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SEASONAL VARIATION AND IDENTIFICATION OF ISBJ DIATOMS IN AL-NAWRAS BAY IN SAUDI ARABIA

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Abstract: This study was implemented during the period from spring 2008 to winter 2009 in Al-Nawras Bay located in Jeddah city, KSA to monitor the seasonal fluctuation of diatoms numbers as affected by the most predominant ecological factors in the bay. Identification of the prevalent diatoms species in the four study sites was achieved. Results showed that 24 diatoms species belonging to 12 genera were detected in the four tested sites. Of those, eight species were found in all four sites, while some species were found in one site but not in the others. For example, the species Navicula sp. 1, Navicula sp. 2, Nitzschia angularis, Nitzschia sp. 3 and Surirella fastuosa were found only in site A, which is the nearest site to a discharge point of mixed ground water in the Bay. Further, both diatoms species Amphora acutiuscula, and Surirella scalaris were found in site B that is located adjacent to site A. Meanwhile, site C contained the three species Amphora sp. 1, Amphora sp. 2 and Campylodiscus sp. The control site D, which is the farthest point from the pollution source, contained six diatom species (i.e., Tricertuim dubium, Amphora sp. 3, Bacteriastrum sp., Diploneis sp., Mastogloia sp. and Surirella hybrida). In terms of nutrient content and diatom numbers, site A recorded the highest number of diatoms and the highest content of nitrate, nitrite, ammonia, phosphate and silicate in comparison with the three other sites. The average number of diatoms in the four sites ranged from 2,343 to 19,188 cell/L with the fewest in site D and the highest in site A. Regarding seasonal variations of diatom numbers, the summer season significantly surpassed the other three seasons where the diatom numbers were 19,514, 9,158, 3,773 and 1,640 cell/L for summer, spring, autumn and winter, respectively. A positive, significant correlation was found between diatom numbers and transparency, nitrate, ammonia, sulphate and chlorophyll b, while a highly significant positive correlation was recorded between diatom numbers and chlorophyll a, chlorophyll c and carotenoids. In contrast, a negative correlation was detected between the diatom numbers and pH.

Keywords: Diatoms, Al-Nawras Bay, seasonal variation, identification, Saudi Arabia

INTRODUCTION

The Red Sea is an unusual marine system. Entirely surrounded by desert, freshwater input is low, and evaporation is high. While as much as 2,000 m deep, the Red Sea is separated from the Gulf of Aden and Indian Ocean by a sill that is never more than 100 m deep, limiting water exchange. Thus it tends to be hypersaline. Its surface currents are dominated by monsoon seasons. Halim gives a very good overview of Red Sea zooplankton and phytoplankton ecology and distribution.

Diatoms from ocean waters around the Arabian Peninsula remain poorly known. Given the historical and economic importance of the Red Sea, it is perhaps particularly significant that its diatom flora is poorly known. There are a few early studies of the Red Sea flora, including Cleve and Ostenfeld & Schmidt, but diatoms of the Red Sea have received little recent attention, and there are few species which have been illustrated via even light microscopy, much less electron microscopy. The few exceptions were not focused on floristic questions, but were concerned either with unusual diatom endosymbionts of foraminifera, or included Red Sea specimens in a larger study of a limited number of species.

Like floristic studies, ecological studies of the diatoms of this unusual basin are rare. Ecological studies indicate that the greatest abundance of diatoms is to be expected in winter months (ca. December to March). Shaikh et al. found that phytoplankton abundance patterns were driven by stratification patterns, with the diatom bloom associated with the onset of stratification.

This study illustrates some dominant, common, or unusual diatoms identified from Al Nawras Bay of the Red Sea near the city of Jeddah in 2008 and 2009. The main objective was to identify diatoms possibly associated with

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potential anthropogenic impact on the bay system.

MATERIALS AND METHODS

The present study was performed during the period from spring 2008 to winter 2009 in the touristic Al-Nawras Bay located in Jeddah city, Saudi Arabia to determine seasonal variation of diatoms as affected by the nutritional content and the other prominent environmental factors prevailing in the Bay. Four locations were selected for physiological, chemical and biological determinations. These locations are: site A, the nearest site to the outlet of a mixed ground and sanitary outflow into Al-Nawras Bay (N21° 35' 12.56", E 39° 06' 22.31'); site B is inside Al-Nawras Bay (N21° 35' 12.56^{*t*}, E 39° 06' 27.06''); site C was on the other side of the Bay (N21° 34' 34.93'', E39'', 06' 32.55''); and site D was chosen as a control because it is far away from the outlet of mixed sanitary water at North of the Bay (N21° 36' 06.57'', E39° 06' 25.06'') (Fig. 1).

Sample collection

Three replicate water samples were collected from the four test sites of Al-Nawras Bay. Nine liters from each sample were filtered through a locally adapted sieve of phytoplankton net (200um pores size). The remaining 10th liter of each sample was used to transfer the collected phytoplankton from the phytoplankton net to a clean plastic bottle for storage in a refrigerator at 4°C for further analysis (Newell & Newell 1977, Sournia 1978a, Parsons et al. 1984, Boney 1989).

Diatom cell count

Ten liters of seawater were filtered through the locally adapted sieve; the collected diatom cells were transferred to a plastic bottle with a small quantity of water and preserved in 10% formalin. For counting diatom cells, one ml of the diatom suspension was applied to Sedgewick Rafter counting cell slide.

Regarding the chemical analyses, three replicates of water samples (one liter/sample) were collected from the surface layer of the four tested sites. Water samples were separated filtered using Whatman Grade GF/C glass-fiber (Whatman 1822-055) filter paper with 0.45 pore size and stored in a 4°C refrigerator for chemical analyses.

Sample preparation for electron microscopic examina - tion

The method described by Sournia (1978b) was followed. Phytoplankton samples were washed separately with distilled water to get rid of salinity. An equal volume of saturated potassium permanganate was added and the samples were incubated for 24 hrs. An equal volume of concentrated hydrochloric acid was added and the mixture was then heated in a water bath for 30 min. at 90oC until the mixture became clear or greenish yellow.

Water temperature, pH, salinity, dissolved oxygen and turbidity assay

2070). Water content of dissolved oxygen was assayed using the conventional Winkler's method and back titration of liberated iodine according to Anderson & Foyen (1969). Turbidity was measured by Easy View Wide Range Light Meter, Ea30.

Nutritional elements assay

For chemical analyses, three replicates of water samples (one liter/sample) were collected from the surface layer of the four tested sites. Water samples were separated filtered using filter paper of 0.45 pores size, then kept in a refrigerator for analyses. Nitrate, nitrite, ammonium, active phosphate and active silicate were estimated according to Parsons et al. (1984).

Determination of chlorophyll a, b & c and carotenoids

The three types of chlorophyll and carotenoid pigments were estimated spectrophotometrically following the methods outlined in Parsons et al. (1984) and Boney (1989) using the wave lengths (nm) of 665, 645 and 630 for chlorophyll a, 645, 665 and 630 for chlorophyll b, 630, 665 and 645 for chlorophyll c, and 480 and 510 for carotenoids.

Statistical analysis

Statistical analyses were carried out according to Duncan (1955) using the Costat program (version 6.303, CoHort, USA, 1998–2004). Least significant differences were determined at 5% according to Gomez & Gomez (1984). In addition, the Pearson correlation coefficient at a confidence limit 95% was used to study the relationship between diatom number and some chemical properties of water samples. The partial coefficient of determination (R2) was estimated for each component to evaluate the relative contribution and to construct the prediction model of the diatoms number (y) according to the equation:

Y = a + b1 x1 + b2 x2 + b3 x3 (Snedecor & Cochran 1982).

RESULTS AND DISCUSSION

Identification of diatoms

The collected diatom samples from the four sites were identified and classified according to Newell & Newell (1977) and Boney (1989). Further, some other references were used to confirm the identity of the diatoms (Cleve-Euler 1952, Simonsen et al. 1980, Barber & Haworth 1981, Sykes 1981, Round et al. 1990, Tomas et al. 1997). The results show that 24 diatoms species belonging to 12 genera were detected in the four test sites A, B, C and D (Table 1). It is noteworthy that eight species of diatoms were found in all four sites i.e., *Amphora bigibba, Amphora coffeaeformis, Chaetoceros lorenzianus, Cocconeis scutellum, Nitzschia clostrerium, Nitzschia* sp.1, *Nitzschia* sp.2 and *Pleurosigma* sp. (Table 1 and Fig. 2a-h).

In contrast, some diatom species were restricted to one site. *Navicula* sp.1, *Navicula* sp.2, *Nitzschia angularis*, *Nitzschia* sp.3 and *Surirella fasthosa* were found only in site A (Table 1 and Fig. 3a-e). *Amphora acutiuscula* and *Surirella scalaris* were detected only in site B (Table 1 and Fig. 4a,b). Site C contained three species i.e., *Amphora* sp.1, *Amphora* sp.2 and *Campylodiscus* sp. (Table 1 and Fig. 5a-c) not found

Water temperature was measured with an ordinary thermometer, salinity was assayed with a hand refractometer, and pH was determined with a pH meter (Jenway model

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elsewhere. Finally, site D included six unique diatoms species i.e., Triceratium dubium, Amphora sp.3, Bacteriastrum sp., Diploneis sp., Mastogloia sp. and Surirella hybrida (Table 1, Fig 6a-f).

Table 2 summarizes the comparison of biological, physical and chemical properties of the four test sites at Al-Nawras Bay. No significant differences were detected between the four sites for pH, temperature, turbidity and chlorophyll b; average values of these properties ranged from 7.86 to 7.97, 29.5 to 30.19°C, 0.92 to 0.95 m and 0.010 to 0.433 mg l⁻¹, respectively.

Regarding salinity of the four sites, both sites C and D recorded the highest values of 39.97 and 40.12 PSU (Practical Salinity Unit), respectively in comparison with salinity at site A that reached 38.08 PSU (Table 2). This is likely due to the fact that sites C and D are distant from the diluting effect of sanitary water.

Site D had the highest value for dissolved oxygen (mg/l), which reached 6.58 in comparison with the other three test sites. In contrast, site A had the highest significant values for content of nitrate, nitrite, ammonia, phosphate, silicate, diatom numbers, chlorophyll a & c, and carotenoids, followed by sites B, C and D, respectively (Table 2). The gradient concentrations of macro-elements and carotenoids are proportional to the location of the four sites in relation to the source of pollution of sanitary water; site A is the nearest one followed by sites B, C, and (D). The only exception is between sites C and D regarding phosphate and silicate content.

The total number of diatoms decreased with increasing distance from the pollution source of sanitary water where site A recorded a significantly higher number in comparison to the other three sites B, C, and the control D (Table 1). The average number of diatoms in the four sites ranged from 1,918 to 2,343 cells/l with the highest number in site A and the lowest in site D. This finding could be explained by the high nutrient content of site A in comparison to the other three tested sites. This explanation is supported by the results that there is a positive correlation coefficient between diatom number and the amount of ammonium, nitrate, phosphate, chlorophyll a, b & c and carotenoids (Table 4 Figs. 7-8).

Seasonal variation of diatom number and the other tested parameters was also detected (Table 3). No significant differences were found for both pH and salinity over the four seasons except for autumn, which recorded a lower value of dissolved oxygen (mg 1-1). For the macro-nutrients (i.e., nitrate, nitrate, ammonia, phosphate and silicate), the highest significant values of these were registered in autumn, followed by spring, summer while winter showed the lowest values.

Summer significantly surpassed the other seasons for diatom number (19,514 cell 1-1), followed by spring (9,158 cell l-1), autumn (3,773 cell l-1) and winter (1,640 cell 1-1) (Table 3). The number of diatoms is closely correlated with the recorded temperature (32.3oC in summer and 27.60C in winter). Furthermore, chlorophyll a, b and c and carotenoid water content increased significantly in summer in comparison with the other three seasons, whereas these differences were not significant between autumn, winter and

spring.

The correlation coefficient between diatom number and the various tested parameters is shown in Table 4. The correlation was not significant between diatom number and salinity, temperature, dissolved oxygen, silicate and nitrate content. In contrast, a positive significant correlation was found between diatom number and transparency, nitrite, ammonia, phosphate and chlorophyll b. Furthermore, a positive significant correlation was recorded between the number of diatoms and chlorophyll a, chlorophyll c and carotenoids. On the other hand, a negative correlation was found between diatom number and pH (Table 4). This finding is consistent with those obtained by Taraldsvik & Myklestad (2000) who found that growth rate of Skeletonema costatum was nearly constant at pH range from 6.5 to 8.5 and declined at pH > 9.0.

The regression coefficients of diatom number with turbidity, temperature, pH and salinity are shown in Figures 7a, b, c and d, respectively. A positive linear regression was detected between the diatom number and turbidity (Fig. 7a) and temperature (Fig. 7b), whereas the regression coefficient of diatom number with pH factor (Fig. 7c) was negative. Moreover, a weak regression coefficient was recorded between diatom number and concentration of salinity (Fig. 7d).

Figure 8 shows the regression coefficient of diatom number with Al-Nawras Bay nutrient content and dissolved oxygen. An obvious positive regression was recorded between the diatom numbers and ammonia (Fig. 8b), nitrate (Fig. 8c), and nitrate (Fig. 8d) concentration. In contrast, a slight regression coefficient was observed between the diatom number and dissolved oxygen, phosphate and silicate.

A high correlation coefficient between diatom number and chlorophyll and carotenoid content was detected (Fig. 9). The highest values were recorded for chlorophyll a (Fig. 9a), chlorophyll c (Fig. 9c) and carotenoids (Fig. 9d), while the lowest value was found for chlorophyll b (Fig. 9b).

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Table 1. Diatom species and their cell counts (cell l-1) in the four test sites of Al-Nawras Bay during the four

| - 5 | ea | ISC | л | ıs. | |
|-----|----|-----|---|-----|--|
| | | | | | |

| Distanceme | | Sit | c (A) | | | Sit | 2 (B) | | | Site | 2 (C) | | | Sit | e (D) | |
|----------------------|-----|------|--------|------|-----|------|-------|------|-----|------|-------|-----|----|-----|-------|-----|
| Diatoms spp. | W | A | S | Sp | W | A | S | Sp | W | A | S | Sp | W | A | S | Sp |
| Amphora bigibba | | 124 | 421 | 143 | 45 | 238 | 123 | 181 | | | 512 | 274 | - | | 62 | 23 |
| Amphora | 3.4 | 173 | 537 | 340 | | | 114 | 213 | | | 192 | 67 | | | 132 | 87 |
| coffeae form is | | | | | | | | | | | | | | | | |
| Chaetoceros | | 473 | 9856 | 2865 | 84 | 423 | 1328 | 765 | | | 592 | 359 | 17 | 18 | 215 | 132 |
| lorenzianus | | | | | | | | | | | | | | | | |
| Cocconeis scutellum | | | 759 | 243 | | | 346 | 56 | 145 | | 412 | 71 | | 15 | 59 | 36 |
| Nitzschia closterium | 984 | 2733 | 4293 | 3435 | | | 4024 | 1257 | 23 | 51 | 145 | 123 | | | 152 | 113 |
| Nitzschia sp.1 | 22 | 58 | 114 | 221 | | | 174 | 62 | | | 84 | 32 | | 20 | 254 | 102 |
| Nitzschia sp.2 | 85 | 155 | 624 | 435 | | | 412 | 167 | 71 | 5.2 | 121 | 57 | - | 2.3 | 422 | 134 |
| Pleurosigma sp. | 132 | 404 | 5229 | 2719 | 163 | 2095 | 4439 | 1065 | | 21 | 213 | 183 | | 13 | 155 | 132 |
| Navicula sp.1 | | | 84 | 2.4 | | | | | | | | | - | | | |
| Navicula sp.2 | | 63 | | 74 | | | | | | | | | | | | |
| Nitzschia angularis | | | 23 | | | | | | | | | | | | - | - |
| Nitzschia sp. | | 3.4 | | | | | | | | | | | - | | | |
| Surirella fastuosa | 36 | 23 | | | | | | | | | | | | | | |
| Amphora acutius cula | | | | | | 43 | | | | | | | - | | | |
| Surirella scalaris | | | | | | | | 40 | | | | | - | | | |
| Amphora sp.1 | | | | | | | | | | | 80 | | | | | |
| Amphora sp.2 | | | | | | | | | | | 173 | | - | | | |
| Campylodiscus sp. | | | | | | | | | | 12 | 62 | 83 | | | | |
| Amphora sp.3 | | | | | | | | | | | | | | | 58 | |
| Bacteriastrum sp. | | | | | - | | | | | | | | 30 | 53 | - | |
| Diploneis sp. | | | | | | | | | | | | | | 11 | | |
| Mastogloia sp. | | | | | | | | | | | | | 23 | | | |
| Surirella hybrida | | | | | | | | - | | | | | - | | 4.5 | |

W, winter; A, autumn; S, summer and Sp., spring

Table 2. Biological, physical and chemical properties of the four test sites of Al-Nawras Bay.

| Parameters | Site (A) | Site (B) | Site (C) | Site (D) | | | | |
|--|-----------|-----------|----------|----------|--|--|--|--|
| Physicochemical parameters | | | | | | | | |
| pH | 7.89 | 7.86 | 7.97 | 7.90 | | | | |
| Salinity (PSU) | 38.08c | 39.29b | 39.97a | 40.12a | | | | |
| Temperature (°C) | 29.5 | 30.19 | 29.95 | 29.60 | | | | |
| Transparency (m) | 0.94 | 0.95 | 0.94 | 0.92 | | | | |
| Dissolved Oxygen (mg l ⁻¹) | 5.87b | 5.82b | 6.10b | 6.58a | | | | |
| Macro-nutrients (mg 1 ⁻¹) | | | | | | | | |
| Nitrate (mg l^{-1}) | 40.47a | 12.90b | 6.37c | 2.96d | | | | |
| Nitrite (mg l^{-1}) | 16.05a | 11.45b | 2.70c | 1.51d | | | | |
| Ammonia (mg l ⁻¹) | 8.31a | 5.13b | 1.86c | 1.78c | | | | |
| Phosphate (mg l ⁻¹) | 21.93a | 11.00b | 8.06d | 9.60c | | | | |
| Silicate (mg l ⁻¹) | 40.40a | 11.13b | 19.03b | 7.45d | | | | |
| Diatoms number (cell 1 ⁻¹), chlorophyll contents and carotenoids | | | | | | | | |
| Diatoms number | 19187.75a | 10125.92b | 2427.92b | 2343.10b | | | | |
| Chlorophyll a (µg l ⁻¹) | 1.653a | 1.053ab | 0.285b | 0.200b | | | | |
| Chlorophyll b ($\mu g l^{-1}$) | 0.204 | 0.433 | 0.010 | 0.025 | | | | |
| Chlorophyll c (μ g l ⁻¹) | 0.735a | 0.733a | 0.150c | 0.133b | | | | |
| Carotenoids ($\mu g l^{-1}$) | 0.573a | 0.283b | 0.102c | 0.089d | | | | |

Mean values followed by the same letter within the treatments are not significantly different (P < 0.05) according to the Duncan's multiple range tests.

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Table 3. Seasonal variation of diatom number, salinity content, pH, dissolved oxygen, macro-nutrients, chlorophyll a, b and c and carotenoids.

| Parameters | Summer | Autumn | Winter | Spring | | | | |
|--|-----------|----------|---------|----------|--|--|--|--|
| Physicochemical parameters | | | | | | | | |
| pH | 7.96 | 8.04 | 7.92 | 7.77 | | | | |
| Salinity (PSU) | 39.87 | 39.27 | 39.22 | 39.10 | | | | |
| Temperature (°C) | 32.30a | 29.14ab | 27.56b | 30.24ab | | | | |
| Transparency (m) | 1.08a | 0.91b | 0.78c | 0.98ab | | | | |
| Dissolved Oxygen (mg l ⁻¹) | 6.27a | 5.07b | 6.11a | 6.92a | | | | |
| Macro-nutrients (mg l^{-1}) | | | | | | | | |
| Nitrate | 13.09b | 23.76a | 11.87c | 13.99b | | | | |
| Nitrite | 9.87a | 10.18a | 1.13b | 10.53a | | | | |
| Ammonia | 4.08b | 6.33a | 1.51c | 5.18b | | | | |
| Phosphate | 7.37b | 17.68a | 6.75b | 18.78a | | | | |
| Silicate | 12.80b | 40.50a | 6.50b | 18.20b | | | | |
| Diatoms number, chlorophyll contents and carotenoids | | | | | | | | |
| Diatoms number | 19514.08a | 3772.58c | 1640.0d | 9158.01b | | | | |
| Chlorophyll a ($\mu g l^{-1}$) | 1.94a | 0.767b | 0.165b | 0.318b | | | | |
| Chlorophyll b ($\mu g l^{-1}$) | 0.478a | 0.164b | 0.023b | 0.007b | | | | |
| Chlorophyll c (μ g l ⁻¹) | 1.143a | 0.327b | 0.196b | 0.087b | | | | |
| Carotenoids ($\mu g l^{-1}$) | 0.633a | 0.216b | 0.079b | 0.119b | | | | |

Mean values followed by the same letter within the treatments are not significantly different (P < 0.05) according to the Duncan's multiple range tests.

Table 4. Correlation coefficient of diatom number (cell l-1) with the tested physical-chemical parameters, chlorophyll a, b, c and carotenoids and macronutrients.

| Parameters | Diatoms number |
|---|-----------------------|
| pH | -0.051 |
| Salinity (PSU) | 0.031 |
| Temperature (°C) | 0.445 |
| Transparency (m) | 0.546* |
| Dissolved Oxygen (mg l^{-1}) | 0.268 |
| Nitrate (mg l^{-1}) | 0.416 |
| Nitrite (mg l^{-1}) | 0.659* |
| Ammonia (mg l^{-1}) | 0.535* |
| Phosphate (mg l^{-1}) | 0.641* |
| Silicate (mg l^{-1}) | 0.146 |
| Chlorophyll a ($\mu g l^{-1}$) | 0.951** |
| Chlorophyll b (μ g l ⁻¹) | 0.616* |
| Chlorophyll c (μ g l ⁻¹) | 0.981** |
| Carotenoids ($\mu g l^{-1}$) | 0.914** |

** Correlation is significant at the 0.01 level, * Correlation is significant at the 0.05 level.



Fig. 1. Satellite photograph of the four study sites (A, B, C and D).











Fig. 2. Scanning Electron micrographs of the diatoms types found in all test sites A, B, C and D.

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Fig 3. Scanning electron micrographs of the diatom types only detected in site A.



Fig 4. Scanning electron micrographs of the two diatom species only detected in site B.





- 40 = 2 606 3x - 69.052 30 30 = 0.1978 2 R2 = 0.288 2 20 -20 10 0 0 os oo i Transparency [m] 26 32 0.6 07 1.1 12 28 30 3 Temperature [C] 50 50 . D 4 40 mNo.X10*3 40 y = 0.1586x + 2.3264 y = -3.4565x + 35.31 9 30 30 C000.0 = 57 R²= 0.0026 20 20 Diato 10 10 Û 0 32 41 44 47 50 35 38 7.5 8.5 3 0 pH Salinity conc. [PSU]
- Fig 7. Regression analysis of diatom number with the physical-chemical properties. R2 is the regression coefficient.

Fig 5. Scanning electron micrographs of the three diatoms species only detected in site C.

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Fig. 8. Regression analysis of diatom number with macro-nutrients content of Al-Nawras Bay. R2 is the regression coefficient.



Fig. 9. Regression analysis of diatom number with chlorophyll a, b and c content of Al-Nawras Bay. R2 is the regression coefficient



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