

MONITORING OF MICROBIAL WATER QUALITY AND SAPROPHYTIC BACTERIAL GENERA OF ABU DHABI COASTAL AREA, U.A.E.

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ABSTRACT

The microbiological assessment of fourteen sampling sites distributed in Abu Dhabi Coast, were surveyed monthly during October 1989 until September 1990 except for February and April 1990. The saprophytic bacteria, salt tolerant saprophytic bacteria, gram-negative bacteria, total and faecal coliforms were determined. In general, the numbers of all determined bacterial groups were found to be correlated with each other and in most cases they fluctuate together. Furthermore, the bacterial flora of the coastal water is markedly affected by water temperature, sampling sites and sampling time. The bacterial content of most sites fluctuated maximally during August and September (late summer). The natural self-purification process of coastal water samples was clearly observed. The predominant saprophytic bacterial genera and the common coliform species occurred in the study area were identified.

INTRODUCTION

ALMOST in all accumulations of water, such as sea, rivers, lakes, gulfs, springs, lagoons, tanks, etc. microorganisms are recorded and are able to live in such environments. The composition of the living communities of coastal water microflora are extremely affected by very different physical and chemical conditions as well as by refuse and sewage in many ways. With domestic sewage high numbers of different microorganisms were introduced into coastal waters, in addition to great amounts of organic and inorganic nutrients which greatly enhanced the growth of microbial mass. On the other hand, pathogens probably get into coastal water with sewage which could become as a source of infection. The coliform and faecal coliform are widely used as indicator organisms for

pollution during analysis of coastal water samples. The faecal coliforms show a high specific positive correlation with faecal contamination, hence they are to be considered as best indicator for the microbial quality of water. The present study was concerned with the estimation of saprophytic bacteria, salt tolerant saprophytic bacteria, Gram-negative bacteria, coliform and faecal coliform numbers, saprophytic bacterial genera and coliform species in coastal water of Abu Dhabi City from October 1989 to September 1990, and this is a part of a large project to assess the sanitary quality of water in UAE coasts.

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MATERIALS AND METHODS

Sampling : Water samples from Abu Dhabi Coast (Fig. 1) were collected between October 1989 and September 1990 from fourteen sites which are designated from 1 to 14. Each site was sampled every month and 138 samples were collected in total. No sampling took place during February and April 1990. Site no. 7 and 14 were not sampled during October 1989. The sampling locations were randomly selected to provide comprehensive coverage of the Abu Dhabi Coast. Water samples were collected in sterile plastic bottles from subsurface layer by a team work from Desert and Marine Environment Research Centre, United Arab Emirates University. Both pH and temperature were recorded for each sample at sampling site. Samples were placed on ice and transported to the laboratory.

Bacterial Count : Saprophytic bacteria (SB) were enumerated on nutrient agar (Difco) supplemented with glucose and yeast extract 5 and 3 (g/l, w/v) respectively (SNA). Salt tolerant saprophytic bacteria (STSB) were also enumerated on the supplemented nutrient agar plus 3% salt (w/v) and termed 3% salt agar (SA). The salt was obtained from the water samples under study by solar evaporation. The standard plate count (SPC) procedure was used in this study which is similar to the standard methods of American Public Health Association (1980). Gram-negative bacteria (GNB) were counted using the same technique on MacConkey agar (MA).

Total coliform (TC) were detected and enumerated by the five-tube fermentation technique (MPN), where 10, 1.0, and 0.1 ml of the sample and its dilutions were inoculated in lactose broth (LB) from Difco. The multiple inoculation modified technique (m-MPN) established by Evans *et al.* (1980) was used

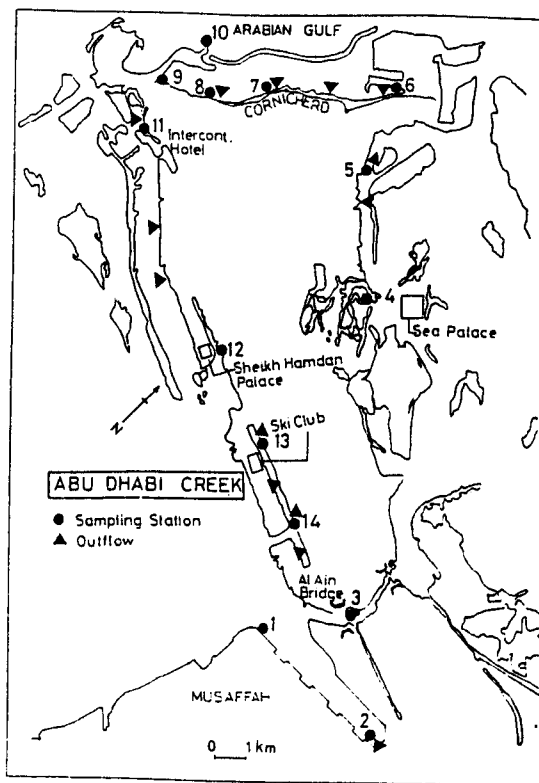


FIG. 1. Sampling sites of the Abu Dhabi coastal area.

for the completion of the steps. The faecal coliforms (FC) were detected and enumerated by inoculating tubes of EC medium from positive presumptive coliform tubes and incubated at 44.5°C for 24 hrs. The gas production was considered as a positive result indicating the presence of faecal coliforms. The EC medium contained (g/l, w/v) : trypticase 20; lactose 5; dipotassium monohydrogen phosphate (K_2HPO_4) 1.4; sodium chloride 5;

bile salts 1.5 and distilled water upto one litre. The medium was dispensed in screw-cap culture tubes with inverted durham vials and autoclaved. The prepared medium was tested by positive control stock culture before using. Some of the water samples were selected randomly and stored under refrigeration or at room temperature for different times. Then, the stored samples were investigated again using the above mentioned microbiological methods.

Identification : 260 morphologically distinct colonies were isolated randomly throughout the study from counting plates, subsequently they were identified to the genus level and 122 coliform isolates were also identified to the species level. A special test to check the presence of *Salmonella*, *Shigella*, *Brucella*, *Bordetella*, *Coxiella*, *Fransciscella*, *Mycobacterium*, *Neisseria* and *Mycoplasma* in the water samples were carried out. The identification procedures and the special tests for the presence of pathogenic bacteria were performed using standard tests, identification keys and manuals (Cowan, 1974; MacFaddin, 1980; Starr *et al.*, 1981; Gordon, 1987; Holt, 1984, 1986). The Gram-negative isolates were reidentified using API-20E (France).

RESULTS

The temperature of water showed obvious seasonal variations which gradually decreased from October 1989 until January 1990, then increased from March 1990 to August 1990 followed by a drop during September 1990 (Fig. 2). The lowest temperature values were recorded during the month of January and ranged from 18.8 to 21.5°C, whereas the highest temperatures were observed in July (ranged between 33.7 and 37.1°C). The water temperatures were found to be different from site to site during the same month.

The pH values recorded during October 1989 until March 1990 were relatively higher than those observed during May 1990 through September 1990, but the seasonal variations of pH between the samples were relatively low. The pH values varied from site to another through the same collection time. The pH of 138 samples which were collected during this study ranged between 8.0 and 8.56.

The standard plate count (SPC) of saprophytic bacteria (SB) in 138 water samples ranged from 0.9×10^4 to 4.2×10^4 CFU/ml (Fig. 2). The highest saprophytic bacterial counts in sites No. 1, 3, 4, 5, 8 and 10 were seen during October. Whereas, the lowest numbers were observed during July (for sites No. 3 and 5). Samples from sites No. 4, 8 and 10 showed the lowest total saprophytic counts during January, March and June respectively. Site No. 1 exhibited the least saprophytic counts during both January and June (Fig. 2). Samples from sites 2, 6, 12, 13 and 14 showed a maximum fluctuation of SB during the same month (August), but the minimum counts were observed in different months (December for site No. 6, 12, 13; January for site No. 2 and November for site No. 14). The samples from sites No. 7 and 11 contained the highest saprophytic count in September, while the site No. 9 showed the highest saprophytic numbers in March. The average saprophytic counts in the 14 surveyed sites of the Abu Dhabi Coast which were calculated monthly from October 1989 until September 1990 were as follows: 24.7, 19.3, 13.4, 13.19, 15.33, 13.39, 14.57, 18.29, 22.43 and 20.82 CFU $\times 10^4$ /ml respectively, whereas no samples were collected during February and April, 1990.

The counts of **STSB** generally correlate with the counts of **SB**, but the later continue to be the highest (Fig. 2). The monthly means

of STSB count in the fourteen studied sites from October 1989 to September 1990 were 2.7, 6.6, 4.9, 5.26, 7.06, 7.54, 8.46, 10.54, 13.86 and 11.5 CFU $\times 10^4$ /ml respectively, but February and April were not sampled.

The GNB counts correlated closely with the saprophytic counts on 3% salt agar, but the latter counts continue to be the highest

were observed during October. The lowest numbers of GNB were recorded during : December of sites No. 6, 9, 11, 13; January

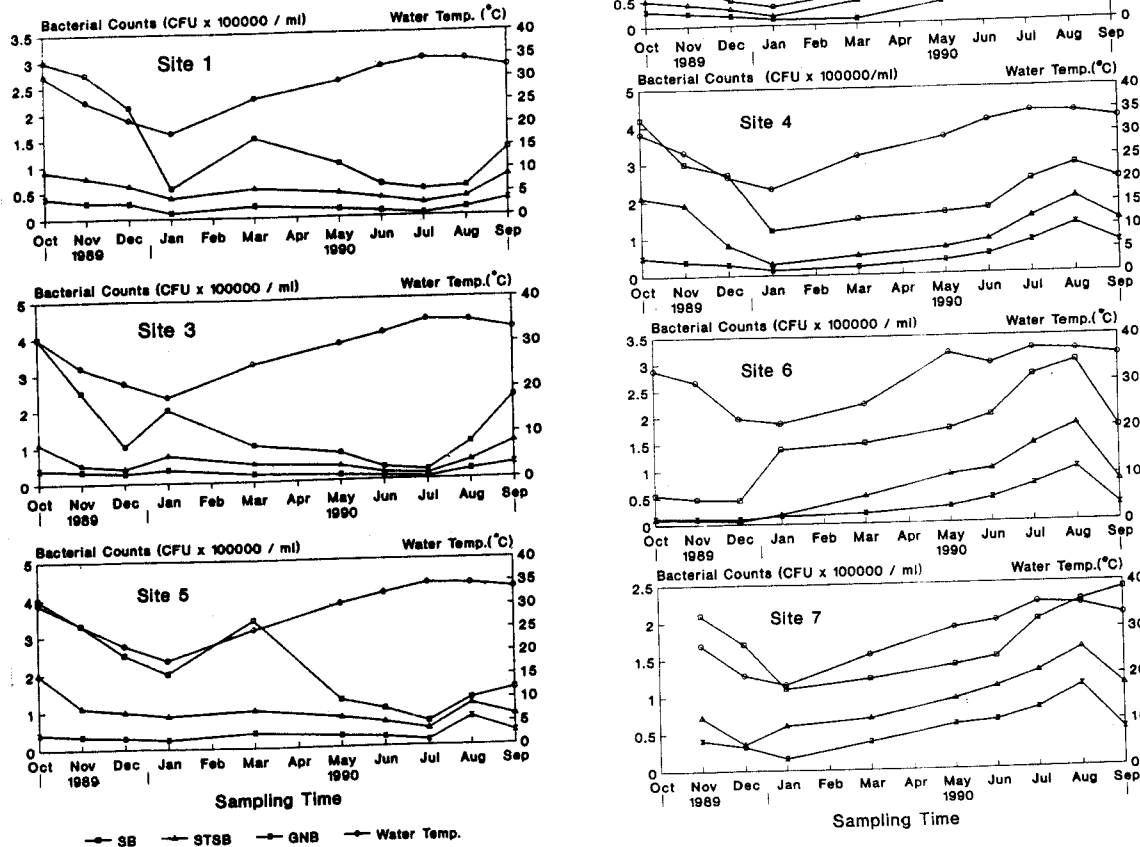


FIG. 2. The distribution of total saprophytic bacteria (SB), salt tolerant saprophytic bacteria (STSB), Gram-negative bacteria (GNB) and water temperature of fourteen sampling sites in Abu Dhabi Coast.

(Fig. 2). The highest number of GNB in samples collected from sites No. 2, 4, 6, 7, 9, 12, 13 and 14 were observed during August and for the samples from sites No. 3, 8, 10 and 11 during September, whereas the maximum fluctuation of the GNB in sites No. 1 and 5

for sites No. 2, 4, 7, 11 and July for sites No. 1, 3, 5. However, the least gram-negative counts were shown during November for samples from sites No. 8, 10 and 14 respectively (Fig. 2). The numbers of GNB ranged between 0.5-4, 1.2-10, 0.5-4, 1.4-13, 1.5-4.2, 0.9-10, 1.6-11,

1.5-7.5, 0.9-5, 0.8-3.5, 1-8.5, 2-10, 0.9-15 and 3.2-16.5 CFU $\times 10^4$ /ml in water samples collected during one year from sites No. 1

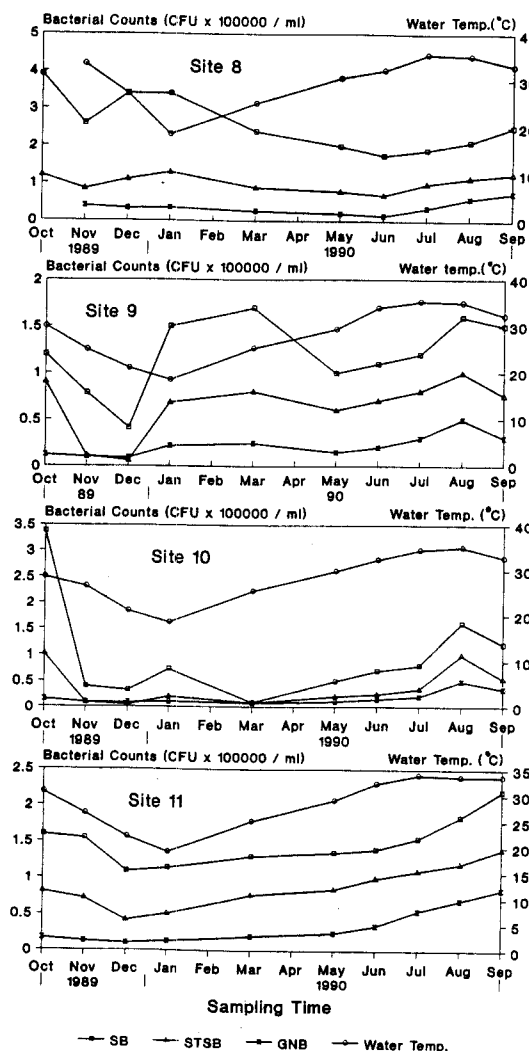
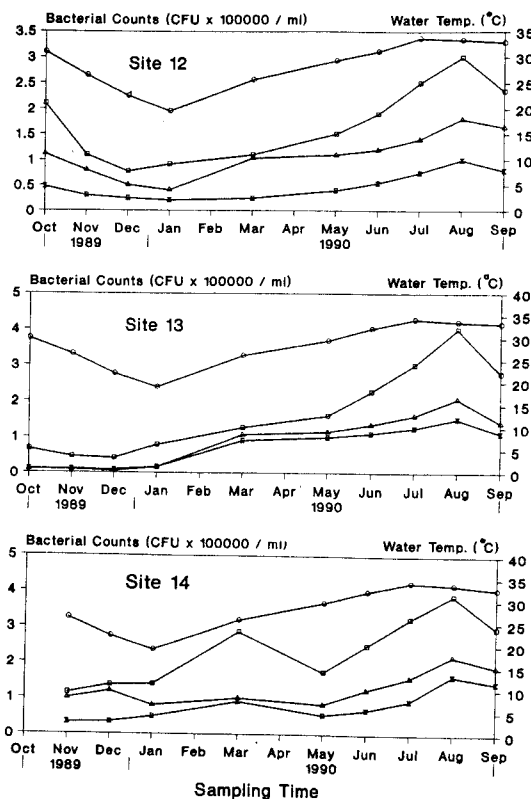


FIG. 2. Continued.

through No. 14 respectively. The average gram-negative count in the fourteen surveyed sites were calculated monthly from October 1984 until September 1990 (February and April were not sampled) were as follows : 2.9, 1.5, 2.16, 2.14, 3.26, 3.5, 4.21, 5.46, 8.39 and 6.32 CFU $\times 10^4$ /ml respectively.

The total as well as faecal coliform counts were also determined in the collected water samples (Table 1). 120 out of 138 samples were found to contain a verified total coliform (about 86.96%), eighteen samples were found to be free from coliform, whereas ninetyfive samples out of 138 were found to contain a verified faecal coliform (about 68.84%). The total coliform were confirmed in all samples collected during May, June, July and September.



They were also verified in all samples collected during the duration of this study from sites No. 4, 5, 8, 11 and 14. The study revealed that 25 water samples were found to contain a total coliform, but they were totally free from faecal coliform. The faecal coliform from site No. 10 were detected only during July, while

the total coliform bacteria were observed only during October, May, June, July and September. This site is considered the least contaminated compared with the other sites.

The identification results (Table 2) showed the presence of 5 dominant Gram-positive and 15 Gram-negative genera. The 122 coliform isolates were : 89 *Escherichia coli*, 9 *Klebsiella pneumoniae*, 8 *Citrobacter freundii*; 8 *Enterobacter agglomerans*, 3 *Enterobacter aerogenes* and 5 unidentified isolates. The pathogenic genera *Salmonella*, *Shigella*, *Brucella*, *Bordetella*, *Coxiella*, *Francisella*,

Mycobacterium, *Neisseria* and *Mycoplasma* were not present in the water of Abu Dhabi Coast.

DISCUSSION

The water temperature recorded during sampling exhibited considerable seasonal variations, which varied between 18.5 and 37.1°C. The temperature of water gradually decreased from October 1989 until January (Winter) 1990 when it reached the lowest level, then it gradually increased during March (Spring) until July (Summer) 1990, where the temperature reached its maximum fluctuation. The temperature recorded in the sampling sites during March until July ranged between 24.9° and 37.1°C, whereas it ranged between 32.4° and 36.7°C during August and September 1990. These levels are relatively high and correlate with weather temperature where the summer is considered the longest season compared with other seasons in this geographical area. The obvious observation is the variation of temperature in different sites during the same sampling date, which is probably due to the activity of the 'biota' and/or the sewage outflow in certain sites.

Relatively limited fluctuations of the pH were found in the fourteen sampling sites in the Abu Dhabi Coast, where it varied between 8.0 and 8.56. In general, the pH slightly increased during October 1989 until December 1989, then decreased slightly during January 1990 through July 1990, while a small rise was observed during August 1990 then dropped again in September 1990. Slight deviations in the results were observed in some sites probably due to the action of some local factors. Lucht (1964) mentioned that the average reaction of many lakes is pH 7, but Dietrich and Kalle (1957) reported that the pH of the surface zone of the sea is 8.2 Rheinheimer (1976) stated that relatively large fluctuations of the pH are found in eutrophic lakes (between 7 and 10).

TABLE 2. The dominant bacterial genera distributed in the Abu Dhabi coastal water and their appearance (%)

Genera	Appearance %
Gram-positive	
<i>Bacillus</i>	30.0
<i>Staphylococcus</i>	6.54
<i>Micrococcus</i>	3.46
<i>Corynebacterium</i>	1.92
<i>Arthrobacter</i>	0.77
Unidentified isolates	8.08
Gram-negative	
<i>Alteromonas</i>	10.77
<i>Pseudomonas</i>	5.38
<i>Alcaligenes</i>	4.62
<i>Enterobacter</i>	4.62
<i>Escherichia</i>	4.52
<i>Vibrio</i>	3.08
<i>Flavobacterium</i>	1.92
<i>Chromobacterium</i>	1.54
<i>Citrobacter</i>	1.54
<i>Aeromonas</i>	1.15
<i>Moraxella</i>	1.15
<i>Photobacterium</i>	0.77
<i>Serratia</i>	0.77
<i>Xanthomonas</i>	0.77
<i>Acinetobacter</i>	0.38
Unidentified isolates	6.15

The slight variation of pH value from site to another during the same sampling date probably means that, the sampling sites may be affected by a different physical, chemical or biological factors.

Much attention is focussed on the role of standard plate count bacteria in water. The SPC is considered by some investigators to be a better indicator of water quality than the coliform index (Geldreich *et al.*, 1972; Muller, 1977; Mood, 1977; Ptak and Ginsbury, 1977). LeChevallier *et al.* (1980) mentioned that, elevated SPC levels indicated a potential health risk posed by opportunistic pathogens. The SPC of SB, STSB and GNB were enumerated in the present study. The bacterial counts of all types were found to correlate generally with each other in the same sampling site and mainly fluctuate at the same season. The bacterial counts also correlated with water temperature. However, with extreme rise in water temperature in some sites the bacterial content diminished.

In most sampling sites the bacterial counts fluctuated maximally during August or September (late summer), but a higher bacterial number in some sampling sites was observed outside this time. This is probably due to a local supply of food and or alien microflora from sewage out-flow or soil. The bacterial content varied from sampling site to another as well as from month to month, but site No. 10 was found to contain the lowest bacterial counts, probably because it is located in open-sea. Rheinheimer (1965, 1970) reported that microorganisms for which the living conditions in a particular water are favourable will quickly multiply at summer temperature, but for other such conditions are unfavourable and will quickly perish, as can be observed with many bacteria from sewage, freshwater or soil when they get into brackish or sea water.

The numbers of SB, STSB and GNB varied between 0.9-42; 0.4-22.5; and 0.5-16.5 CFU $\times 10^4$ /ml respectively. This reflects the extreme fluctuation which occurred between the different bacterial groups. In the North Sea and the Baltic, the saprophytic number is between ten thousands and several hundred thousands. In the adjoining area, they are between one thousand and some ten of thousands, and at a somewhat greater distance from the coast (several nautical miles) less than one hundred to several thousands (Rheinheimer, 1976). In general, the number of SB continues to be the highest compared with the number of other bacterial groups, followed STSB and finally the GNB.

The sewage out-flow in and/or neighbouring the sampling sites is unknown if it is properly treated or not. However, this does not change the principle of its extreme effect on the microbial contents of the coastal water. It makes the nutritional conditions highly favourable, so that bacteria not only accumulate in the coastal water, but can also multiply vigorously. Furthermore, the bacterial content may change or be affected by many other physical, chemical and biological factors such as sun light, pressure, turbidity, salinity, wind, cloud cover, waves, depth, currents, pH, temperature, dissolved gases, inorganic substances, organic substances, biota and others.

Some of the creeks or bays distributed in Abu Dhabi Coast were found to be loaded with coliforms. In general, the MPN of coliforms and/or faecal coliforms correlated with the number of other bacterial groups. In general, the high incidence of coliforms matched with the presence of high counts of SPC bacteria. But, the presence of extremely high numbers of SPC bacteria minimized the numbers of coliforms, and this is probably due to the masked detection. All samples collected during

the summer (May, June, July, August and September) were found to contain a verified total coliform except for one sample. Furthermore all samples collected during July and August (except site No. 2) were loaded with faecal coliforms. These organisms were most probably introduced into the coastal water with sewer out-flow.

The sunshine during the spring helps the phytoplankton to flourish. However, death of phytoplankton may start with elevated temperature of the summer leading to the production of much nutrients and creating favourable conditions. Occasionally in such environments, the introduced faecal and/or total coliforms become predominant. The use of certain locations as recreational sites during the long summer months will contribute to increased incidents of faecal and/or total coliform.

Twentyfive samples were found to be free from faecal coliforms, but they were found to contain verified total coliforms. The sanitary quality of coastal waters is best indicated by faecal coliforms, because the latter exhibit a highly specific positive correlation with the contamination by faecal material from warm blooded animals.

An interesting observation involving specific dilutions of some water samples with a positive verified faecal and/or total coliform, whilst the undiluted sample continue to be negative. This masked result in the undiluted samples is probably due to the extremely high numbers of non-coliform bacteria present in the samples which interfere with the indicator organisms. Many investigators stated that, the high number of non-indicator organisms in samples could result in the failure recovery of the indicator organisms (Weaver and Boiter, 1951; Reitler and Seligmann; 1977, Ahmed *et al.*, 1967; Geldreich *et al.*, 1972; Evans *et al.*, 1980; Hsu and Williams, 1982; Pagel *et al.*, 1982).

Preservation of the collected water samples at room temperature or under refrigeration caused the loss of the majority of its bacterial content. When the samples were kept in the refrigerator (4-5°C), its total coliforms as well as faecal coliforms totally disappeared through a period of one to three months and furthermore its bacterial flora was markedly reduced. Likely, similar results were obtained through a period of 12 and 45 days, if samples were stored at room temperature (25-35°C). This is probably due to the natural self-purification of water. Rheinheimer (1976) mentioned that, by the breakdown of organic refuse, microorganisms contribute decisively to the natural self-purification of waters. During these processes the concentration of nutrients is diminished and eventually the bacterial content decreases. Rheinheimer further mentioned that 'waters differ a great deal in their power of self purification'.

Five Gram-positive and fifteen Gram-negative genera were commonly present in Abu Dhabi coastal area during the duration of the study. Most frequent was the genus *Bacillus* (30%) followed by *Aeromonas* (10.77%), *Staphylococcus* (6.54%) and *Pseudomonas* (5.38). *Acinetobacter*, *Arthrobacter*, *Photobacterium*, *Serratia* and *Xanthomonas* were observed in very small numbers. *Escherichia coli* was represented the highest number of coliforms (73% out of total coliform isolates), whereas *Enterobacter aerogenus* was represented the least number of coliform isolates (2.5%) in the water samples. Study concerning the detection of some pathogenic bacteria in water samples revealed that the genera *Salmonella*, *Shigella*, *Brucella*, *Bordetella*, *Coxiella*, *Francisella*, *Mycobacterium*, *Neisseria* and *Mycoplasma* were totally absent. In general, the bacterial genera present in the water showed the occurrence of some strains that are usually associated with sewer out-flow.

In conclusion the risk of polluted coastal water is not restricted in causing disease for both aquatic plants and animals, but is a great hazard associated with the

public health. Consequently the sewage refuse must be well treated before delivery into the coastal water. Treatment not only by eliminating the microbial flora, but also by eliminating or greatly minimizing the extensive nutrient-load of the sewage-refuse, or the sewage drainage

should be totally excluded from coastal water. By this manner the polluted coastal sites probably become purified via the natural self-purification and the expected period needed for this process would be maximum of three months.

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