

## Seasonal Incidence of Some Heterotrophic Aerobic Marine Bacteria in an *Avicennia marina* Habitat along a Qatari Coast

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**Abstract:** Total bacterial counts and five different groups in sea water were investigated from May 1998 to May 1999 at two *Avicennia marina* L. inter tidal sites 1 and 2 and a control *A. marina* free site 3. The highest total bacterial counts in sites 1 and 2 waters were recorded during June and September 1998 respectively. However, site 2 exhibited higher total counts and showed an additional smaller total count peak during November. Proteolytic, cellulolytic, amylolytic and lipolytic bacterial group counts followed similar trend in sites 1 and 2. Although amylolytic and proteolytic counts were higher in site 1. Lowest numbers were recorded during the winter months. Site 3 recorded peak counts for the different groups during June-July and were of the same order as site 1 but lower than site 2 except for the proteolytic group which was higher than that for sites 1 and 2. Spore formers in all sites were several orders of magnitude lower than other groups. In general, the study showed that bacterial counts were higher during warmer months. Water temperatures had great influence on bacterial counts compared with other environmental parameters studied.

**Key words:** Marine bacteria, waters, seasonal incidence, Qatar

### Introduction

The invisible activity in the mangrove ecosystem could be estimated for example by just scooping few grams of mangrove soil or transferring few milliliters of surrounding sea water and examining it microscopically and studying different bacterial densities and activities (Anonymous, 1982). One would be amazed to find greater than  $10^5$ - $10^9$  cfu per gram of soil or 1ml of water. These bacteria are capable of keeping the system in equilibrium if not disturbed. This indigenous marine bacteria helps in breaking down leaf litter and other natural sources of nutrients (Twilley, 1985) of plant and animal origin, thus balancing the biological system from environmental point of view. In the mangrove system nothing is wasted, nutrient rich leaves are biodegraded by microorganisms or eaten by other forms of life living on the mangrove forest floor surrounding waters.

Studies of mangroves in different parts of the world have shown that thousands of tones of mangrove litter are introduced into sea waters each year (O'Grandy, 1986), hence the bacterial numbers in the waters is as dense as those in the mangrove mud.

Searching literature indicates great scarcity of information on the abundance and seasonal distribution of some aerobic heterotrophic marine bacteria along the Arabian Gulf Coastal waters including the Qatari coast (Mahasneh, 2000). Hydrolytic marine bacteria is not an exception and if any is available it is restricted in its scope (Mahasneh and Sayed, 1997). This article is a report on the seasonal distribution and population densities of aerobic heterotrophic marine bacteria in *A. marina* surrounding waters in two selected *A. marina* sites and a third *A. marina* free control site. Some physicochemical parameters of the marine environments are also recorded, these included water and atmospheric temperatures, water-pH and salinity.

### Materials and Methods

**Study sites and sample collection:** Water samples were collected from 3 different sites (Fig.1). Sites 1 (Al-Khor) and 2 (Dakhira) are *A. marina* L. forests, the third site 3 which is a sandy beach devoid of *A. marina* trees, was assigned as the control site.

Site 1 is located at a mangrove (*A. marina*) forest in the inter-tidal zone near Ras-Al-Matbach. The neighbouring terrestrial

area is covered with halophytic plants and Sabkhas. The site floor is rather sandy sediment at Ras-Al-Matbach, north east of Al-Khor is heavily populated by *A. marina* trees of varying sizes and ages. The soil is rich in sandy deposits with abundant fragments of shells with no obvious anaerobiosis of soil. Site 2 (Dakhira) is also located at a mangrove forest south east of Dakhira at about 7km north of site 1. The site floor is sloppy gray sediment characterized by anaerobiosis activity exemplified by soil blackening. The site exhibits lower density of *A. marina* trees compared to site 1. The neighbouring terrestrial area is heavily covered with halophytes. The control site 3 is about 6km to the north-west of site 1 and is a sandy very clear beach with no mangrove trees; the neighbouring terrestrial environment is inhabited by low density halophytic plants.

Samples of sea water were collected monthly in sterile 500 ml plastic bottles. *A. marina* leaves were collected in sterile sampling bags, and were placed in an ice box and were transported to the laboratory within 2-3 hours of collection for analysis.

For bacterial analysis of water samples, 1 ml of each sample was serially diluted in aged sterile sea water and 0.1 ml aliquots of appropriate dilutions were plated onto suitable media plates in duplicate at least as detailed below.

For bacterial spores determination, water samples were treated at 80°C for 20 minutes and samples were then plated onto suitable plates of marine agar.

Bacterial counts were then carried out using the media, temperatures and incubation times listed below:

- Marine agar plates for total viable marine bacteria and spore formers.
- Marine agar plates supplemented with 2% (w/v) cellulose to enumerate cellulase producers.
- Marine agar plates supplemented with 2% (w/v) skimmed milk to detect bacterial colonies producing the protease enzyme.
- Marine agar plates supplemented with 2% (w/v) starch to detect amylase producers.
- Marine agar plates with added 2% (v/v) Tween-80 to detect lipase producers.

All samples were plated in duplicates at least, incubated at 30 ± 1°C for 2-4 days and numbers were then recorded. Results of enumeration are reported as cfu.ml<sup>-1</sup> of water

samples and culture media were prepared.

**Environmental parameters:** Water and atmospheric temperatures, pH and salinity was measured. Analysis of variance was applied to the overall bacterial groups mean values recorded in the different stations. (Nie *et al.*, 1975)

### Results and Discussion

The annual mean atmospheric temperatures for site 1 was 31.3°C with minimum of 18°C in the cooler months of December and January (Table 1). The temperature rose upto 45°C in June. The atmospheric annual mean temperature for station 2 was 30.2°C with a minimum of 20°C in January-February and a maximum of 43°C in June-July. The control site 3 annual mean atmospheric temperature was 32.6°C with a minimum of 21°C recorded in January rising to 45°C in June. Seasonal atmospheric temperatures for the study sites indicated a wide fluctuation (0-20°C) between winter and summer months. Sea water temperatures (Table 1) for the different sites showed annual mean values of 26.4°, 27.2° and 28.0°C for sites 1, 2 and 3 respectively with minimum of 20, 21 and 21° C respectively and were recorded during December-January. However, maximum water temperatures were also recorded respectively and values of 35, 34 and 37°C for sites 1, 2 and 3 were observed in July-September. The annual mean salinities were 48.0, 41.5 and 40.2 g L<sup>-1</sup> for sites 1, 2 and 3. The sea water annual mean pH values were 7.8, 7.81 and 7.65 respectively for stations 1, 2 and 3. Mahasneh and Al-sayed, (1994 and 1997) reported rather similar values of water temperature, salinities and pH values of Bahrain near shore and open sea waters.

Different sites water temperatures (Table 1) did not show great variations between sites, (P < 0.05) where the highest temperatures were recorded during the summer months of June-July, and the lowest were recorded during winter months of December-January. The seasonal pattern of sea water followed more or less the same trend of atmospheric temperatures for all sites. It is also evident that no seasonal variations in water salinity and pH values were observed between the different sites 1, 2 and 3. However, site 2 salinities were lower than values recorded for the two other sites. Tendency of increased salinity values was observed during the months of April, May and June for both sites 1 and 2. Whereas the control site 3 showed a more homogenous and rather lower salinity values all through the study period. Conversely speaking and looking at the above presented data, it is fair to say that rather little, if any, significant variability for atmospheric and water temperatures, salinity, and pH were observed among the three different sites 1, 2 and 3. This situation is not an exception in the shallow Arabian Gulf waters known with little water currents (Mahasneh and Al-sayed, 1994, 1997 and Madany *et al.*, 1986). Day *et al.*, (1996) did not observe seasonal variations in water and soil salinity water and soil salinity in a south eastern Mexican mangrove forest. Mahasneh and Al-sayed, 1997 reported annual mean salinities for Bahrain pelagic and near shore waters in the order of 46 ± 1.53 with maximum of 48 and minimum of 40 g L<sup>-1</sup>. These variations as it is the case in this study were not as it appears linked to neither water nor atmospheric temperature and no significant correlations were observed. This may be understood if we realize that the annual precipitation and runoffs to the sea is negligible by any rate (Mahasneh and Al-sayed 1994, 1997 and Madany *et al.*, 1986). Salintilan (1997) reported the decline of the above ground biomass communities of *A. marina* with increasing salinity. Meyer-Reil *et al.*, 1980, reported similar observation

as this report when studying interrelationships between microbiological and chemical parameters in a marine environment. However, no great fluctuations in salinity values were observed in this study (Table 1), though the mere effect of salinity alone on the *A. marina* communities was not in the scope of this study.

**Bacterial abundance and activity:** Bacterial heterotrophic activity in marine environments has been of increasing interest to ecologists (Mahasneh and Al-sayed, 1997, Mahasneh *et al.*, 1985 and Strickland, 1973). Most bacteriological studies of marine environments have examined only few parameters and therefore have yielded a partial understanding of the interrelationships between chemical, physical and microbiological parameters, (Meyer-Reil *et al.*, 1978 and Benosoussan, 1980)

Table 2 summarizes the distribution and abundance (monthly mean ± S.D) of viable aerobic heterotrophic total (Total), protease (Prot), amylase (Amy), cellulase (Celu), lipase (Lipa) and spore forming (Spor) bacteria in Al-Khor waters site 1, Dakhira site 2 and control site 3 (Fig.1). Rather significant (P < 0.05) seasonal differences in mean values were found between different groups of bacteria within the same site. Similarly less significant differences were found between different groups of bacteria in the different sites (F<sub>(2,4)</sub> = 19.69, P < 0.001) with marked reduction in numbers of different groups of bacteria in control site 3 which is devoid of *A. marina* trees compared with the two other *A. marina* forested sites. However little significant mean value differences were observed for bacterial groups within each site (F<sub>(2,4)</sub> = 2.94, P < 0.05) and control site 3. Rather significant (P < 0.05) seasonal differences in mean values were found between different groups of bacteria within the same site. Similarly less significant differences were found between different groups of bacteria in the different sites (F<sub>(2,4)</sub> = 19.69, P < 0.001) with marked reduction in numbers of different groups of bacteria in control site 3 which is devoid of *A. marina* trees compared with the two other *A. marina* forested sites. However little significant mean value differences were observed for bacterial groups within each site (F<sub>(2,4)</sub> = 2.94, P < 0.05). Mahasneh *et al.*, 1985 and Wallberg *et al.* (1999) found similar trend for aerobic heterotrophic bacteria in the gulf of Aqaba and in a tropical coastal ecosystem respectively. In Al-Khor site 1, total viable aerobic heterotrophic bacterial counts reached maximum 4.85 × 10<sup>6</sup> cfu ml<sup>-1</sup> of water during June 98 and a minimum of 1.2 × 10<sup>4</sup> during November 98 and March 99. Among other bacterial groups studied were the protease and cellulase producing bacteria, these two groups followed a similar trend to that of the total bacterial counts, where both bacterial groups recorded maximum counts in June 98 (7.1 × 10<sup>5</sup> and 2 × 10<sup>5</sup>) cfu ml<sup>-1</sup> respectively. Minimum counts were recorded during November 98 and were in the order of 1.3 × 10<sup>3</sup> for both groups. Amylase and lipase producing bacteria recorded maximum counts (5.9 × 10<sup>7</sup> and 6 × 10<sup>5</sup>) cfu ml<sup>-1</sup> respectively both in June 98 and minimum counts were recorded in December and November 98 and were of the order of protease producers (1.5 × 10<sup>3</sup>) and 5 × 10<sup>2</sup> for lipase producers. The lowest recorded bacterial counts in these sites were of the aerobic spore formers which were highest (3 × 10<sup>4</sup> cfu ml<sup>-1</sup>) during June 98 and stayed almost in the order of 2 × 10<sup>2</sup> to 3 × 10<sup>2</sup> through the study period. The distribution, abundance and biological activity of bacteria in aquatic environments have been reported to be closely related to the physical properties and organic carbon content of the ecosystem (Meyer-Reil, 1978, Dale, 1974 and Martinez, 1996). It is observed

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Table 1: Study sites and some environmental parameters: annual mean  $\pm$  S.D. (Maximum and minimum values recorded)

Site and location	Water depth (M)	Water	Atmospheric Temp. (°C)	Salinity g L <sup>-1</sup>	Water pH
(1) Al-Khor 25°40' 00N 51°38' 85E	1.5	28.4 $\pm$ 2.68 (20-35)	31.35 $\pm$ 2.95 (18-45)	48 $\pm$ 3.45 (42-60)	7.8 $\pm$ 0.18 (7.8-8.2)
(2) Dakhira 25°54' 32N 51°35' 00E	1.5	27.25 $\pm$ 2.65 (21-34)	30.20 $\pm$ 1.75 (20-43)	41.5 $\pm$ 1.62 (41-50)	7.81 $\pm$ 0.31 (7.8-8.25)
(3) Control 55°20' 38N 51°32' 00E	1.5	28.0 $\pm$ 2.25 (21-37)	32.6 $\pm$ 3.95 (21-45)	40.25 $\pm$ 1.48 (40-45)	7.85 $\pm$ 0.15 (7.85-8.1)

Table 2: Distribution of different bacteria (cfu.ml<sup>-1</sup>) of the study site waters. Monthly log means  $\pm$  SD for the period May 98 to May 99. Producers of amylase (Amy), Cellulase (Celu), Protease (Prot), Lipase (Lipa), Spores (Spor) and total bacterial counts (Tota L). Numbers in brackets indicate minimum and maximum numbers recorded during the study.

Site	Bacteria					
	Tota	Amy	Celu	Prot	Lip	Spor
Al-Khor(1)	4.7 $\pm$ 1.53 (1.3-8.8)	5.02 $\pm$ 1.4 (3-7.78)	4.3 $\pm$ 0.91 (3.04-5.81)	5.02 $\pm$ 1.70 (2.7-8.88)	4.59 $\pm$ 1.34 (2.3-6.9)	3.02 $\pm$ 0.93 (1.3-4.49)
Dakhira(2)	5.57 $\pm$ 2.17 (3.28-10.06)	4.25 $\pm$ 1.08 (2.48-6.49)	4.60 $\pm$ 1.15 (2.78-6.72)	4.46 $\pm$ 1.5 (2-8)	4.57 $\pm$ 1.20 (2.48-6.7)	3.91 $\pm$ 1.38 (2-6.53)
Control(3)	4.4 $\pm$ 1.52 (1-8.62)	4.42 $\pm$ 0.99 (2.95-6.61)	4.32 $\pm$ 0.92 (3-0.64)	8.5 $\pm$ 1.6 (3-8.53)	4.4 $\pm$ 1.29 (3-6.6)	2.9 $\pm$ 1.6 (1-6.6)

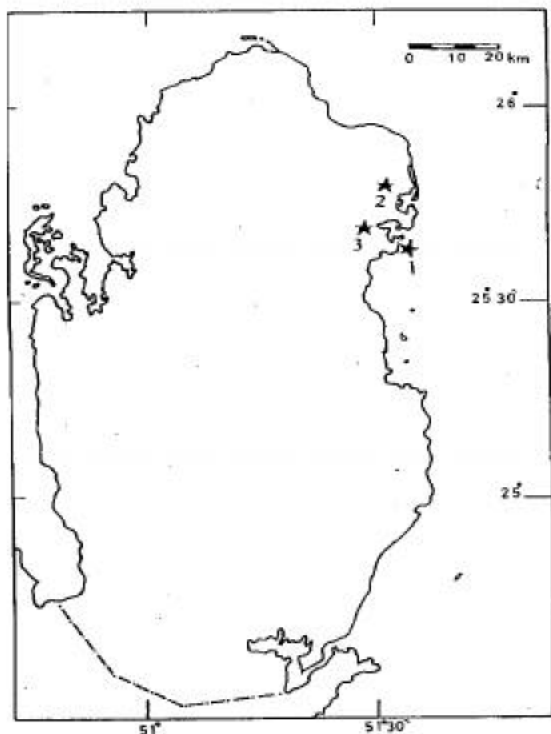


Fig. 1: Map of Qatar showing the study sites Al-Khor 1, Dakhira 2 and Control 3.

generally, that minimum bacterial counts of the different groups were found in winter and maximum counts in summer and this was very clear in the cases of total, protease and cellulase producing bacteria. Similar seasonal trends have been

recorded in marine environments (Martinez *et al.*, 1996, Mayer-Reil *et al.*, 1980 and Wilson and Stevenson, 1980). Water temperature seasonal fluctuations may participate in inducing such variations in bacterial counts. The nature of Al-Khor site 1, being *A. marina* forest site favors the stability of the different bacterial populations in waters. This may be linked to the abundance of available organic matter as a result of decomposing leaf-litter of *A. marina* and other organic detrital materials. The period of low bacterial density among the different groups could be associated with an increase in the number of detritivores such as meiofauna which feed mainly on bacteria (Wahbeh and Mahasneh, 1984). However, it is reported that decomposing mangrove litter with phytoplankton, bacterial and secondary production is important mainly for sustaining the microbial food chain and nutrient regeneration (Wafar *et al.*, 1997). In Dakhira site 2 which is a less dense forest of *A. marina*, summary of the bacterial abundance and densities are presented in Table 2. Total aerobic viable bacteria reached maximum higher counts ( $2 \times 10^{11}$  cfu ml) during September and minimum of  $2 \times 10^9$  cfu ml<sup>-1</sup> during November and the counts remained of the low side up to April 99, where it reached  $10.5 \times 10^9$  cfu ml<sup>-1</sup>, then declined again. This abundance indicates the presence of two peaks of bacterial number. These peaks coincide with the beginning of Autumn and Spring seasons where productivity may bloom in oceanic water similar to the Arabian Gulf shallow waters. At the higher production rate of such environments, the amounts of released photo assimilated carbon is probably higher and so would the abundance of bacteria (Mazure and Field, 1980 and Biddle and Fletcher, 1995). Mahasneh and Al-Sayed (1997) studied the seasonal incidence of some marine bacteria in Bahrain pelagic and near shore waters and found that water temperatures have greater influence on distribution of different bacteria compared with other environmental factors such as salinity, pH and dissolved oxygen. Moles *et al.* (1988), studied the density of certain marine bacteria in the Atlantic coast and observed marked increase of numbers from May through October followed by a gradual reduction during November and December where it

remained there from January through mid March and a sharp increase from April to summer levels prevailed.

Protease producing bacteria reached maximum numbers of  $10^8$  cfu ml<sup>-1</sup> during June and declined to  $3 \times 10^2$  ml<sup>-1</sup> during November and stayed around  $10^4$  from November to May. Amylase producers reached peak number of  $1.7 \times 10^7$  during July declining to  $7.5 \times 10^5$  in September and from November to May numbers stayed more or less in order of  $3 \times 10^3$  to  $9 \times 10^4$ . Cellulase and lipase producers in Dakhira site 2 followed somehow regular pattern of distribution reaching maximum of about  $4 \times 10^6$  during June and September and declined to stay in the range of  $10^3$  to  $10^4$  cfu ml<sup>-1</sup> with minimum numbers during November 98 and February 99. Spore formers followed similar pattern of distribution as that of Al-Khor site 1 where numbers in general were rather low ( $10^2$  to  $3.8 \times 10^3$  cfu ml<sup>-1</sup>) with no real variation in the mean values through the study period (Table 2). Numerous studies have quantified the activities of hydrolytic ectoenzymes in marine environments and showed that it is an ubiquitous reflection of phenotype of bacterial population in seas. Martinez *et al.* (1996) studying protease and lipase producers among pelagic marine bacteria, they found activities along a broad range suggesting shifts in the dominant species of bacteria at a given time and space, could strongly influence the rates and pattern of polymer and particle hydrolysis in sea water. This agrees with the notion that only small part of the microbial community is active at any given time (Muzure and Field, 1980 and Biddle and Fletcher *et al.*, 1995). Ferguson *et al.*, 1998 demonstrated that within 16-32h at 25°C, significant changes in sea water occurred in bacterial total numbers, culturable numbers, cell volume, turn over of amino acids and predominant groups and species. Factors such as time, temperature, organic content and light seem to especially affect the bacterial community. The control site 3 is a very fine sandy beach, devoid of *A. marina* trees, no indication of anaerobiosis in the sea-bed. Total bacterial counts ml<sup>-1</sup> of water in the control site reached a peak of  $9 \times 10^9$  in June and July (Table 2) which is of the same order for Al-Khor site 1. However these counts were lower than that recorded in Dakhira site 2, although the highest of  $5 \times 10^{10}$  to  $10^{11}$  cfu ml<sup>-1</sup> in this site was reached in September towards the end of summer. This coincided with algal blooms and heavy growth of some sea-grasses, a situation which was unique to this site but not Al-Khor or Dakhira water.

For protease producing bacteria in the control site, highest counts were recorded in June  $5 \times 10^8$  cfu ml<sup>-1</sup> and September  $5 \times 10^5$  cfu ml<sup>-1</sup> (Table 2) and numbers fluctuated between lowest of  $8 \times 10^2$  cfu ml<sup>-1</sup> in November and an average of  $2 \times 10^4$  to  $5.5 \times 10^5$  from July 98 to May 99, a trend which has been observed for the other two sites. A common observation in the three sites is that concerning viable aerobic heterotrophic protease producers which seems to be more abundant than other bacterial groups, which may indicate available protein substrates in the waters. Becquevort *et al.* (1998) reported concomitant evolution of ectoprotease producers with phytoplankton blooms in coastal waters of the North Sea. Colwell and Morita (1972) reported higher proteolytic bacteria in marine habitat as compared to fresh water and soil habitats. Cellulase producing bacteria in the control site (Table 2) were of the same order for Dakhira site 2, but both were lower than that in the Al-Khor site 1, where the maximum counts recorded ml<sup>-1</sup> of water in these sites were  $5 \times 10^6$  cfu ml<sup>-1</sup> in June compared to  $9 \times 10^9$  cfu ml<sup>-1</sup> for Al-Khor site 1. Amylase and lipase producing bacterial counts of the control site 3 did not show significant variations compared with Al-Khor and Dakhira sites. However these counts were slightly lower. This

may be explained by the nature of the control site which is devoid of *A. marina* forest with what this may impact upon bacterial abundance and activity. Spore formers also were very low in the order of  $10^2$  (Table 2) ml<sup>-1</sup>, which is similar to the other two sites.

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