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A model to predict HAB occurrence near desalination plants in the Red Sea

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ABSTRACT

Normal growth of phytoplankton means gradual growth that allows mixed community to grow and allow a gradual succession of other phytoplankton species with a balanced and high biodiversity. In the contrary, a rapid, large-scale growth of certain species of phytoplankton is defined as harmful algal bloom (HAB). HABs cause water quality issues in fresh, brackish, and marine waters worldwide. In the Red Sea, HABs hinder normal operation of desalination plants and can even lead to their temporary shutdown. This research has already detailed how wastewater discharge from Red Sea desalination plants helps trigger HABs. The primary objective for this paper is to help manage and even mitigate this problem through development of a model to be used along with satellite images and physicochemical monitoring analysis as an early warning system for HABs near desalination plants in the Red Sea. To develop the model, physicochemical data were collected by sampling three coastal areas near desalination plants at Jeddah, Al Shoaibah, and Al Qunfudhah from December 2014 to November 2016. A total of 1,944 water samples were collected. To understand the unique environment of the seawater around the desalination plant that may trigger HABs, 15 parameters were measured. These parameters were divided into two groups, growth indicators (phytoplankton count, chlorophyll, phycoerythrin, dissolved oxygen, and turbidity) and those that might trigger the growth (temperature, conductivity, salinity, pH, total dissolved solid [TDS], nitrate, nitrite, ammonium, phosphorous, and silica). It was found that the highest concentrations of chlorophyll at Al Qunfudhah often exceeded the critical threshold level of 2 to 5 μg/L, which indicates the presence of an algal bloom. However, at Jeddah and Al Shoaibah chlorophyll concentration did not exceed this level. The results showed that higher temperature, salinity, TDS, and pH may promote some HAB species in environments that promote less phytoplankton diversity. Nitrate, phosphorus, and silica were the major nutrients that can trigger HABs. Prediction equations were developed using data from those parameters that promote HAB occurrence.

Keywords: Physicochemical factors; HAB; Algae; Red Sea; Desalination plant; Algal bloom; Saudi Arabia

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1. Introduction

Red tides are rapid and extensive growths (blooms) of some microscopic planktonic algae that are capable of discoloring the surface of the sea from red (red tides) to yellow, green, brown, or blue depending on the type of alga and its depth and concentration [1–3]. Although the conditions for blooms are not fully understood, the phenomenon is influenced by climatic and hydrographic circumstances plus human activities [4,5].

Extensive growths sometimes appear during changes in weather conditions but important contributing causes are variations in upwelling, temperature, transparency, turbulence, or salinity of the water as well as the concentration of dissolved nutrients, wind, or surface illumination [1,6,7]. A stratified surface layer above colder, nutrient-rich waters can be a direct cause of red tides that often occur when marine water temperature rises or freshwater runoff creates such stratification [5,8–10]. A high consumption rate of nutrients in the upper water layer due to the fast-growing algae will cause the depletion of these nutrients, leaving nitrogen and phosphorus highest below the interface of the layers, called the pycnocline [11].

Dinoflagellates, a mobile alga, have an advantage over the immobile algae that cannot swim down to the pycnocline to access needed nutrients at night and sunlight during the day. Therefore, dinoflagellate blooms are likely to occur in surface waters that are poor in nutrients [7,12,13]. The resting cyst of some dinoflagellates sinks to the bottom of the sea and becomes the seed of a new bloom after germination when favorable growth conditions return [14]. Bloom reoccurrence is most likely associated with the cysts propagation that was accumulated in the sediments from previous blooms. This process may mainly be governed by ecological factors [15].

Ecological features of the Arabian Gulf, combined with contamination from oil tanker ballast water containing invasive algal species that are not normally present in this environment, and anthropogenic activity that lead to nutrient enrichment are the factors that increase red tide problems in the Arabian Gulf [16]. The Arabian Gulf is a low, semi-encircled area, with severe anthropogenic stresses and extreme natural environmental conditions. The rate of evaporation in the Arabian Gulf exceeds both rainfall and inputs of coastal freshwater by a factor of 10. This fact along with a low rate of flushing every year [17], and high water temperatures that rise to over 32°C [18], leads to a high salinity of up to 44.30‰ [19]. During a 1998 red tide near Kuwait, a high biomass (55.4–262.7 μg/L chlorophyll a) was reported, which were some of the highest values ever reported [20].

These red tides led to changes in the ratios of carbon assimilation and structure of the phytoplankton, nanoplankton, and picoplankton. The red tide problem in the Arabian Gulf comes mainly from species of dinoflagellates [21]. The essential mechanisms of the 2008–2009 Arabian Gulf and Gulf of Oman red tide outbreak [22] included increased nutrient loading of the coastal waters from domestic and industrial sources, natural climatological, and oceanographic forces, and invasion of this species through ballast water discharge.

The effect of harmful algal blooms (HABs) on water desalination processes is a problem for strategic industries such as drinking water, especially in countries that rely on it to ensure their freshwater needs. Saudi Arabia and approximately 150 countries worldwide operate desalination plants to produce drinking water from seawater, and it is expected that the desalination capacity will be increased in the near future [23]. During algal bloom episodes, up to a 40% reduction in desalination plant production can occur along with a lower quality of desalinated drinking water from taste and odor problems and even low levels of HAB toxins in the potable water. These toxin levels can be at health risk levels comparable with those that are a risk from shellfish and fish consumption [23,24]. In addition blockage of intake filters, fouling of surfaces, and damage to costly reverse osmosis (RO) membranes will impair desalination plant operation from the large numbers of algal cells. These more severe HAB events will lead to a temporary shutdown of the desalination plant just like other problems such as electricity shutdowns as happened in March 2009 at the Ghaleelah RO desalination plant of Ras Al Khaimah, UAE. Also, other desalination plants, such as those in Kalba, Fujairah, and Khor Fakkan, were also shut down for a few months during HAB events that have occurred over the past few years [25].

Pace et al. [26] showed that modeling of phytoplankton blooms used for management and basic research may help in controlling or minimizing this problem.

Remote sensing is advantageous for identification of spatiotemporal patterns in the location of offshore HABs [27]. These blooms are characterized by sea surface discoloration and high chlorophyll a concentrations which can be studied using satellite remote sensing such as the Sea-viewing Wide Field-of-view Sensor [28], Moderate Resolution Imaging Spectroradiometer (MODIS), or Medium Resolution Imaging Spectrometer [29].

The primary objective is to help manage and even mitigate these HAB problems adjacent to desalination plants in the Red Sea by determining the factors influencing HAB formation. These factors will be used to develop an HAB prediction model using satellite images and specific routine physicochemical analysis as an early warning system for monitoring HABs in front of the desalination plant in the Red Sea. The longer term goals are to develop programs for management and mitigation of HABs in the Red Sea especially as they relate to the operation of sea water desalination plants.

2. Materials and methods

2.1. Study sites

Ship-based surveys were used to monitor algae at three sites offshore of the western coast of Saudi Arabia (east Red Sea coast). The selected three sites are adjacent to desalination plants located at Jeddah (Latitude: N21°32′58.7″, Longitude: E39°06′35.6″), Al Shoaibah (100 km south of Jeddah, Latitude: N20°39′57.0″, Longitude: E39°30′00.9″) ,and Al Qunfudhah (420 km south of Jeddah, Latitude: N19°04′20.1″, Longitude: E1°09′38.6″). Water sampling consisted of three replicates from three different depths (surface, –5, and –10 m) and at three stations (150, 750, and 1,250 m offshore from the desalination plants) at each site. J-1, S-1, and Q-1 are the abbreviations given to the samples for the first station at each site. For each trip, the total water samples collected were 81 along with 18 plankton net samples. A total of 24 field trips were performed during the period of December 2014 to November 2016 collecting a total of 1,944 water samples and 432 net samples [30].

2.2. Sampling during field trips

Two plankton nets (50 μm mesh) were used to collect phytoplankton at the three stations at each site. 20-min surface tows were used for each station with three replicates from each tow. Plankton net samples were used for toxicity tests and algal identification. Water samples (1.7 L) were taken using a Niskin water sampler. One liter was preserved in amber colored bottles with 4% iodine and used for phytoplankton identification and enumeration. The remaining 700 mL (not preserved) was retained for chemical analysis of nitrogen (ammonium, nitrite, and nitrate), phosphorous, and silica in the laboratory [30–32].

2.3. Phytoplankton enumeration and identification

The preserved 1 L samples were concentrated by sedimentation in graduated cylinders according to Dolan and Marasse [33]. After 4–8-d settling period, the top 950 mL of the sample was slowly siphoned off. The remaining concentrated 50 mL was counted under an inverted microscope after settling for 24 h using Utermöhl's method [34]. The entire surface of the settling chamber was examined at 400× magnification.

Identification of phytoplankton taxa was done according to Balech [35], Dodge [36], Gómez [37], Halse and Syvertsen [38], Huber-Pestalozzi [39], Steidinger [40], Tomas [41], and Tregouboff and Rose [42].

2.4. HAB toxigenic species definitions

HAB species are those capable of rapid and extensive growths and HAB toxigenic species are those known to produce potent toxins. HAB and HAB toxigenic species were defined according to IOC-UNESCO [43] Taxonomic Reference List of Harmful Micro Algae.

2.5. Physicochemical analysis

During the 24 months of this study, over 1,900 data points (81 samples per month) for each of the physicochemical analysis were obtained. A-YSI EXO2 Sonde was used to measure temperature (°C), conductivity (μS/cm), salinity (psu), pH, dissolved oxygen (DO) (mg/L) measured as optical dissolved oxygen (ODO), chlorophyll (total algae μg/L), phycoerythrin as a measure of cyanobacteria (blue green algae) biomass (BGA-PE) (μg/L), total dissolved solid (TDS) (mg/L), dissolved organic matter (QSU), and turbidity (FNU). In addition, laboratory analysis was carried out for nitrate, nitrite, ammonium, phosphorous, and silica (mg/L) as they are major nutrients that play a key role for phytoplankton growth. All chemical analysis of these nutrients was analyzed by YSI kits and with an YSI 9500 Photometer (Yellow Spring, OH, USA), according to the manufacturer protocols [44].

2.6. Descriptive statistics

To work with large data sets, means, standard error and skewness (a test for distribution as a measure of the asymmetry of the probability distribution) were computed using the means procedure available in SAS software [45]. To find possible correlations among measured parameters within the different studied ecosystems, Pearson correlation coefficients (r values) were estimated between all characteristics measured on ship cruises (across month, site, station, and depth) using Excel Analysis Tool Pak.

2.7. Analysis of variance

To determine significance of the different ecosystems on the observed values, data were subjected to analysis of variance (ANOVA) using the general linear model procedure on SAS software. The statistical model used was as follows:

$$\mathrm{Y}_{ijklmn} = m + \mathrm{Y}_i + \mathrm{Se}_j + \mathrm{Si}_k + \mathrm{St}_l + D_m + \left(\mathrm{SeSi}\right)_{jk} + \left(\mathrm{SeSt}\right)_{jl} + \left(\mathrm{SeD}\right)_{jm} + (\mathrm{SiSt})_{kl} + (\mathrm{SiD})_{km} + (\mathrm{StD})_{lm} + e_{ijklmn} \quad (1)$$

where Y_{ijklmn} is the observed valued on the n-th replicate, in the m-th depth, in the l-th station, in the k-th site, in the j-th season, at the i-th year; m is the overall means; Y_i is the effect of the i-th year; Se_j is the effect of the j-th season; Si_k is the effect of the k-th site; St_l is the effect of the l-th station; D_m is the effect of the m-th depth; $(\mathrm{SeSi})_{jk}$, $(\mathrm{SeSt})_{jl}$ etc. are the two-way interactions; and e_{ijklmn} is the random error associated with the ijklmn-th observation.

Duncan's multiple range test is a more appropriate procedure for mean separation and is appropriate for large ecological sets of data. Just as in real-world practice the global null hypothesis H0 = "All means are equal" is often false and thus traditional statistics overprotect a probably false null hypothesis against Type I errors. For this reason, Duncan's multiple range test was chosen [46] for mean separation. Differences with $p < 0.05$ were considered significant.

2.8. Prediction equation

Stepwise regression is normally used in fitting regression models where a variable is considered for addition to or subtraction from the set of explanatory variables. The best R^2 was used in the final model [47]. SAS software was used to perform the procedure [45]. Data for each depth within each station at each site were used separately. Total phytoplankton counts were used as the dependent variable, while NO_3, PO_4, temperature, pH, conductivity, and TDS traits were the independent variables. Quadratic and cubic from the independent variables were included as well as the square root in some cases. Only the significant regression coefficient ($p < 0.05$) will be considered in the equations reported here. The statistical model used was as follows:

$$\text{Total phytoplankto}n = \beta_\circ + \beta_1 x_1 + \beta_2 x_1{}^2 + \beta_3 x_1{}^3 + \beta_4 x_2 + ... + e \quad (2)$$

where y_i is the response (dependent); β_0 is the intercept value; β_1, β_2 and β_n are the regression coefficients; x_1, x_2, …,. x_n are the independent variables; and e_i is the random error

3. Results and discussion

3.1. Descriptive statistics

Descriptive statistics for measured parameters averaged across the three sites, three stations, three depths, and 24 months are provided in Table 1. Total phytoplankton averaged 1.06×10^4 cells/L across the three sites, stations, and depths for the period from December 2014 to November 2016. The maximum concentration of phytoplankton reached 442,300 cells/L with 381, 900 cells/L from HAB toxigenic species in the September 2016 samples from Al Qunfudhah. An average of 0.55 μg/L chlorophyll a and 0.63 μg/L BGA-PE) were from blue green algae (BGA), while fluorescence fDOM averaged 1.24 μg/L. Total nitrogen (N) averaged 2.06 mg/L, of which 1.07 mg/L was from nitrate and 0.99 mg/L was from ammonium. Phosphate concentrations averaged 0.06 mg/L, while silicon dioxide averaged 0.26 mg/L. The average DO was 5.73 mg/L and temperature averaged 27.25°C. Conductivity, TDS, and salinity averaged 62, 671 μS/cm, 38, 625 mg/L, and 39.61 psu, respectively. Finally the average pH was 8.13. Additional details about variability in these measured parameters by month, station, site, and depth are provided in the following sections of this report.

An ANOVA was conducted on measured parameters to detect significance across month, site, station, and depth (Table 2). The results showed that variability across seasons (from December 2014 to November 2016) was significant

Table 1
Descriptive statistics for measured parameters

	Chlorophyll a, μg/L	Total HAB, cells/L	Turbidity, FNU	Salinity, psu	pH	NH_4^+, mg/L
Mean	0.55	1.06×10^4	0.74	39.61	8.13	0.99
±SE	0.02	0.07×10^4	0.02	0.03	0.0001	0.01
	BGA-PE, μg/L	HAB toxigenic, cells/L	ODO, mg/L	Conductivity, μS/cm	SiO_2, mg/L	NO_3^-, mg/L
Mean	0.63	0.54×10^4	5.73	62,671.56	0.26	1.07
±SE	0.02	0.01×10^4	0.015	110.10	0.06	0.01
	fDOM QSU	HAB non-toxigenic, cells/L	Temperature, °C	TDS, mg/L	PO_4^-, mg/L	NO_2^-, mg/L
Mean	1.24	0.52×10^4	27.75	38,652.16	0.06	0.004
±SE	0.02	0.01×10^4	0.07	31.73	0.0001	0.0001

SE, Standard error.

Table 2
Level of significance from ANOVA for measured variables across year, season, sites, stations, and depths

	Variables	Y	Se	Si	St	D	Se[a]Si	Se[a]St	Se[a]D	Si[a]St	Si[a]D	St[a]D
1	Total phytoplankton	[c]	[c]	[c]	[b]	[c]	[c]	NS	[c]	NS	NS	NS
2	Toxigenic phytoplankton	[c]	[c]	[c]	NS	NS	[c]	NS	[a]	NS	NS	NS
3	Non-toxigenic phytoplankton	NS	[c]	[c]	[c]	[c]	[c]	NS	[c]	NS	NS	NS
4	Chlorophyll a	[c]	[c]	[c]	NS	[c]	[c]	NS	NS	NS	NS	NS
5	BGA	[c]	[c]	[c]	NS	NS	[c]	NS	NS	[a]	NS	NS
6	DO	[c]	[c]	[c]	NS	NS	[c]	[c]	NS	[b]	NS	NS
7	Turbidity	[c]	[c]	[c]	NS	[a]	NS	NS	NS	NS	[a]	NS
8	Conductivity	[c]	[c]	[c]	NS	NS	[c]	NS	NS	NS	NS	NS
9	Salinity	[c]	[c]	NS	NS	NS	[c]	NS	NS	NS	NS	NS
10	TDS	[c]	[c]	NS	NS	NS	[c]	NS	NS	NS	NS	NS
11	pH	[c]	[c]	[a]	[b]	NS	[c]	NS	NS	NS	NS	NS
12	Temperature	[c]	[c]	[c]	NS	NS	[c]	NS	NS	NS	NS	NS
13	fDOM	[c]	[c]	[c]	NS	[a]	[c]	NS	NS	NS	NS	NS
14	NH_4^+	[c]	[c]	[c]	[b]	[a]	[c]	NS	NS	NS	NS	NS
15	NO_3^-	[c]	[c]	[c]	[c]	[b]	[c]	NS	NS	NS	NS	NS
16	NO_2^-	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
17	PO_4^-	[b]	[c]	[a]	[b]	NS	[c]	NS	NS	NS	NS	NS
18	SiO_2	[a]	[c]	[c]	NS	NS	[c]	NS	NS	NS	NS	NS

Y, Year; Se, season; Si, site; St, stations; D, depth; NS, not significant.
Levels of significance are indicated as follows:
[a] $p < 0.05$
[b] $p < 0.01$
[c] $p < 0.001$

($p < 0.001$) for all measured parameters except NO_2^-. Variability across sites (Jeddah, Al Shoaibah, and Al Qunfudhah) was significant ($p < 0.001$) for all parameters except for TDS, salinity, and nitrite. Variability across stations (distance from shore 150, 750, and 1,250 m) was only significant for total phytoplankton, non-toxigenic phytoplankton, pH, NH_4^+, NO_3^-, and PO_4^-, which were significant at the 0.01 (1%) level. Also, variability across depths (0, –5, and –10 m) was significant for total phytoplankton, non-toxigenic phytoplankton, and chlorophyll a at $p < 0.001$, as well as turbidity, fDOM, NH_4^+, and NO_3^- at $p < 0.05$. Two-factor interactions between season and station for almost all traits were significant at $p < 0.001$, an indication that data need to be examined within each station.

Pearson correlation coefficients (r values) between measured parameters for near shore surface at Jeddah, Al Shoaibah and Al Qunfudhah are presented in Table 3. After the statistics for possible correlations at all sites, stations, and depths were ran, it was found that the most useful and clearly interpreted results were those for the near-shore surface stations. That is because different ecosystems at each plant station as well as the unique ecosystem of the near shore station were used due to brine release from the RO process. Of most interest are correlations between phytoplankton (total, toxigenic, or non-toxigenic) and other measured parameters. Only ammonium had no correlation with phytoplankton. The other 14 measured parameters either had significant or low correlations but were not significant for phytoplankton. Correlations between nitrate, nitrite, or phosphorus and phytoplankton were negative, while correlations between temperature, conductivity, salinity, TDS, pH, turbidity, chlorophyll, BGA-PE, or fDOM and total phytoplankton were positive. Silica or DO had positive and negative correlation with phytoplankton. Plausible reasons for these correlations are discussed.

3.2. Horizontal and vertical variation of phytoplankton concentrations

Over the study period, total phytoplankton concentrations increase from Jeddah to Al Qunfudhah (Fig. 1). In fact, the mean phytoplankton concentration increased from 6.1×10^3 cells/ L in the Jeddah samples to 1.8×10^4 cells/L in the Al Qunfudhah samples. This result was also confirmed by the satelite images which showed that blooms increased from north to south. The HAB toxigenic and non-toxigenic species (Fig. 1) in all three sites showed no significant differences (i.e., no site effect on the occurrence of HAB toxigenic species). However, higher number of toxigenic species was

Table 3
Pearson correlation coefficients (r values) between measured parameters average for the near shore surface stations over all months and sites

Measured parameters	Jeddah			Al Shoaibah			Al Qunfudhah		
	Phytoplankton			Phytoplankton			Phytoplankton		
	Total	Toxigenic	Non-toxigenic	Total	Toxigenic	Non-toxigenic	Total	Toxigenic	Non-toxigenic
Total phytoplankton									
Toxigenic phytoplankton	0.827[b]			0.658[b]			0.992[b]		
Non-toxigenic phytoplankton	0.888[b]	0.477[b]		0.966[b]	0.443[b]		0.009	–0.121	
Ammonium, mg/L	–0.060	–0.052	–0.051	–0.121	–0.084	–0.115	–0.035	–0.028	–0.057
Nitrate, mg/L	–0.086	–0.034	–0.107	–0.201	–0.068	–0.216	–0.094	–0.073	–0.158
Nitrite, mg/L	–0.316[b]	–0.172	–0.353[b]	0.019	0.074	–0.003	–0.036	–0.040	0.029
Phosphate, mg/L	–0.088	–0.190	0.018	–0.180	–0.106	–0.178	–0.106	–0.090	–0.117
Silica, mg/L	–0.067	0.007	–0.112	0.014	–0.219	0.091	0.048	–0.007	0.420[b]
Temperature, °C	0.070	0.211	–0.063	0.397[b]	0.179	0.412[b]	0.094	0.113	–0.152
Conductivity, μS/cm	0.012	0.215	–0.156	0.416[b]	0.167	0.438[b]	0.211	0.202	0.057
TDS, mg/L	0.179	0.127	0.177	0.197	–0.038	0.248[a]	0.232	0.220	0.080
Salinity, psu	0.167	0.114	0.167	0.196	–0.010	0.238[a]	0.247[a]	0.233[a]	0.096
ODO, mg/L	0.192	–0.157	0.429[b]	–0.361[b]	–0.315[b]	–0.323[b]	–0.152	–0.113	–0.293[a]
pH	–0.067	0.064	–0.157	0.166	0.057	0.178	0.174	0.156	0.127
Turbidity, FNU	–0.079	–0.007	–0.118	0.080	0.356[b]	–0.026	0.275[a]	0.293[a]	–0.151
Chlorophyll a, μg/L	0.143	0.021	0.206	–0.015	0.362[b]	–0.142	0.267[a]	0.205	0.465[b]
BGA-PE, μg/L	0.168	0.100	0.180	0.190	0.502[b]	0.056	–0.094	–0.081	–0.096
fDOM, QSU	0.100	0.005	0.153	–0.110	–0.307[b]	–0.026	–0.013	0.010	–0.169

[a]Correlation is significant at the 0.05 level (two-tailed).
[b]Correlation is significant at the 0.01 level (two-tailed).

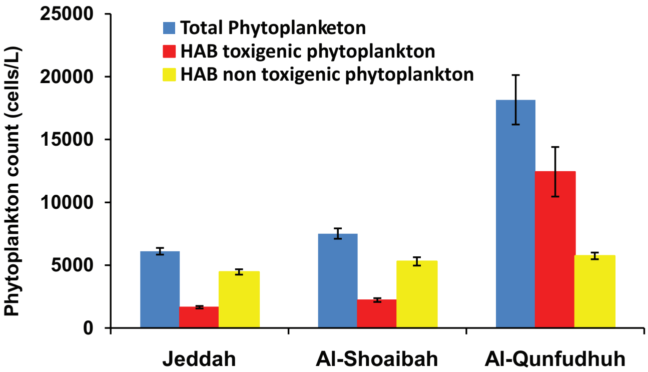

Fig. 1. North to south variation of total phytoplankton concentrations and HAB toxigenic vs. non-toxigenic phytoplankton during the study period.

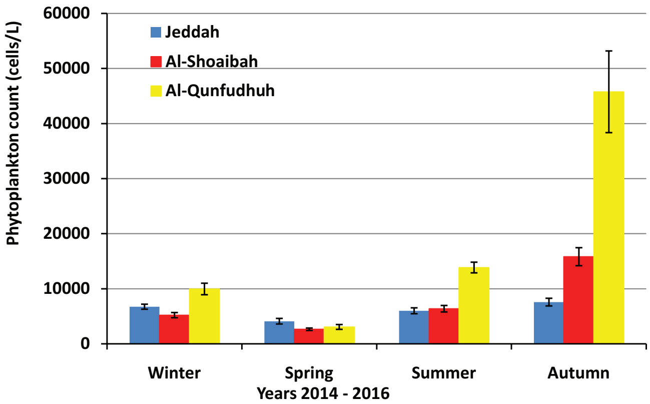

Fig. 2. Seasonal variation of phytoplankton concentrations during the study period in Jeddah, Al Shoaibah, and Al Qunfudhah sites.

observed at Al Qunfudhah. Utilization of particulate nitrogen and phosphorus sources (mixo-phagotrophy) may favor the occurrence of toxigenic species when inorganic concentrations (osmotrophy) are limited [48,49].

Total phytoplankton decreased significantly from (1.2×10^4 cells/L) in Site 1 to (0.7×10^4 cells/L) in Site 3 while greater numbers were found closer to shore. This may be due to the higher concentrations of nutrients especially phosphate at the coastal stations [50]. Significant positive correlation between phosphate and total phytoplankton was detected on the coastal stations. There were no significant differences between Station 1 and Station 2.

There was a significant trend in phytoplankton numbers with depth at the three stations. Greater numbers were found near the surface. Phytoplankton concentrations decreased from the surface (1.3×10^4 cells/ L) to –10 m (0.7×10^4 cells/ L). This result was found at all sites and during the entire study period. Furthermore, HAB toxigenic phytoplankton concentrations showed a decrease from the surface to –10 m. However this decrease in toxigenic HAB is not due to the depth effect but rather to the total lower number of phytoplankton at the –10 m depth because ratios remained about the same. It is not only the depth that controls phytoplankton growth in the submerged water column but rather the incident light intensity that is affected by background turbidity and turbulent mixing rates [51]. That is why it is important to understand the type of environment in a particular ecosystem. High turbidity and turbulent mixing rates will decrease light intensity downward and decrease the depth for phytoplankton growth.

3.3. Seasonal variation of phytoplankton concentrations

During the study period, the phytoplankton community showed the highest concentrations in autumn (Fig. 2). This period is distinguished by a moderate temperature, salinity, and nutrients which are the most common parameters controlling phytoplankton proliferation [50]. In the previous paper, it was suggested that low diversity may be the main factor that triggers HAB occurrence in this particular ecosystem adjacent to desalination plants [30]. Lower diversity during a bloom event followed by higher diversity after bloom decline was also observed in permanently eutrophic waters of a South African estuary [52]. Other environmental factors may help to initiate such conditions. To understand environmental factors that may trigger the bloom, samples were analyzed for physicochemical parameters. The 15 measured parameters could be divided into two groups, the first being phytoplankton growth indicators and the second the parameters that could trigger growth. In the first group, there are three parameters (chlorophyll a, BGA-PE, and DO) that could be used as indicators for phytoplankton growth. The 12 parameters in the second group, that may affect the phytoplankton growth, are fDOM, TDS, turbidity, temperature, conductivity, salinity, and pH along with the major nutrients represented by ammonium, nitrate, nitrite, phosphorus, and silica.

3.4. Seasonal variation of parameters affecting growth

Twelve parameters were investigated in this study to understand the unique environment around the desalination plant that may trigger an increase in phytoplankton concentration. Out of these 12 parameters only 7 (TDS, temperature, conductivity, pH, PO_4^-, NO_3^-, and SiO_2) will be discussed in detail as these have correlations with HAB phytoplankton and were used in the prediction equation.

3.4.1. TDS variations during the study period

TDS is mainly an expression of inorganic salts other than organic matter and other dissolved materials in the aquatic ecosystem [53]. TDS levels in water bodies are affected most by atmospheric precipitation, the geology of the drainage, and the balance between water evaporation and precipitation [54]. Toxicity occurs as a consequence of increasing salinity due to increasing TDS that alters the aquatic system ionic composition. Such increases in salinity cause changes in the biotic communities and affect less-tolerant species [55]. A negative correlation was reported between chlorophyll a concentrations (as an indication of phytoplankton growth) and concentrations of Na^+, Mg^{2+}, SO_4^{2-}, HCO_3^-, and CO_3^{2-} [56]. Contrary to previous reports, a positive correlation between TDS and phytoplankton counts were reported at all sites of this study (Table 3). This might be an indication that more tolerant species and strains are adapting to the higher TDS. This positive correlation is confirmed by the data illustrated in Fig. 3 where higher concentration of TDS in autumn (compared with winter and spring) is observed during the highest phytoplankton counts (Fig. 2).

3.4.2. Conductivity and salinity seasonal variations

Conductivity and salinity are key factors in the Red Sea aquatic habitat because it helps formulate nutrient availability for marine organisms. Although some reports indicate that conductivity measurements are more precise for this comparison [57], in this study, conductivity and salinity showed the same clear trend during the study period at all three sites, but conductivity was chosen to use (Fig. 4). At all sites, conductivity recorded in summer and autumn seasons were higher than that recorded during winter and spring seasons, and this is in agreement with the observed phytoplankton growth pattern (Fig. 3). This finding was also observed with TDS. This means that TDS and conductivity could be the key factors that directly shape phytoplankton bloom patterns in the Red Sea. Also conductivity showed a positive correlation with phytoplankton counts as observed with TDS (Table 3). These findings showed that higher TDS and conductivity enhance phytoplankton growth. This could only be explained if higher TDS and conductivity are favorable conditions for some species by decreasing species competition in less favorable environments for other species. Although dinoflagellates and diatoms favor mid-to-high salinities in an estuary [58], at high salinities lower species diversity is usually observed [30] which is due to salinities lethality to many phytoplankton in an estuarine environment [59]. This lethality is mainly caused by osmotic stress on cells, uptake or loss of ions, effects on the cellular ionic ratio, increased respiratory activity, and inhibitory effects on physiological processes in phytoplankton [60,61]. It should be noted that phytoplankton adapted to constant salinity may not resist drastic changes in salinity [62]. Increasing TDS, conductivity, and salinity may be a consequence of increasing temperature for a given season (Fig. 5).

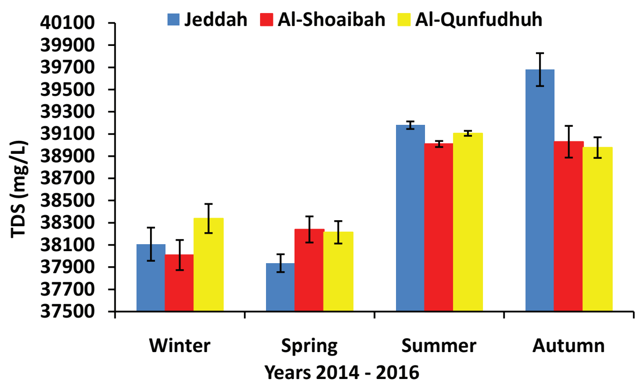

Fig. 3. TDS profile in mg/L during the study period in Jeddah, Al Shoaibah, and Al Qunfudhah sites.

3.4.3. Temperature seasonal variations

Temperature is a key factor in marine systems [63] that affects other physicochemical parameters. In this study, higher TDS, conductivity, salinity, and pH were observed as temperature rose in summer and autumn (Fig. 5) leading to higher phytoplankton counts in autumn (Fig. 2). It seems that temperature is a key factor that directly affects phytoplankton growth in this study as higher temperature narrow phytoplankton changeability that lower the diversity [63,64]. However, temperature is an indirect factor that reshapes other critical factors such as conductivity and nutrients, which in turn directly control phytoplankton growth.

3.4.4. Seasonal pH variations

The pH value was a parameter which gave good correlation with phytoplankton growth. The pH ranged from 7.84 to 8.95 during the study period at all sites. In this study, a general pattern at all three sites was higher pH in autumn compared with winter (Fig. 6). This pattern coincided with the phytoplankton growth pattern (Fig. 2). In general, different species of marine phytoplankton favor near neutral to alkaline pH and growth is significantly reduced at a pH around 6 or at a pH above 10 [65]. Also, reports of high dinoflagellates counts in marine ecosystems were correlated with higher pH values with cell counts increasing at a pH higher than 8.5 [66]. The lower pH value observed in this study during winter, following the high phytoplankton count in autumn, may be due to zooplankton grazing activity and bacterial activity on phytoplankton cells

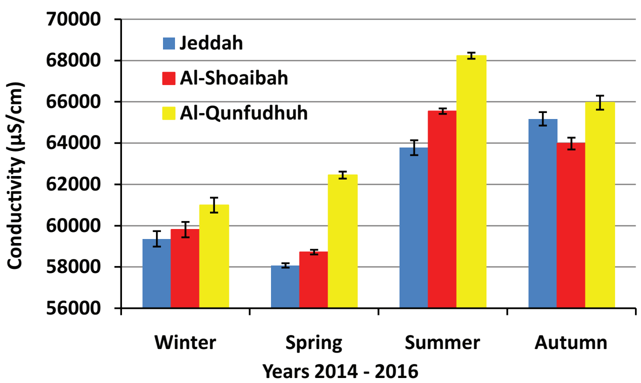

Fig. 4. Conductivity profile in µS/cm during the study period in Jeddah, Al Shoaibah, and Al Qunfudhah sites.

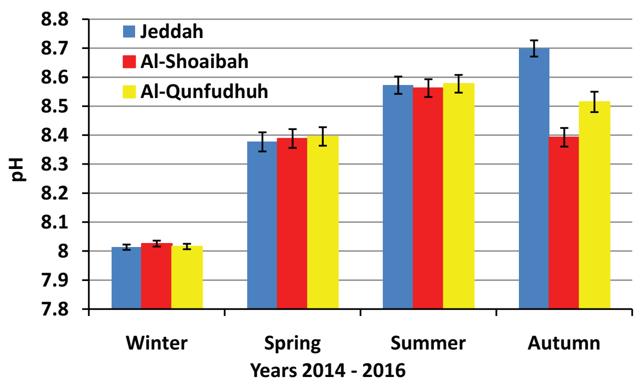

Fig. 5. Temperature profile in degree Celsius (°C) during the study period in Jeddah, Al Shoaibah, and Al Qunfudhah sites.

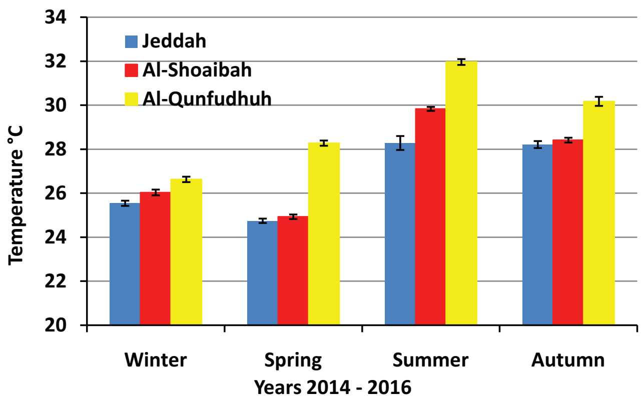

Fig. 6. The profile of pH during the study period in Jeddah, Al Shoaibah, and Al Qunfudhah sites.

or cell debris followed by higher CO_2 concentrations due to respiration leading to lower pH values [67].

3.4.5. Nitrogen profiles during seasonal variations

Nitrogen in aquatic ecosystems is present mainly as dissolved nitrogen (N_2), and in ionic form as ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), and urea ($CO(NH_2)^2$) [68,69]. In this study, ammonium was positively correlated with pH. Seawater pH alters the different forms of ammonium. About 95% of ammonium at the average pH of seawater is in the form of NH_4^+ (the cationic form of ammonium) and about 50% of ammonium is in the (toxic) gaseous form (NH_3) when pH rises to ~9.5 in seawater [70]. Maximum cell concentrations of *Gymnodinium* sp. (>6 × 10^6 cells/L) were preceded by more than a 20-fold increase in mean inorganic nitrogen concentration (up to 60 µM) and elevated inorganic phosphate concentrations. These combined factors along with an increase of inorganic and organic nutrients within the bloom, points to coastal nutrient eutrophication as likely the controlling factor for bloom development and maintenance [21]. Normally, lower levels of nitrogen and phosphorus should be observed during high phytoplankton count events due to their rapid uptake [71]. This was only true with the lower nitrate level at Al Qunfudhah during summer and autumn months (Fig. 7(a)), which indicates a higher consumption of nitrate during high phytoplankton growth events in autumn and that the maximum nitrate level during the spring season was consumed during summer and autumn. No obvious seasonal variation trends for ammonium and nitrite concentrations (Figs. 7(b) and (c)) were observed except for the higher concentration of nitrite at Al Qunfudhah in autumn (Fig. 7(c)). This means that either there is a surplus of ammonium in these environments or that the nitrate is the limiting factor. The fact that both nitrate and phosphorus is the limiting factors for several HAB events has been reported [52]. These findings were confirmed by the negative correlations of nitrate with phytoplankton counts and that no correlations were observed for ammonium and nitrite with regard to phytoplankton counts (Table 3). Again this phenomenon of the nitrate negative correlation with phytoplankton was reported during the peak of the bloom [52].

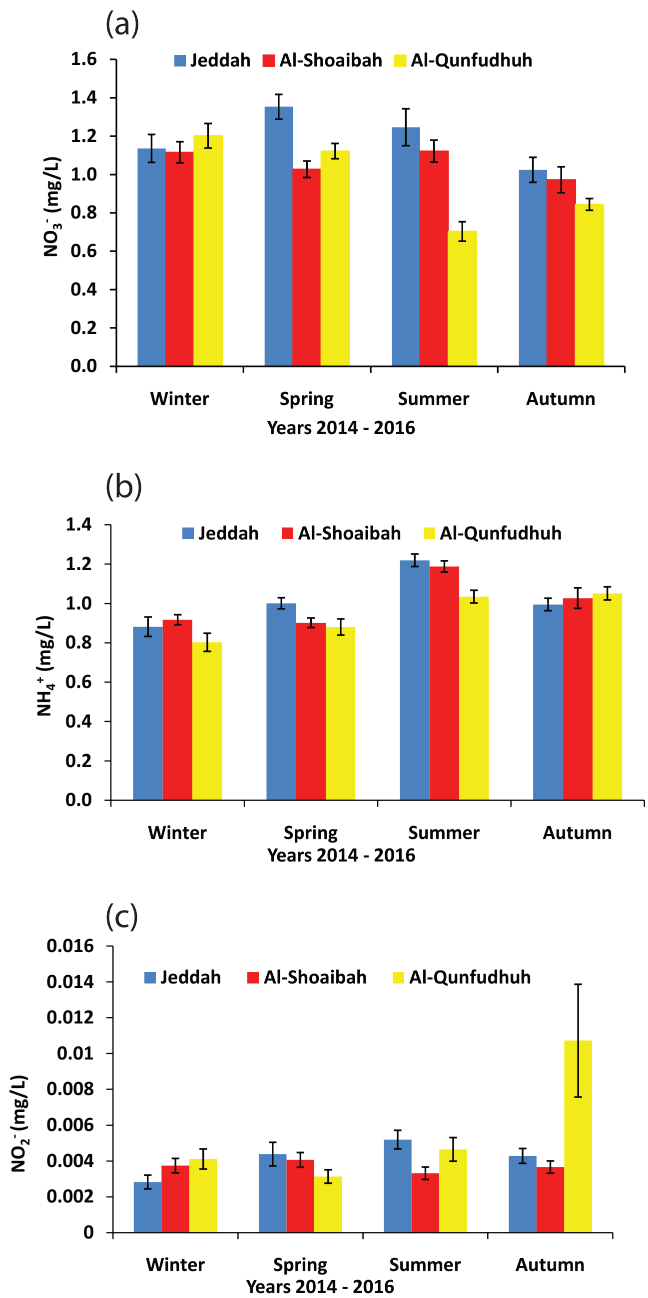

Fig. 7. (a) NO_3^-, (b) NH_4^+, and (c) NO_2^- level profiles in mg/L during the study period in Jeddah, Al Shoaibah, and Al Qunfudhah sites.

3.4.6. Phosphorus profiles during seasonal variations

The same trend as nitrate was observed for the phosphate profile at Jeddah and Al Qunfudhah (Fig. 8) where it reached its maximum level during the spring season and was consumed by biological activity during summer and autumn. This trend was not observed at Al Shoaibah (Fig. 8). Analysis of phosphate at different depth layers did not show a clear trend; however, deep layers tend to have a higher concentration of phosphate compared with the surface which means that the major source of phosphorus might be from sediment influx. Both dissolved and particulate forms of phosphorus

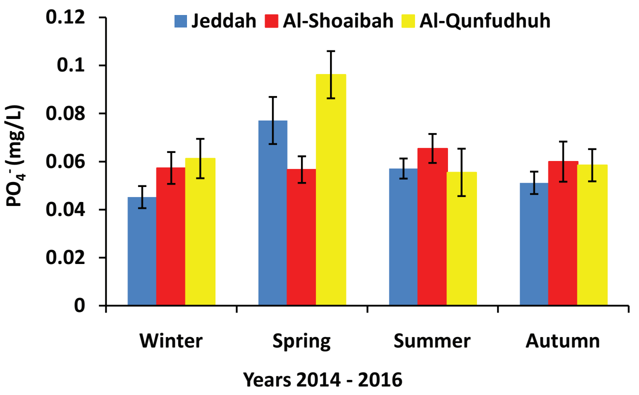

Fig. 8. PO_4^- level profile in mg/L during the study period in Jeddah, Al Shoaibah, and Al Qunfudhah sites.

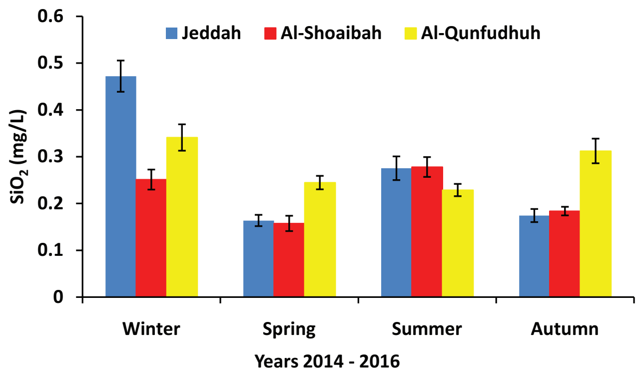

Fig. 9. SiO_2 level profile in mg/L during the study period in Jeddah, Al Shoaibah and Al Qunfudhah sites.

are usually found in aquatic systems. The adsorption and desorption of phosphate from mineral surfaces form a buffering mechanism that regulates dissolved phosphate concentrations in rivers and estuaries [72]. The trend toward lower nutrient concentration during the peak of phytoplankton growth was clearly shown for nitrate and phosphorus levels during autumn (Figs. 7 and 8). Although Lemley et al. [52] indicated that the limiting key factor for HABs during blooms in the Sundays Estuary of South Africa was identified as nitrate and phosphate availability, they also observed nitrate and phosphate depletion during the peak of the bloom.

Benthic recycling accounts for about 72% of the annual phosphorus inputs in some ecosystems [73]. Another major sink and recycling process for phosphorus in sea water is the phytoplankton activity that plays a role as sinks during their growth profile and as a source of phosphorus during their decline phase and decomposition. That is why nitrate and phosphate depletion is the key factor for bloom duration [52]. Therefore, a wide variation in phosphorus level in seawater is observed. Baturin [74] showed that variations in phosphorus level ranged from 1 to 300 µg/L.

3.4.7. Silica profiles during seasonal variations

The highest level of silica was recorded during winter at Jeddah and Al Qunfudhah (Fig. 9). Silica showed a seasonal trend in Jeddah similar to Al Shoaibah. A cyclic trend in silica pattern was observed at these two sites where an increased concentration of silica in winter was consumed during the spring and increased again in summer and winter. This pattern of silica consumption in autumn, where higher phytoplankton counts (Fig. 2) occurred, was confirmed by the negative correlation (Table 3) found at Al Shoaibah, meaning that the logarithmic phase of the growth were being observed at that time. However, a different pattern was observed for Al Qunfudhah where higher concentrations of silica were recorded for winter and autumn (Fig. 9). During autumn, a bloom of the non-toxigenic phytoplankton diatom *Nitzschia longissima* was observed and had the highest cell count number (4.4×10^{-5} cells/L) during this study [30]. The high concentration of silica found in autumn (Fig. 9) was confirmed by the significant positive correlation (Table 3) between silica and the bloom during autumn at Al Qunfudhah. This means that either the stationary or decline phase of the bloom with increased cell decomposition and consequent release of silica were being observed. This also means that the highest number recorded in this study may underestimate the real cell numbers during the peak of the bloom.

3.5. Regression analysis

In this section of the report, a regression analysis is used to understand the trend of the data in response to each parameter in order to run a stepwise regression. The final goal of such analysis is to come up with a prediction equation based on the sea truth data. Only a significant regression of the first, second, or third order was used in this analysis of the parameters affecting phytoplankton growth. Among the 12 parameters that could trigger the growth, only 9 parameters were chosen that have significant regression analysis as turbidity and fDOM regression analysis were not significant. Conductivity was used instead of salinity because conductivity proved to give a more precise measurement [38]. The nine most important parameters used in this analysis are ammonium, nitrate, nitrite, phosphate, silica, conductivity (instead of salinity), TDS, pH, and temperature. The significant regressions for conductivity and salinity were first order in all sites. The phytoplankton count increased with increasing conductivity from 55,000, 53,500, and 53,000 µS/cm to reach its maximum count at 69,500, 68,000, and 67,000 µS/cm at Al Qunfudhah, Al Shoaibah, and Jeddah, respectively. The same trend was observed with regard to salinity where a higher level was observed at higher salinity for all sites. The highest levels were recorded at 41.1, 40.4, and 40.2 psu at Al Qunfudhah, Al Shoaibah, and Jeddah, respectively.

The significant regression for TDS in Jeddah was second order and the highest cell count was 39,400 mg/L, whereas at Al Qunfudhah and Al Shoaibah the TDS observed for the highest cell count was 40,000 and 39,400 mg/L, respectively, in a first-order relationship. Again increasing pH led to higher phytoplankton counts at all sites. A significant second-order relationship between pH and phytoplankton count was detected at Al Shoaibah where two peaks of the highest count were at pH 8.2 and 8.95. In Al Qunfudhah and Jeddah, the significant relationship with phytoplankton count was linear with the highest count at pH 8.2 for Jeddah and 8.9 for Al Qunfudhah. Higher temperature increased the phytoplankton count. A significant second-order regression relationship was observed at Jeddah where the highest count was at 27.5 and at 30.5°C. Significant linear regressions were detected in Al Qunfudhah and Al Shoaibah where the highest phytoplankton counts were at 32.5 and 31.5°C, respectively.

All of the nitrogen forms (NH_4^+, NO_3^-, and NO_2^-) had a significant second-order regression in Jeddah with two high count peaks at 0.92 and 1.28 mg/L for ammonium and 1.0 and 1.57 mg/L for nitrate and 0.014 and 0.026 mg/L for nitrite. At Al Qunfudhah and Al Shoaibah, the relationships for nitrate and nitrite were also second-order regression and were first order for ammonium. The nitrate at Al Shoaibah was 0.373 and 2.02 mg/L and at Al Qunfudhah it was 0.96 and 1.79 mg/L. The nitrites were 0.002 and 0.015 mg/L for Al Shoaibah and for Al Qunfudhah they were 0.004 and 0.008 mg/L. The first-order regression for ammonium showed 0.75 and 1.44 mg/L for Al Shoaibah and Al Qunfudhah, respectively.

Phosphate relationships were linear at Jeddah and Al Shoaibah at concentrations of 0.11 and 0.01 mg/L, while in Al Qunfudhah it was second order and the concentrations were 0.07 and 0.1 mg/L. Regressions of a second order were observed for silica at Al Shoaibah (0.14 and 0.28 mg/L) and Al Qunfudhah (0.26 and 0.8 mg/L). Jeddah showed a first-order relationship at a concentration of 0.38 mg/L. In conclusion, it could be that higher temperature, salinity, TDS, and pH are the factors that favor HAB species that can tolerate such conditions in a less competitive environment.

Nutrients are a second major factor that may trigger HABs, however, these limiting factors are hard to specify without controlled lab experiments as unexpected results were found during this study. No correlation between HAB counts and ammonium, a negative correlation in the case of nitrate, nitrite, and phosphorus, plus a negative and positive correlation for silica were found. This means it depends on the cell growth phase that are sampling at, where higher nutrient concentrations will be found at the beginning of the log phase and at the stationary and decline phase during cell decomposition. Lower nutrient concentration is expected at the middle to the end of the log phase. This is true in those cases where there is no continuous influx in that environment as is the case during sewage dumping. There was no sewage dumping in the different areas around the water desalination plants at all three sites during the study. Therefore, the annual seasonal variations due to thermocline formation in summer and autumn and the mixing process as well as the freshwater runoff during the rainy season are the major factors that control nutrient influx at the three desalination plants near shore environment. Table 4 lists the different parameter numbers that define the environment during times in which higher phytoplankton counts occurred during this study.

3.6. Prediction equation based on the sea truth data

Because of the highly significant differences among sites, stations, and depths, data for each depth within each station and site were used to develop a prediction equation. Equations were constructed to predict the total HAB count. Data were subjected to the stepwise procedure available in SAS software [45]. The model used included total HAB count as the dependent variable, while SiO_2, NO_3^-, NO_2^-, PO_4^-, temperature, conductivity, TDS, and pH were the independent variables. Quadratic and cubic of almost all of the independent variables were included as well as square root in some cases. Examining the 27 resulting equations through the coefficient of determination (denoted as R^2: a number that indicates the proportion of the variance in the dependent variable that is predictable from the independent variable) revealed that Depth 2 of Station 2 allows the best prediction equation, depending on its R^2 (Table 5). Only these equations for the three sites will be discussed. As illustrated in Table 2, the three sites were significantly differed for all measured parameters except for TDS, NO_2^-, and salinity. Therefore, it was dealt with three different ecosystems that should have three different prediction equations. Several reports indicate that bloom events at different ecosystems have different limiting key factors that trigger the bloom occurrence which means that each ecosystem has to have its own predicting model depending on the major key factors that affect bloom initiation [26,33,52,75].

Prediction equation based on the sea truth data:

$$y_i = \beta_\circ + \beta_1 x_1 + \beta_2 x_1{}^2 + \beta_3 x_1{}^3 + \beta_4 x_2 + ... + e \quad (3)$$

where y_i is the dependent variable (total phytoplankton); β_0 is the intercept value; β_1, β_2.. β_n is the regression

Table 4
The different parameters values in the ecosystem during HAB events during the course of this study from December 2014 to November 2016

Parameter	Jeddah	Al Shoaibah	Al Qunfudhah
Temperature, °C	27.5 and 30.5	31.5	32.5
Conductivity, μS/cm	67,000	68,000	69,500
Salinity, psu	40.2	40.4	41.1
TDS, mg/L	39,400	39,400	40,000
pH	8.2	8.2 and 8.95	8.95
Ammonium, mg/L	0.92 and 1.28	0.75	1.44
Nitrate, mg/L	1.0 and 1.57	0.373 and 2.02	0.96 and 1.79
Nitrite, mg/L	0.014 and 0.026	0.002 and 0.015	0.004 and 0.008
Phosphate, mg/L	0.11	0.01	0.07 and 0.1
Silica, mg/L	0.38	0.14 and 0.28	0.26 and 0.8

Table 5
The R^2 values for different 27 prediction equations, where an equation was predicted using the data of each depth from each site at each station

Site	Station 1			Station 2			Station 3		
	Depth 1	Depth 2	Depth 3	Depth 1	Depth 2	Depth 3	Depth 1	Depth 2	Depth 3
1	0.45	0.46	0.49	0.71	0.84	0.49	0.68	0.54	0.49
2	0.71	0.86	0.51	0.77	0.84	0.83	0.53	0.71	0.70
3	0.59	0.29	0.47	0.68	0.76	0.60	0.70	0.74	0.65

coefficient; x_1 is the independent variable NO_3^-; x_2 is PO_4^-; x_3 is temperature; x_4 is conductivity; x_5 TDS; x_6 pH; and e_i is the random error

Only significant ($p < 0.05$ or less) regressions are included in the equations. For simplifying and making the prediction equations easer, only the following traits were included for predicting the equations (NO_3^-, PO_4^-, temperature, conductivity, and pH). However, TDS was added for Site 3. As mentioned before only significant regression coefficients are reported.

Site 1 Station 2, Depth 2 ($R^2 = 0.8610$)

$$\begin{aligned}\text{Total phytoplankton} = {} & 4\text{E+}7 + 3.9\text{E+}3\,(x_1)^2 - 1.7\text{E+}3\,(x_1)^3 \\ & + 1.1\text{E+}6\,(x_3) - 3.9\text{E+}4\,(x_3)^2 \\ & + 4.7\text{E+}2\,(x_3)^3 - 1.1\text{E+}3\,(x_4) \\ & + 0.01\,(x_4)^2 - 9.88833\text{E}{-}8\,(x_4)^3 \\ & - 6.7\text{E+}6\,(x_6) + 4.1\text{E+}5\,(x_6)^2\end{aligned} \tag{4}$$

Site 2 Station 2, Depth 2 ($R^2 = 0.8428$)

$$\begin{aligned}\text{Total phytoplankton} = {} & -3.2\text{E+}7 - 4.8\text{E+}4\,(x_1) + 3.9\text{E+}\,4\,(x_1)^2 \\ & - 9.1\text{E+}3\,(x_1)^3 + 9.7\text{E+}5\,(x_3) \\ & - 3.6\text{E+}10\,(x_3)^2 + 4.5\text{E+}2\,(x_3)^3 \\ & +5.9\text{E+}6\,(x_6) - 3.7\text{E+}5\,(x_6)^2\end{aligned} \tag{5}$$

Site 3 Station 2, Depth 2 ($R^2 = 0.7064$)

$$\begin{aligned}\text{Total phytoplankton} = {} & 6.5\text{E+}7 + 1.2\text{E+}6\,(x_2) - 1.2\text{E+}7\,(x_2)^2 \\ & + 2.3\text{E+}7\,(x_2)^3 + 3.4\text{E+}3\,(x_3) \\ & - 8\text{E+}1\,(x_3)^3 - 3.1\text{E+}3\,(x_4) \\ & + 0.04\,(x_4)^2 - 2.2\,(x_4)^3 + 8.5\,(x_5)^3 \\ & + 1.6\text{E+}3\,(x_6)^3\end{aligned} \tag{6}$$

During a bloom, lower diversity with higher cell counts for the species responsible is usually reported to be a characteristic feature of a bloom [49]. In his definition of what is a bloom, Smayda [49] explained that it is not necessary to reach a high cell count or high abundance for a species to be a bloom. Events with cell count of 10^4 cells/L (or lower) resulting from background growth of 10^2 is considered to be a bloom. Accordingly, for an early warning alert for desalination plant authorities' management efforts, the threshold for the phytoplankton count was set 10^4 cells/L to be considered as a bloom. This conservative threshold will help desalination plant authorities have enough time to manage the bloom. Prediction models of phytoplankton blooms often contain critical ecosystem thresholds that may signal bloom triggering and can be used for management and control [26].

These prediction equations for each site employ numbers for each factor in the equation with the resulting number being the expected phytoplankton count for the given conditions. When this threshold count is exceeded an alert should be declared allowing management actions within the desalination plant. In real practice, this threshold value could be manipulated according to other biological factors accompanying the phytoplankton bloom that might also threaten the desalination plant.

According to Pace et al. [26], advance warning of blooms may be expected using pigment observations in lake water and calculating resilience indicators. Stopping nutrient input after preset indicator threshold was reached, reversed the bloom in their experiment. In this proposed prediction models, a phytoplankton critical threshold was set that could be predicted in advance using certain ecological factors based on this 2-year study.

4. Conclusions

In conclusion, following phytoplankton profiles over the study period from December 2014 to November 2016 was succeeded. The different phytoplankton species around the desalination plants in the selected sites were reported and the HAB physicochemical factors that influence this unique environment were identified. The sea truth field trips showed that the HAB season is in autumn, which was predicted based on historical monthly composite MODIS AQUA satellite images from NASA showing chlorophyll a concentrations increased from Jeddah to Jazan beginning in June and peaked in September–February. The field samples showed that phytoplankton increased from Jeddah to Al Qunfudhah and decreased from the coast to the open sea sites and from the surface to the depth of 10 m. Dinoflagellates were the dominant species at all sites. Higher temperature, conductivity, salinity, TDS, and pH were correlated with the presence of certain HAB species. A hypothesis for the reason behind the increasing number of certain species in this condition is due to the tolerance of these species to unfavorable environments compared with the larger number of species unable to grow in such conditions. Nitrate, phosphorus, and silica were the major nutrients that correlated with promotion of HAB species. Equations to predict those conditions favoring HAB occurrence were able to be generated using those parameters that promote HAB occurrence. An early warning system for algal blooms could be based on the proposed prediction equations and daily satellite imagery for the Red Sea.

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