—	+	+	PSEUDOMONAS I OR II
46	—	+	BROWN	STR. RODS SINGLE	+	O	+	—	—	—	+	+	PSEUDOMONAS I OR II
47	—	+	BROWN	STR. RODS, SINGLE	+	O	+	+	—	—	+	+	PSEUDOMONAS I OR II
AB 41	—	+	YELLOW	STR. RODS, SINGLE	+	NO ACTION	—	NO GROWTH					PSEUDOMONAS IV
42	—	—	YELLOW	STR. RODS, SINGLE	+	NO ACTION	—	+	+	—	+	+	FLAVOBACTERIUM
43	—	+	ORANGE	STR. RODS, SINGLE	+	O	—	NO GROWTH					PSEUDOMONAS I OR II
44	—	+	ORANGE	STR. RODS, SINGLE	+	O	+	+	—	—	+	—	PSEUDOMONAS I OR II
45	—	—	ORANGE	STR. RODS, SINGLE AND PAIRS	+	O	+	—	—	—	+	—	FLAVOBACTERIUM
46	—	—	GREY	COCCO-BACILLI, SINGLE, PAIRS & CHAINS	+	O	—	+	+	+	+	+	ACHROMOBACTER / ALCALIGENES
47	—	—	BROWN	SHORT TO LONG RODS, SINGLE	+	O	—	+	+	—	+	+	FLAVOBACTERIUM
BA 41	—	—	YELLOW	LONG, STR. AND CURVED RODS, SINGLE	+	O	+	—	—	—	—	—	FLAVOBACTERIUM
42	+	+	YELLOW	STR. RODS, SINGLE	—	NO ACTION	—	NO GROWTH					GRAM-POSITIVE
43	—	—	ORANGE	COCCO-BACILLI, SINGLE, PAIRS	+	O	—	+	+	—	+	—	ACHROMOBACTER / ALCALIGENES
44	—	+	BROWN	STR. RODS, SINGLE	+	O	+	—	+	—	+	—	PSEUDOMONAS I OR II
45	—	—	YELLOW	SLENDER, STR. RODS, SINGLE	+	O	+	—	—	—	+	—	FLAVOBACTERIUM
46	+	+	BROWN	STR. RODS, SINGLE	+	O+F	+	—	—	—	+	+	GRAM-POSITIVE
BB 41	—	S	YELLOW	LONG, STR. RODS, SINGLE	+	O	+	—	—	—	+	—	SPIRILLUM
42	—	+	YELLOW	LONG, STR. RODS, SINGLE	+	O	+	+	—	—	+	+	PSEUDOMONAS I OR II
43	—	—	ORANGE	CURVED RODS, SINGLE, PAIRS	+	O	+	+	+	+	+	+	FLAVOBACTERIUM
44	—	+	BROWN	LONG, STR. AND CURVED RODS, SINGLE	+	O	+	—	—	—	+	+	PSEUDOMONAS I OR II
45	—	+	BROWN	STR. RODS, SINGLE	+	O	+	NO GROWTH					PSEUDOMONAS I OR II
46	—	+	PINK	STR. RODS, SINGLE	+	O	+	—	—	—	+	—	PSEUDOMONAS I OR II
47	—	+	BLACK	STR. RODS, SINGLE	+	O	+	LIMITED GROWTH INSENSITIVE TO CHLORAMPHENICOL					?
CA 41	—	+	YELLOW	STR. RODS, SINGLE	+	O	+	NO GROWTH					PSEUDOMONAS I OR II
42	—	—	COLOUR-LESS	COCCO-BACILLI	+	O	+	NO GROWTH					ACHROMOBACTER / ALCALIGENES
43	—	+	BROWN	STR. RODS, SINGLE	+	O	+	—	—	—	+	+	PSEUDOMONAS I OR II
44	—	+	BROWN	STR. RODS, SINGLE	+	O	+	—	—	—	+	—	PSEUDOMONAS I OR II
46	—	—	BROWN	STR. RODS, SINGLE	+	NO GROWTH	+	NO GROWTH					FLAVOBACTERIUM
47	—	+	BROWN	STR. RODS, SINGLE	+	O	+	+	—	—	+	+	PSEUDOMONAS I OR II
CB 41	—	S	COLOUR-LESS	STR. RODS, SINGLE AND PAIRS	+	O	+	+	—	—	+	+	SPIRILLUM
42	—	—	COLOUR-LESS	STOUT, HEAVY RODS, STR.	+	O	+	—	—	—	+	+	ACHROMOBACTER / ALCALIGENES
43	—	+	COLOUR-LESS	LONG RODS, SINGLE	+	O	+	NO GROWTH					PSEUDOMONAS I OR II
44	—	S	COLOUR-LESS	STR. RODS, SINGLE, PAIRS AND CHAINS	+	O	+	—	—	—	+	+	SPIRILLUM
45	—	+	COLOUR-LESS	STR. RODS, SINGLE	+	O	+	+	—	—	+	+	PSEUDOMONAS I OR II
46	—	+	BLACK	STR. RODS, SINGLE	+	O	+	+	—	—	—	—	?

SAMPLING DATE : 28th JANUARY, 1976

ISOLATE NUMBER	GRAM REACTION	MOTILITY	COLONY COLOUR	MORPHOLOGY	KOVACS OXIDASE	MARINE OXID - FERM. MEDIUM	GROWTH AT 37 C	ANTIBIOTICS					GENUS
								PENICILLIN	OXYTETRA-CYCLINE	VIBRIOSTAT 0/129	CHLORAM-PHENICOL	STREPTO-MYCIN	
AA 31	—	—	ORANGE	STOUT, OVAL, SPHERICAL RODS, SINGLE	?	O+F	—	—	+	—	+	+	FLAVOBACTERIUM
AB 41	—	—	CREAM	STR. AND CURVED RODS, SINGLE	+	ALKALINE	—	+	—	—	+	+	ACHROMOBACTER/ ALCALIGENES
42	—	—	ORANGE	LARGE, STR. RODS, SINGLE	+	O+F	—	—	—	—	+	+	FLAVOBACTERIUM
43	—	+	CREAM	STR. RODS, SINGLE	+	ALKALINE	+	—	—	—	+	—	PSEUDOMONAS III
44	—	—	ORANGE	STR. RODS, SINGLE	+	O+F	—	—	—	—	+	+	FLAVOBACTERIUM
BA 41	—	—	BROWN	LARGE STR. AND CURVED RODS, SINGLE	+	O+F	—	—	—	—	+	+	FLAVOBACTERIUM
42	—	+	COLOUR-LESS	STR. SIMPLE RODS	+	O	—	—	—	—	+	+	PSEUDOMONAS I OR II
43	—	+	ORANGE	SLENDER RODS, SINGLE	+	O	+	—	—	—	—	—	PSEUDOMONAS I OR II
44	—	+	COLOUR-LESS	LARGE, STR OR CURVED RODS, SINGLE	+	O	+	—	—	—	+	+	PSEUDOMONAS I OR II
45	—	+	COLOUR-LESS	STR. RODS, SINGLE	+	O	—	—	—	—	+	+	PSEUDOMONAS I OR II
46	—	+	BROWN	STOUT, OVAL AND STR. RODS, SINGLE & PAIRS	+	O+F	—	—	—	—	+	+	FLAVOBACTERIUM
47	—	+	COLOUR-LESS	SHORT RODS, SINGLE OR PAIRS	+	O	+	—	—	—	+	+	PSEUDOMONAS I OR II
48	—	—	COLOUR-LESS	SHORT, STOUT RODS, SINGLE OR PAIRS	+	ALKALINE	—	—	—	—	+	+	ACHROMOBACTER/ ALCALIGENES
BB 41	—	S	GREY	SINGLE RODS	+	NO REACTION	—	+	—	—	+	+	SPIRILLUM
42	—	—	BROWN	STOUT, OVAL RODS, SINGLE	+	O+F	—	—	+	—	+	+	FLAVOBACTERIUM
43	—		COLOUR-LESS		—								CULTURE LOST
44	—	—	COLOUR-LESS	SHORT, STOUT RODS, PAIRS	+	ALKALINE	—	—	—	—	+	+	ACHROMOBACTER/ ALCALIGENES
45	—	+	COLOUR-LESS	SLENDER, CURVED RODS, SINGLE	+	O	—	—	—	—	+	+	PSEUDOMONAS I OR II
46	—	+	COLOUR-LESS	LARGE, STR. AND CURVED RODS, SINGLE	+	O	—	—	—	—	+	+	PSEUDOMONAS I OR II
47	—	+	BROWN	LARGE RODS, SINGLE AND PAIRS	+	O	—	—	—	—	+	+	PSEUDOMONAS I OR II
48	—	+	BROWN	LARGE RODS, MOSTLY SINGLE	+	O	—	—	—	—	+	+	PSEUDOMONAS I OR II
CA 31	—	+	BROWN	STR. ROD, SINGLE, SOME ENORMOUS, MALFORMED	+	O+F	—	—	—	—	+	+	FLAVOBACTERIUM
32	—	—	ORANGE	SHORT RODS, SINGLE	+	O	—	+	—	—	+	+	FLAVOBACTERIUM
33	—	+	YELLOW	LARGE STR. RODS, SINGLE PAIRS, STAR CLUSTERS	+	O+F	+	+	+	—	+	+	PARACOLON
34	—	+	BROWN	STR. RODS, SINGLE, GROTESQUE SHAPES	+	O	—	—	—	—	+	+	PSEUDOMONAS I OR II
35	—	+	COLOUR-LESS	LARGE STR. RODS, OVAL SINGLE, MANY STAR CLUSTERS	—	O	+	+	—	—	+	+	PARACOLON
36	—	+	YELLOW	LARGE, STR. RODS, SINGLE	—	O	+	+	—	—	+	+	PARACOLON
37	—	+	ORANGE	LARGE, STR. RODS, SINGLE, STAR CLUSTERS	—	O	+	—	—	—	+	+	PARACOLON
38	—	—	COLOUR-LESS	BACILLUS-LIKE RODS	—	O	+	+	—	—	+	—	PARACOLON
CB 41	+	—	YELLOW	LARGE COCCI IN PAIRS AND TETRADS	+	NO ACTION	—	+	—	—	+	+	GRAM-POSITIVE
42			ORANGE										CULTURE LOST
43	+	—	YELLOW	COCCI, SINGLE, PAIRS AND TETRADS	+	O+F	—	—	—	—	+	+	GRAM-POSITIVE
44	—	+	YELLOW	STR. RODS, SINGLE AND PAIRS	—	F	+	+	—	—	+	+	PARACOLON
45	—	—	BROWN	BACILLUS-LIKE ROD	+	O	+	+	+	—	+	+	FLAVOBACTERIUM
46	+	—	COLOUR-LESS	ENORMOUS RODS UP TO 25 μ, STR & OTHER FORMS	—	O	+	+	+	+	+	+	GRAM-POSITIVE
47	—	+	YELLOW	STR. & CURVED RODS, SINGLE AND PAIRS	—	O+F	+	—	—	—	+	—	PARACOLON
48	—	+	BROWN	SHORT, STOUT RODS, SINGLE	+	O	+	—	+	—	+	+	PSEUDOMONAS I OR II