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Species composition and abundance of dinoflagellates from the coastal waters of Pakistan

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ABSTRACT

Objective: To assess the community structure, seasonal dynamics of dinoflagellates population with environmental conditions in the nutrient-rich and polluted coastal waters off, Karachi.

Methods: Sampling sites were located from Karachi harbour (Station A) and Mouth of Manora Channel (Station B). Total 180 replicate samples were collected at 1-m depth through Niskin bottle sampler (1.7 L) and fixed with 2% lugol's preservative then examined under light inverted microscopy, scanning electron and epiflourescence microscope.

Results: The environmental conditions, such as temperature [(20–27) ˚C], salinity [(35–40) psu], chlorophyll a [(1–103) µg/L], pH (6.03–8.13) and dissolve oxygen [(0.7–5.5) mg/L] were recorded from both stations. A total of 96 species were identified into potential harmful toxic/ non-toxic bloom forming species and cysts producer. Total dinoflagellate cells between two coastal sites were much concentrated at the adjacent area of mouth of Manora Channel compare to harbor site. The dinoflagellate cell concentration ranging from 20 to ~55 000 cells/L and the maximum values observed in two season, (~55 000 cells/L) in autumn and (~ 3 000 cells/L) in winter season. *Gyrodinium* sp. was the predominant taxa with the maximum abundance (48 166 cells/L) observed in autumn season following by *Scrippsiella trochoidea* (1 200 cells/L), *Alexandrium ostenfeldii* (3 000 cells/L) in winter season, and *Ceratium furca (*640 cells/L), *Protoperidinium steinii* (780 cells/L), *Ceratium fusus* (906 cells/L), *Pyrophacus steinii* (840 cells/L), *Gonyaulax spinifera* (666 cells/L), *Alexandrium tamarense* (520 cells/L) and *Dinophysis caudata* (393 cells/L) in summer and spring season. Statistically, abundance of dinoflagellates correlated significant to chlorophyll a with chlorophyll a and temperature but inverse relation to salinity and pH observed from both sites.

Conclusions: The present study reports on the prevalence and significance of harmful algae bloom forming taxa in the area which would be available for the coastal zone managers and fishery industry to inform them of possible threat and damage that can be caused by any blooms to, for example, fishery industry, and environmental and human health.

1. Introduction

Dinoflagellates are a group of important primary producers in phytoplanktons community which have adaptive ecology, including multiple habitat, large species richness[1,2]. Dinoflagellates play an important role in sequestering anthropogenically-derived carbon from biosphere[3]. Therefore, they are linked with other abiotic and biotic components of the ecosystem. During high upwelling conditions, inorganic nutrients (N, S and P) and lights are the favourable factors for the growth of dinoflagellates and other flagellates in the Arabian Sea[4-7]. These nutrients recycle by zooplankton which limit the silicon concentration in the water and effect or decline the growth of diatoms but the high rate of N can increase the production of dinoflagellates. In this condition, the cells can proliferate and increase the cellular chemical composition (toxin synthesis). Presently, more than 2 000 dinoflagellate species have been reported in the world and 200 species are toxic which responsible for harmful algal blooms in the coastal areas[1,8]. Moreover, they are correlated with coral reef system and are one reason led to the mortality of mammals[9] and reef fishes[10,11] and caused health issues.

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Pakistan coastal area is situated at the coastal belt of Northern Arabian Sea, to the north along the Bombay, Kutch and Saurashtra and at the south of Iran and Gulf of Oman, are known as highly productive zones[12]. Arabian Sea has complex water masses structure and important dynamic property to give different shape to phytoplankton ecology in this region[13]. The circulation pattern of monsoon gyres have highly effects on diatoms and dinoflagellates population, dominate one community to other during SW monsoon period to NE monsoon period[14,15]. During the NE monsoon season, the nutrients shift to the upper-column creating favorable condition for dinoflagellate growth. The spring inter-moonsoon eddies and recirculation factor increases which enhance the phytoplankton growth to some extend[16]. These initiate the blooms during upwelling and nutrient rich cold waters in northern Arabian Sea[17]. Such blooms has been caused the large fish killings in Balochistan coast[18] and Karachi coastal waters of Pakistan[19]. Taylor[20] has extensive work on distribution of dinoflagellates including some sites of Pakistani waters. Most literature deal largely with taxonomy from the coastal sites of Pakistan such as Manora Channel and Korengi Creek[21-26] and Baluchistan coast[27]. Recently some studies have been carried out on ecology, biodiversity and taxonomy[27-34], biovolume and carbon biomass[35-37], growth rate[38,39] of diatoms and dinoflagellates. These available reports indicated that the dyanamics studies of dinoflagellates are scarce from the Pakistan waters and present research is based on a comprehensive study on the seasonal changes of dinoflagellates population in coastal waters of Pakistan which focuses on 1) community structure, 2) prevalence of potentially harmful species, 2) diversity and abundance of dinoflagellates and develop the biodiversity strategy to fisheries industries in Pakistan.

2. Materials and methods

In May 2002-July 2003, 180 triplicate samples were collected from two sites, Karachi Harbor Station A (24˚49.77' N, 66˚57.85' E) and the mouth of Manora Channel Station B (24˚47.93' N, 66˚58.87' E) in Karachi, Pakistan (Figure 1). Samples were collected through Niskin bottle sampler at 1-m depth and fixed with Lugol's preservative. Samples were examined using Utermöhl techniques[40]. Samples were observed using a light microscope (model) after 24 h settling for concentration (50 mL). Dinoflagellates were identified using light, epifluorescence and scanning electron microscopy. For examination of thecal plates, cells were stained with 1% calcofluor white MR2 (Sigma, St Louis, MO) and observed under UV excitation of fluorescence microscopy. The physio-chemical parameters, *e.g.,* temperature, salinity, chlorophyll a, pH and dissolve oxygen (DO), were noted through using spectrophotometer, (Hanna Kit) by methods[41], and pH recorded by using probe (Hanna, HI9023, Italy).

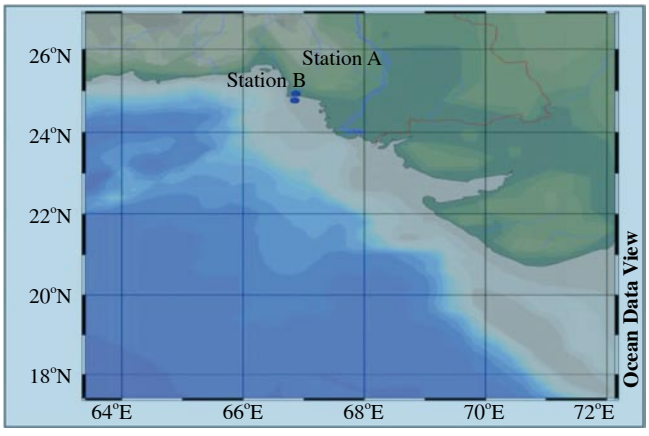

Figure 1. Sampling stations (A, B) located at Manora Channel, Karachi.

For SEM, preserved cells were desalted using a 10% step gradient of freshwater and dehydrated by using a series of acetone (10%–100%). Samples were coated with gold platinum and dried using a Denton sputter-edge coater (Denton Vacuum, Moorestown, NJ) and examined with a JEOL JSM-5600LV (JEOL, Tokyo, Japan) scanning electron microscope.

3. Results

3.1. Physico-chemical parameters in 2002-2003

Temperature, salinity, chlorophyll a, DO and pH from both sites are shown in Figure 2. The temperature [(20–31) ˚C] and salinity [(32–41) psu] had similar ranges at both stations and low temperature was corresponding to high salinity values (T < 20 ˚C to S > 41 psu) in colder months and high temperature corresponding to low salinity (T < 31˚C to S > 34 psu) in summer months (Figures 2a and 2b).

Chlorophyll a values observed with different seasonal trend at both sites which ranged from 2 to 74 µg/L at Station A and 0.48 to 103 µg/L at Station B (Figure 2c). The maximum chlorophyll a values was observed in autumn season at Station A and in summer at Station B (Figure 2c). Maximum chlorophyll a values corresponding to ~30 ˚C and ~35 psu and low chlorophyll a value corresponding to ~30 ˚C and ~38 psu (Figure 2c).

DO and pH values has shown similar trend at both sites which ranged from 0.7 to 5.6 mg/L and 6.34 to 8.13, respectively (Figure 2d–2e). The low DO values were observed minimum in autumn at Station A and in summer at Station B and high DO values were observed in winter. The maximum DO value corresponding to high pH values (DO > 4.0; pH > 8.05) and low DO corresponding to low pH values (DO > 2.25; pH > 6.74) (Figures 2d and 2e). The low pH values was observed on July 2, 2002 and high value was observed on (Mar 1) at Station A and low values was observed during (Aug 1, Sep 1 2002) and high value was observed during (Mar 2, Apr 1) (Figure 2e).

3.2. Dinoflagellate community structure and abundance

A total of 96 taxa identified including 30 taxa were toxin producing species, 26 taxa as potential harmful bloom causing species, 9 taxa as icthyotoxic producing species, 24 taxa as cyts producing species (Table 1) and illustrated by light/ fluorescence and scanning micrography (Figure 3). *A. ostenfeldii*, *D. caudata*, *C. furca*, *C. fusus*, *G. spinifera*, *S. trochoidea*, *P. steinii*, were frequently observed and *Py. steinii*, *G. spirale*, *A. tamarense*, *P. depressum*, *Gyrodinium* sp., *H.* cf. *circularisquama* were occasionally occuring species and more than 40 species were observed rare in the study period(Table 2). Dinoflagellates showed 87%–94% population dominated to the total phytoplanktons in autumn season (October) and 34% in winter season (January 2003). Total cell concentration of dinoflagellate ranged from 20 to 55 726 cells/L which increases toward to adjacent sea area Station B and lower cell concentration 17 000 cells/L to the near shore site, Station A. The low abundance ~600 cells/L observed during summer season (June 2002 to July 2002) from both sites (Figure 4). The dominance index estimated for the dominant taxa *Gyrodinium* sp (87.00%), *A. ostenfeldii* (5.00%), *C. fusus* (1.70%), *Py. steinii* (1.40%), *A. tamarense* (0.94%), *P. divergens*, *G. spinifera*, *D. caudata* (0.67%) corresponding to peak abundance in autumn season and *A. ostenfeldii* (32.00%), *S. trochoidea* (28.00%) and *C. furca* (18.00%) during second peak in winter season.

3.3. Seasonal distribution and abundance of dinoflagellate

Seasonal distribution and abundance of dinoflagellates based on the abundant species, *e.g. A. ostenfeldii*, *D. caudata*, *C. furca* were present during the whole year (May 2002–July 2003) from both sites. Most of these dominant species have high abundance values distributed to the

Table 1
List of dinoflagellate species identified from two stations of Manora Channel, 2002–2003.

Toxic species	Nontoxic species	Icthyotoxic species	Cyst producer
Alexandrium minutum	*Ceraitum furca*	*Akashiwo sanguinea*	*Protoperidinium divergens*
Alexandrium ostenfeldii	*Ceraitum lineatum*	*Cochlodinium fulvescens*	*Protoperidinium depressum*
Alexandrium tamarense	*Ceraitum fusus*	*Gyrodinium spirale*	*Protoperidinium curvipes*
Alexandrium tamiyavanichii	*Ceraitum inflatum*	*Gyrodinium* sp.	*Protoperidinium oceanicum*
Alexandrium concavum	*Ceraitum tripose*	*Heterocapsa* cf. *circularisquama*	*Protoperidinium oblongum*
Gonyaulax spinifera	*Ceraitum contrarium*	*Noctiluca scintillans*	*Protoperidinium pentagonum*
Gonyaulax polygramma	*Ceraitum minutum*	*P. balticum*	*Protoperidinium bipes*
Gonyaulax verior	*Ceraitum trichocerose*	*P. minimum*	*Protoperidinium brevipes*
Gonyaulax digitalis	*Ceraitum macroceros* var. *macroceros*	*Prorocentrum donghaiense*	*Protoperidinium simulum*
Lingulodinium polyedrum	*Ceraitum massilliense*		*Protoperidinium excentricum*
Protoceratium reticulatum	*Ceraitum pelluchellum*		*Protoperidinium* cf. *avellana*
Ostreopsis cf. *ovata*	*Ceraitum lunula*		*Protoperidinium longipes*
Gymnodinium catenatum	*Ceraitum breve*		*Protoperidinium quarnerense*
Gymnodinium sp.	*Ceraitum hexicantum*		*Protoperidinium leonis*
Dinophysis caudata	*Ceraitum kofoidii*		*Protoperidinium minutum*
Dinophysis acuminata	*Prorocentrum micans*		*Protoperidinium subinerme*
Dinophysis tripos	*Prorocentrum gracile*		*Protoperidinium granii*
Dinophysis miles	*Prorocentrum arcuatum*		*Protoperidinium cerasum*
Dinophysis fortii	*Prorocentrum scutellum*		*Protoperidinium ovatum*
Dinophysis acuta	*Prorocentrum sigmoides*		*Preperidinium meunieri*
Dinophysis mitra	*Prorocentrum compressum*		*Pyrophacus steinii*
Dinophysis roduntata	*Protoperidinium steinii*		*Scrippsiella trochoidea*
Dinophysis dense	*Diplopsalis lenticula*		*Scrippsiella* sp2
Dinophysis infundibulata	*Katodinium glaucum*		*Scrippsiella* cf. *spinifera*
Prorocentrum balticum	*Torodinium cf. teredo*		
Prorocentrum lima	*Triadinium polyedricum*		
Prorocentrum faustaie			
Prorocentrum emarginatum			
Karenia cf. *mikimotoi*			
Oxytoxum cristatum			

Alexandrium minutum: *A. minutum*; *Alexandrium ostenfeldii*: *A. ostenfeldii*; *Alexandrium tamarense*: *A. tamarense*; *Alexandrium tamiyavanichii*: *A. tamiyavanichii*; *Gonyaulax spinifera*: *G. spinifera*; *Gonyaulax polygramma*: *G. polygramma*; *Gonyaulax verior*: *G. verior*; *Gonyaulax digitalis*: *G. digitalis*; *Protoceratium reticulatum*: *P. reticulatum*; *Gymnodinium catenatum*: *G. catenatum*; *Dinophysis caudata*: *D. caudata*; *Dinophysis acuminata*: *D. acuminata*; *Dinophysis miles*: *D. miles*; *Dinophysis fortii*: *D. fortii*; *Dinophysis acuta*: *D. acuta*; *Dinophysis dense*: *D. dense*; *Prorocentrum balticum*: *P. balticum*; *Prorocentrum minimum*: *P. minimum*; *Ceraitum furca*: *C. furca*; *Ceraitum lineatum*: *C. lineatum*; *Ceraitum fusus*: *C. fusus*; *Ceraitum tripose*: *C. tripose*; *Ceraitum macroceros* var. *macroceros*: *C. macroceros* var. *macroceros*; *Protoperidinium steinii* : *P. steinii*; *Gyrodinium spirale*: *G. spirale*; *Heterocapsa* cf. *circularisquama*: *H.* cf. *circularisquama*; *Protoperidinium divergens*: *P. divergens*; *Protoperidinium depressum*: *P. depressum*; *Protoperidinium oceanicum*: *P. oceanicum*; *Protoperidinium oblongum*: *P. oblongum*; *Protoperidinium pentagonum*: *P. pentagonum*; *Protoperidinium brevipes*: *P. brevipes*; *Protoperidinium longipes*: *P. longipes*; *Protoperidinium minutum*: *P. minutum*; *Protoperidinium ovatum*: *P. ovatum*; *Pyrophacus steinii*: *Py. steinii*; *Scrippsiella trochoidea*: *S. trochoidea*.

Table 2
Occurance and distribution of dinoflagellate species from Station A and Station B.

Frequency	Species	Station A	Station B
Dominant	*A. ostenfeldii*	++	++
	C. furca	++	++
	D. caudata	++	++
	P. minimum/ P. balticum	++	++
	Prorocentrum donghaiense	++	++
	Gyrodinium sp	++	++
	S. trochoidea	++	++
	A. tamarense	++	-
	A. tamiyaunichivi	-	++
	C. fusus	++	++
	C. inflatum	++	++
	C. lineatum	++	++
Occasional	*D. acuminata*	++	++
	G. spinifera	++	++
	G. spirale	++	++
	G. digitalis	-	++
	H. cf. *circularisquama*	++	++
	P. steinii	++	++
	P. divergens	++	++
	P. depressum	++	++
	P. conicavum	++	-
	Phyrophacus steinii	++	++
	Oblea roduntata	++	++

(*continued on right column*)

Table 2 *(continued)*

Frequency	Species	Station A	Station B
Rare	*Akashiwo sanguinea*	++	++
	A. minutum	++	-
	A. conicavum	++	-
	C. tripose	++	++
	Ceraitum contrarium	-	++
	Ceraitum hexicantum	-	++
	Ceraitum breve	-	++
	C. blechii	-	++
	C. extensum	-	++
	C. euarcuatum	-	++
	Ceraitum minutum	-	++
	C. macroceros var macroceros	-	++
	Ceraitum kofoidii	++	-
	Ceraitum massilliense	-	++
	Ceraitum pelluchellum	-	++
	Ceraitum lunula	-	++
	D. acuta	++	-
	D. fortii	++	-
	D. miles	-	++
	D. mitra	-	++
	Dinophysis roduntata	++	-
	G. vievor	++	-
	Goniodoma	++	-
	Gymnodinium catanetum	++	-
	Karenia sp.	-	++

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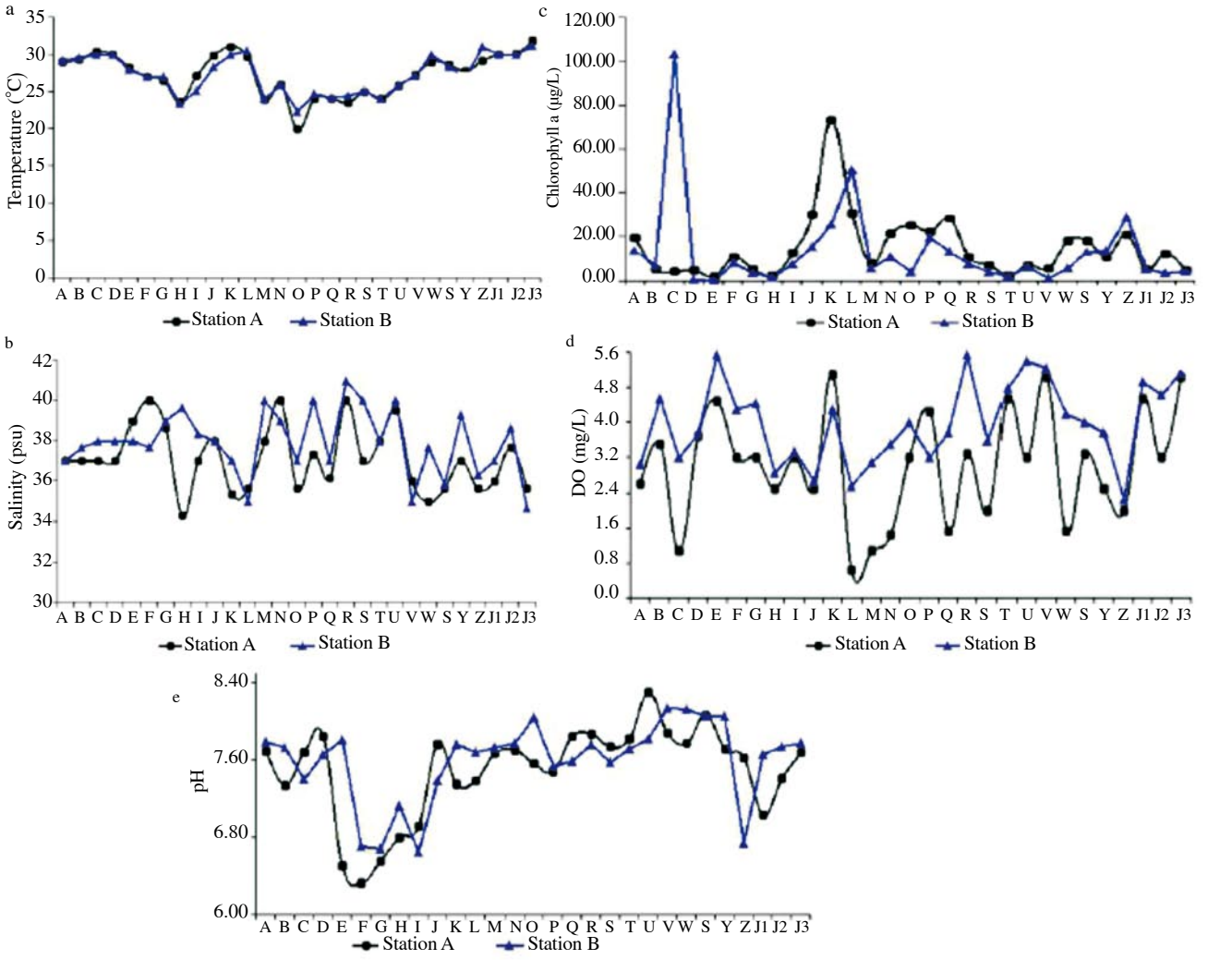

Figure 2. Seasonal distribution of physical parameters, temperature (a), salinity (b), chlorophyll a (c), DO (d), pH (e) from Station A and Station B, Karachi. A: 1 May 2002; B: 2 May 2002; C: 1 Jun 2002; D: 2 Jun 2002; E: 1 Jul 2002; F: 2 Jul 2002; G: 1 Aug 2002; H: 2 Aug 2002; I: 1 Sep 2002; J: 2 Sep 2002; K: 1 Oct 2002; L: 2 Oct 2002; M: 1 Nov 2002; N: 2 Nov 2002; O: 1 Dec 2002; P: 2 Dec 2002; Q: 1 Jan 2003; R: 2 Jan 2003; S: 1 Feb 2003; T: 2 Feb 2003; U: 1 Mar 2003; V: 2 Mar 2003; W: 1 Apr 2003; S: 2 Apr 2003; Y: 1 May 2003; Z: 2 May 2003; J1: 1 Jun 2003; J2: 2 Jun 2003; J3: 1 Jul 2003.

Table 2 *(continued)*

Frequency	Species	Station A	Station B
Rare	*Katodinium gulucum*	++	-
	Oxyphsis	++	-
	Oxyhirrus marina	-	++
	P. reticulatum	++	++
	Protoperidinium curvipes	++	-
	P. oceanicum	++	-
	P. oblongum	++	-
	P. excentricum	++	-
	P. cf. *avellena*	++	-
	P. minutum	++	-
	Protoperidinium subinerme	++	-
	Protoperidinium granii	++	-
	P. ovatum	-	++
	P. pentagonum	-	++
	P. brevipes	-	++
	P. simulus	-	++
	P. longipes	-	++
	P. lenois	-	++
	P. cerasus	-	++
	Scrippsiella sp2	-	++
	Torodinium cf. *teredo*	-	++
	Periperidinium munierier	-	++

Dominant: 1 000–50 000 cells/L; Occasion: 1 000–250 cells/L; Rare: 100–20 cells/L; -: Absent; ++: Presence.

adjacent sea area, Station B and slight low abundance to near shore sites Station A such as *A. ostenfeldii* was the abundant species observed in all months from both sites, it occurs with the cell concentration ranges from 20–3 000 cells/L with maximum values observed in autumn season (October 2002) and 1 440 cells/L in winter season (January 2003) (Figure 5A). The maximum values was corresponding to high chlorophyll a values (50.51 ug/L), temperature, salinity, pH and low DO (T > 30.50 °C, S > 35.00, pH > 7.68 and DO < 2.55) in October at Station B and low chlorophyll a values (28.70 µg/L), temperature, salinity, pH and low DO (T > 24.5 °C, S > 36.00 pH > 7.86 and DO < 1.55) in January 2003 at Station A.

C. furca was second most frequent occuring species with the mean values 20–640 cells/L observed the maximum concentration in February 2003 at Station B and 73 cells/L in January 2003 at Station A (Figure 5B).These maximum values are corresponding to low chlorophyll a values, temperature, salinity, pH and low DO (T > 24.5 °C, S > 36.00, chlorophyll a > 28.70, pH > 7.86 and DO < 1.55) .

C. fusus was third frequent occuring species of Station B but only observed in 6 months at Station A. The abundance values ranging from 20–940 cells/L with maximum concentration observed in October 2002 at Station B (Figure 5C) which corresponding to high chlorophyll a values, temperature, salinity, pH and low DO (T > 30.50 °C, S > 35.00, chlorophyll a > 50.51, pH >7.68 and DO < 2.55).

S. trochoidea has shown the distribution in 9 months from both sites

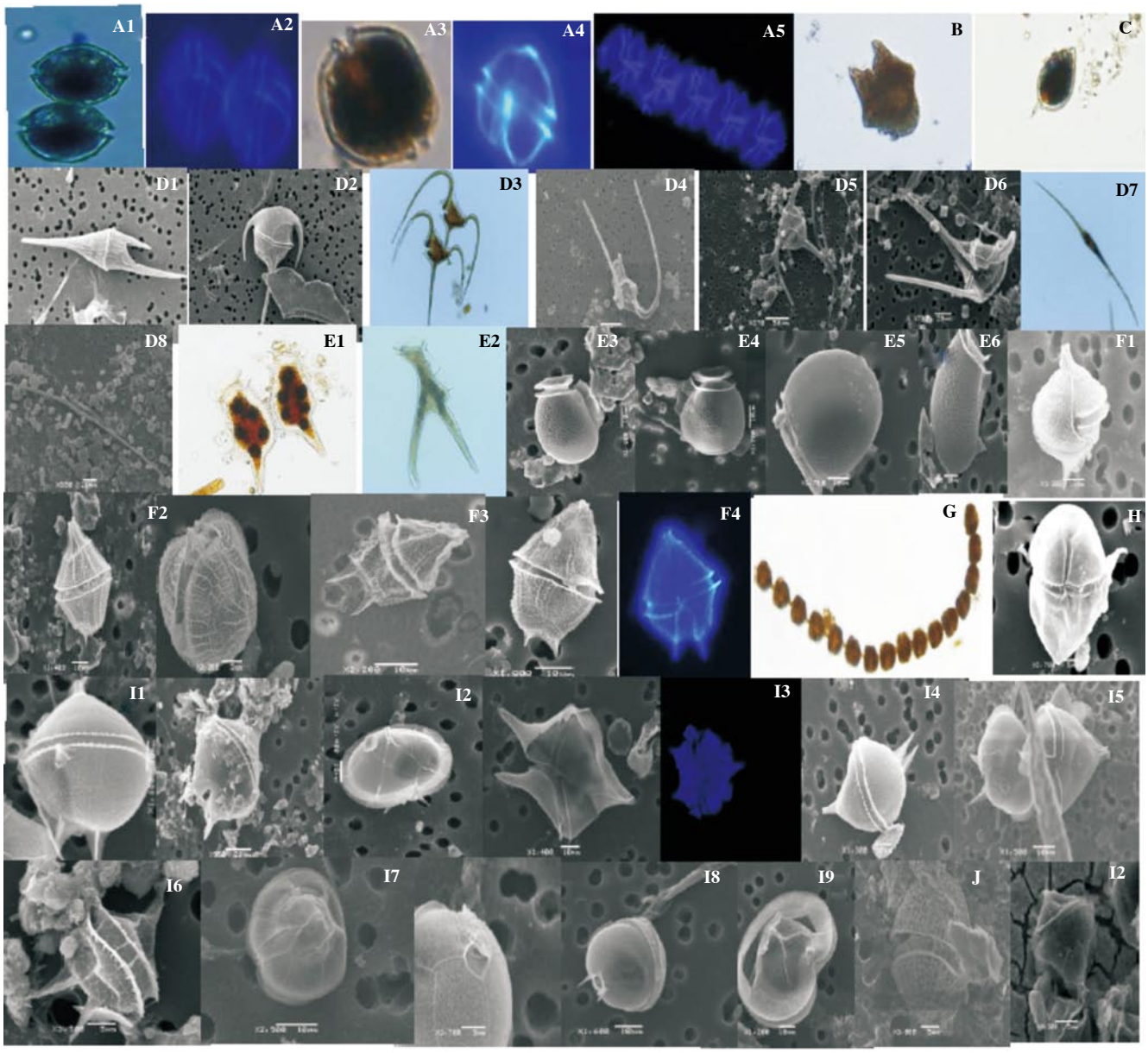

Figure 3. Light/ fluorescent micrographs of *A. ostenfeldii* (A1-A2), *A. minutum* (A3), *A. tamarense* (A4), *A. tamiyavanich* (A5), *Akashiwo sanghainea* (B), *Prorocentrum micans* (C), Scanning/ light micropgraphs of *Ceraitum furca* (D1), *C. tripos*e (D2), *C. macroceros* var. *macroceros* (D3), *C. longipes* (D4), *C.* var. *reflexa* (D5-D6), *C. fusus* (D7), *Ceraitum inflantum* (D8), Scanning/ light micropgraphs of *D. caudata* (E1), *D. miles* (E2), *D. acuminata* (E3), *D. infundibulus* (E4), *D. rotundata* (E5), *D. fortii* (E6), *G. spinifera* (F1), *G. polygramma* (F2), *G. verior* (F3-F4), *G. catenatum* (G), *S. trochoidea* (H), *Protoperidinium stenii* (I1), *P. depressum* (I2), *P. divergens* (I3), *P. pellucidum* (I4), *P. granelii* (I5), *P. longipes* (I6), *P. lenois* (I7), *P. minimum* (I7-I8), *P. subrineme* (I9), *P. quenrequence* (I20), *P. reticulatum* (J).

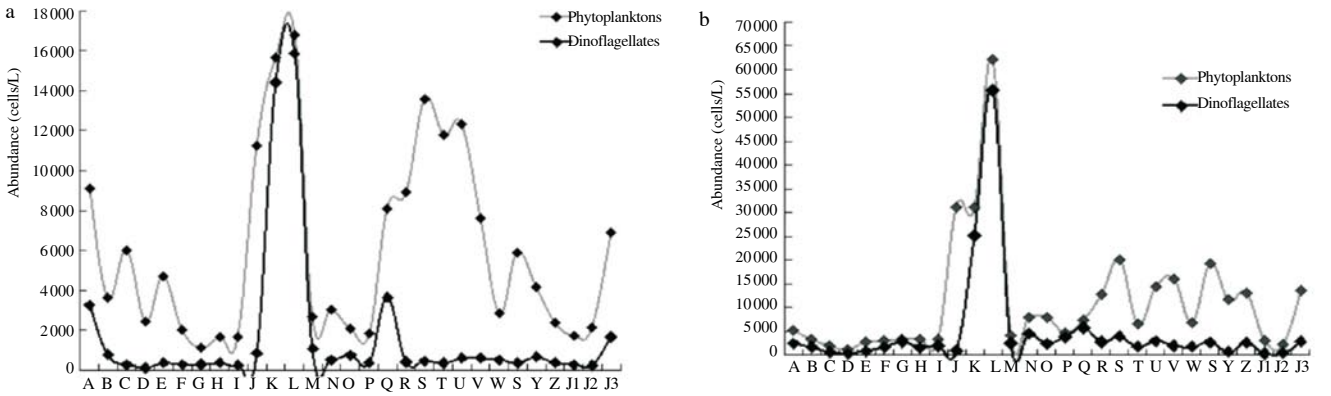

Figure 4. Seasonal distribution and abundance cells/L of dinoflagellates from Station A (a) and from Station B (b), Manora Channel 2002–2003.
A: 1 May 2002; B: 2 May 2002; C: 1 Jun 2002; D: 2 Jun 2002; E: 1 Jul 2002; F: 2 Jul 2002; G: 1 Aug 2002; H: 2 Aug 2002; I: 1 Sep 2002; J: 2 Sep 2002; K: 1 Oct 2002; L: 2 Oct 2002; M: 1 Nov 2002; N: 2 Nov 2002; O: 1 Dec 2002; P: 2 Dec 2002; Q: 1 Jan 2003; R: 2 Jan 2003; S: 1 Feb 2003; T: 2 Feb 2003; U: 1 Mar 2003; V: 2 Mar 2003; W: 1 Apr 2003; S: 2 Apr 2003; Y: 1 May 2003; Z: 2 May 2003; J1: 1 Jun 2003; J2: 2 Jun 2003; J3: 1 Jul 2003.

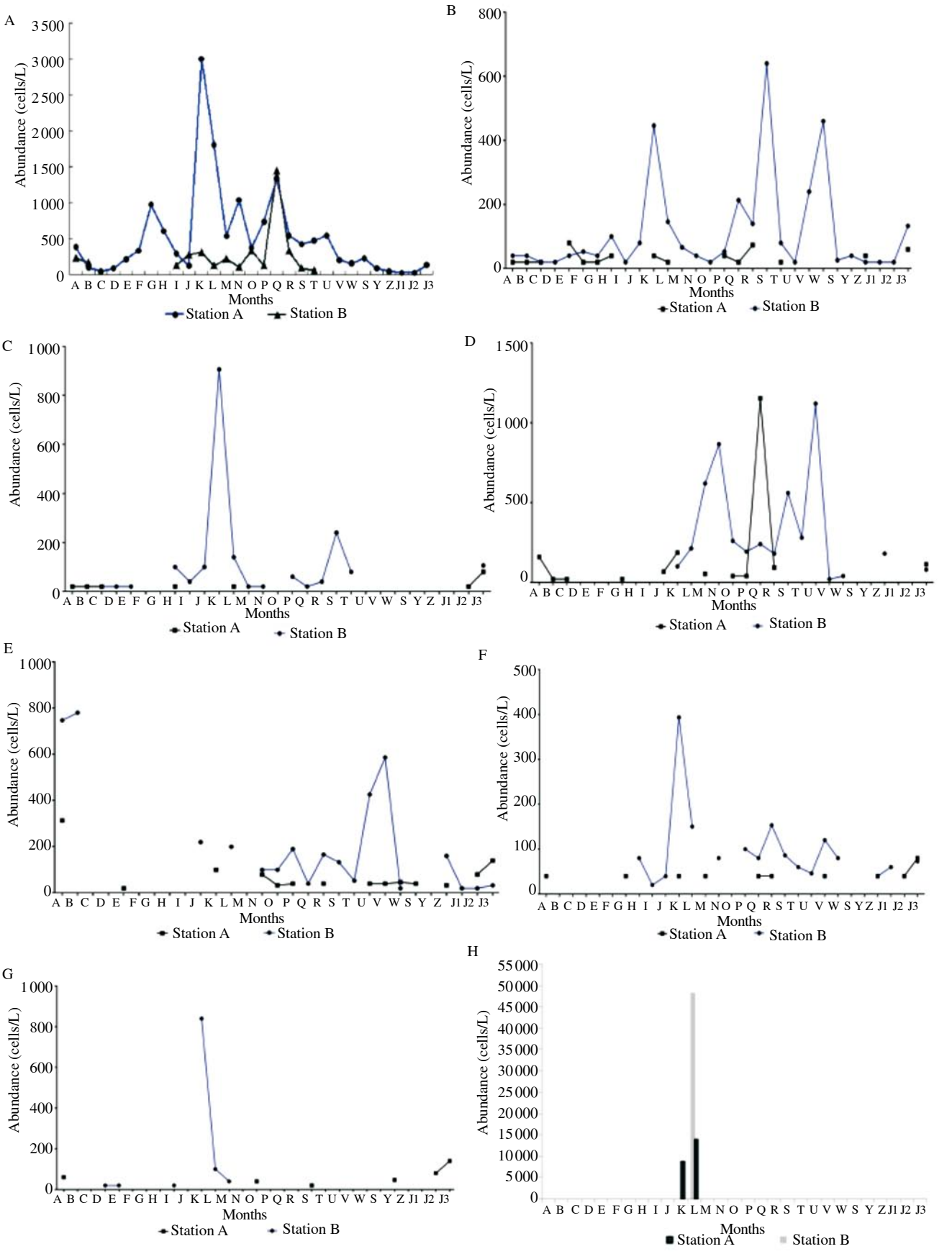

Figure 5. Seasonal distribution and abundance of dominant and rare species from Station A and Station B.

A: *A. ostenfeldii*; B: *C.furca*; C: *C. fusus*; D: *S. trochoidea*; E: *P. steinii*; F: *D. caudata*; G: *Py. steinii*; H: *Gyrodinium* sp.

A: 1 May 2002; B: 2 May 2002; C: 1 Jun 2002; D: 2 Jun 2002; E: 1 Jul 2002; F: 2 Jul 2002; G: 1 Aug 2002; H: 2 Aug 2002; I: 1 Sep 2002; J: 2 Sep 2002; K: 1 Oct 2002; L: 2 Oct 2002; M: 1 Nov 2002; N: 2 Nov 2002; O: 1 Dec 2002; P: 2 Dec 2002; Q: 1 Jan 2003; R: 2 Jan 2003; S: 1 Feb 2003; T: 2 Feb 2003; U: 1 Mar 2003; V: 2 Mar 2003; W: 1 Apr 2003; S: 2 Apr 2003; Y: 1 May 2003; Z: 2 May 2003; J1: 1 Jun 2003; J2: 2 Jun 2003; J3: 1 Jul 2003.

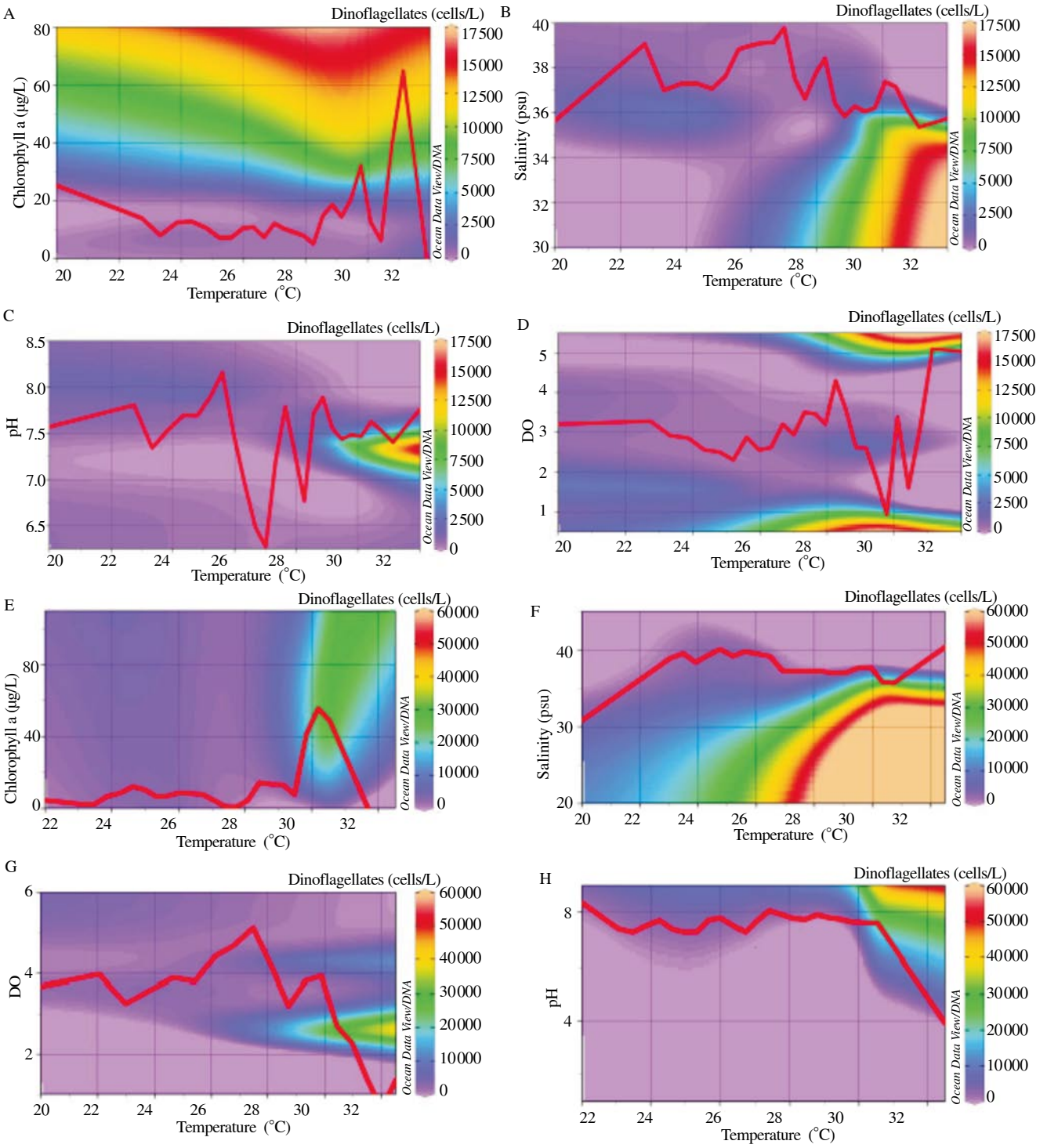

Figure 6. ODV graph showing the relationship between abundance of dinoflagellates cells and water parameters from both sites, Station A (A–D) and Station B (E–H).

from August to January and July 2003 at Station A and 9 months from October to July 2003 at Station B (Figure 5D). The abundance values ranging from 20–1 153 cells/L with maximum concentration observed during winter (January) at Station A which corresponding to low chlorophyll a values, temperature, salinity, pH and low DO (T > 24.5 °C, S > 36.00, chlorophyll a > 28.70, pH > 7.86 and DO < 1.55) and during summer month (March) observed at Station B which corresponding to low chlorophyll a values, temperature, with high salinity, pH and low DO (T > 25.5 °C, S > 39.00, chlorophyll a > 7.70, pH > 8.31 and DO < 3.2).

P. steinii has show the frequently distribution observed at Station B and occassionaly occurred in 3 months at the Station B. The abundance values ranging from 20 to 780 cells/L with maximum concentration observed during the summer months (May 2002) (Figure 5E) which corresponding to low chlorophyll a values, temperature, with high salinity, pH and low DO (T > 29.5 °C, S > 37.00, chlorophyll a > 19.53, pH > 7.61 and DO < 3.05) at Station B.

D. caudata has shown the freqent distribution but with low abundance ranging from 20–393 cells/L observed with maximum concentration during October 2002 at Station B (Figure 5F). The distribution of *G. spinifera* also observed in 7 months with cell abundance ranging from 20–666 and maximum concentration observed during autumn month (October 2002) at Station B which corresponding to high chlorophyll a values, temperature, salinity, pH and low DO (T > 30.50 °C, S

> 35.00, chlorophyll a > 50.51, pH > 7.68 and DO < 2.55). *H.* cf. *circularisquama* has frequent distribution for 7 months at Station B and occasionally distributed only in 4 months at Station A. The abundance values ranging from 20–250 cells/L with maximum concentration observed during summer (March–April) at StationB corresponding to low chlorophyll a values, temperature, with high salinity, pH and low DO (T > 25.5 ˚C, S > 39.00, chlorophyll a > 7.70, pH > 8.31 and DO < 3.2).

The distribution and abundance of *Py. steinii* were observed in 7 months from both stations, it has cell concentration ranges from 20–840 cells/L with maximum values observed during (October 2002) at Station B and the lowest values 170 cells/L observed in Station A (Figures 5G and 5F). The highest cell concentration corresponding to high chlorophyll a values, temperature, salinity, pH and low DO (T > 30.50 °C, S > 35.00, chlorophyll a > 50.51, pH >7.68 and DO < 2.55). *A. tamarense* distributed from 8 months (May–June 2002, August to February) at Station A and abundance values ranges from 20–520 cells/L at Station A with maximum cell concentration occurred during October. *P. depressum* distributed also in 7 months (June–October 2002, April–May 2003) at Station B and abundance values ranges from 20 cells to 280 cells/L observed with maximum cell concentration during October 2002 which corresponding to high chlorophyll a values, temperature, salinity, pH and low DO (T > 30.50 °C, S > 35.00, chlorophyll a > 50.51, pH > 7.68 and DO < 2.55).

G. spirale distributed occasionally during the months (May 2002, February and May–July 2003) from both sites. The abundance values ranges from 20–187 cells/L with the maximum cell concentration was observed during summer (May 2002) at Station A which corresponding to low chlorophyll a values, temperature, with high salinity, pH and low DO (T > 29.5 ˚C, S > 37.00, chlorophyll a > 19.53, pH > 7.61 and DO < 3.05) at Station A.

Other unidentified *Gyrodinium* sp. has pronounced abundance in the month of October 2002 and it has appeared one time in whole year. The abundance of the *Gyrodinium* sp. was recorded to bloom status during the October 2002 that ranges from 20–48 166 cells/L at Station B and 8 853–14 053 cells/L at Station A (Figure 5H). The maximum abundance was corresponding to high chlorophyll a values, temperature, salinity, pH and low DO (T > 30.50 °C, S > 35.00, chlorophyll a > 50.51, pH >7.68 and DO < 2.55).

Majority of species were observed rarely with the low abundance values ranges from 20–225 cells/L including *A. tamiyavanichii* (225 cells/L), *Alexandrium concavum* (113 cells/L), *G. catenatum* (306 cells/L) in July 2003, *C. inflatum* (160 cells/L), *C. lineatum* (106 cells/L) and *C. tripos* (54 cells/L) observed in October and 20–40 cells/L values observed for the *A. minutum*, *D. fortii*, *D. mitra, D. rotundata*, *D. dense*, *G. catenatum*, *G. digitalis*, *G. verior*, *G. grindely*, *G. polygramma*, *Lingulodinium polyedrum*, *P. ovatum*, *P. grandii, Protoperidinium leonis*, *P. minutum*, *P. avellanum*, *P. excentricum*, *P. simulus*, *Protoperidinium bipes*, *P. oblongum*, *P. oceanicum* at station A and *C. lineatum*, *C. inflatum*, *C. tripose*, *P. pentagonum*, *P. brevipes*, *P. longipes*, *P. cercuas* at Station B.

3.4. Statistical analysis

Total abundance of dinoflagellates cells were corresponding to low Chloropyll a, DO and high temperature , salinity and pH values from both sites (Figure 6). The estimates of the Pearson correlation between total dinoflagellate cells and water parameters had shown the negative values with salinity ~ (r = –0.23-0.31), DO ~ (r = –0.26–0.31) at both sites. The positive correlation values estimated from Chlorophyll a ~ (r = 0.72–0.36), temperature ~ (r = 0.26–0.27) and pH (r = 0.16) from station A and station B at P > 0.01–0.005 (Tables 3 and 4).

4. Discussion

The present study is dealt with the dinoflagellates abundance and distribution in the coastal waters of Pakistan. In this seasonal study, the high cells densities [(25 200–55 726) cells/L] recorded in October 2002 which is good agreement with the previous phytoplankton studies in the Arabian Sea[12] and the coast of India Ocean[42]. Qasim[12] has described the abundance of phytoplankton increases in September to October and decreases in February to March. This is possible because of the monsoonal season which increased nutrient concentrations which leads to a proliferation the dinoflagellates. In October 2002, the heterotrophic *Gyrodinium* sp. displayed very high cell abundance (48 166 cells/L) in this area (Figure 5). The abundance of this species recorded in range as reported from the other coastal waters, *G. corsicum* (43 000 cells/L) in Mediterranean Sea[43] and *G. aureolum* (9.9×10^5 cells/L) in Canada[44]. The abundance of *Gyrodinium* sp. also present in range as reported to the other Gymnidinoids species for example *Karenia sellisformis* syn (*Gymnodinium* spp.) had maximum densities (6×10^6 cells/L, October 1999)[45] and *Cochlodinium polykrikoides* had maximum densities ($1.1–2.1 \times 10^7$ cells/L, 21 to 23 October 2008[46]. These species were reported in the same months

Table 3

Pearson correlation values calculated between physio-chemical parameters and total dinoflagellates at Station A.

Station A	Temperature (°C)	Salinity (psu)	Chlorophyll a (μg/L)	pH	DO (mg/L)	Dinoflagellates (cells/L)
Temperature (°C)	–	–0.211	0.142	–0.069	0.125	0.274
Salinity (psu)	–0.211	–	–0.265	–0.096	–0.004	–0.313*
Chlorophyll a (μg/L)	0.142	–0.260	–	0.124	–0.023	0.729**
pH	–0.069	–0.096	0.124	–	–0.193	–0.014
DO (mg/L)	0.125	–0.004	–0.023	–0.193	–	–0.080
Dinoflagellates (cells/L)	0.270	–0.313*	0.729**	–0.014	–0.080	–

*: Significant value at P < 0.05; **: Highly significant value at P < 0.000 1.

Table 4

Pearson correlation values calculated between physio-chemical parameters and total dinoflagellates at Station B.

Station B	Temperature (°C)	Salinity (psu)	Chlorophyll a (μg/L)	pH	DO (mg/L)	Dinoflagellates (cells/L)
Temperature (°C)	–	–0.211	0.319*	–0.076	0.001	0.205
Salinity (psu)	–0.213	–	–0.134	–0.040	0.313*	–0.230
Chlorophyll a (μg/L)	0.319*	–0.134	–	–0.306	–0.420*	0.362*
pH	–0.076	–0.040	–0.306	–	0.420*	0.162
DO (mg/L)	0.001	0.313*	–0.420*	0.420*	–	–0.260
Total dinoflagellates (cells/L)	0.205	–0.230	0.360*	0.162	–0.260	–

*: Significant value at P < 0.05; **: Highly significant value at P < 0.000 1.

from different areas of the Arabian Sea and are associated with fish kills and damaged aquaculture industries. *Karenia mikimotoi* had maximum densities (1 550 × 10^4 cells/L; October 2009) have been reported to water discoloration in India[42]. To date, the other heterotrophic species was *Noctiluca scintillans* has been high cells densities (2.4 × 10^6 cells/L) during green tides in Pakistan[19]. Many *Gyrodinium* sp. are reported to graze the chain forming diatoms species including *Skeletonerna costatum*[47]. The abundance peak of *Gyrodinium* sp. in Pakistani waters occurs concomitantly with low abundance of diatoms[31].

A. ostenfeldii [(paralytic shellfish poisoning (PSP)] species was present in single or two cells with high cells densities (3 000 cells/L) in Pakistan which calls for rigorous monitoring in terms of health and safety of seafood as some *Alexandrium* species even at lower abundance can be harmful, and as a result shellfish harvest and consumption has been banned, for example, due to presence of *A. tamiyavanichii* (at 200–4 300 cells/L) in Japan[48] and *A. ostenfeldii* (1 000 cells/L) in St. Lawrence, the Mediterranean Sea[49]. Another chain forming PSP species, *G. catenatum*, recorded in very low numbers in this study which have been reported from other coastal areas such as in India[50] and New Zealand[51] were implicated in PSP incidences. Putative diarrhetic shellfish poisoning (DSP) producing species (such as, *D. caudata*, *Dinophysis tripos*, *D. miles*, *D. fortii*, *D. dense*, *D. mitra*, *D. rotundata*, *D. acuta*, *D. acuminata*) are also recorded in this study in low cell abundance, only *D. caudata* had high cells densities. Low cells densities of DSP producers have been implicated shell fish toxicity in the Black Sea[52], the United States waters[53], South East Asia[54], India[55] and also has been responsible to closed culture and wild shellfish stock in Europe since 1979[56,57]. Yessotoxin producing species also reported from the coast of Pakistan[58]. One of the yessotoxin producing species *G. polygramma* is a common bloom forming species from inshore waters of Karachi Pakistan[59]. *G. spinifera* had high cells densities in present study. The presence of the toxic species of DSP and yessotoxin producing species can cause the future toxicity at low cells densities for example > 20 cells/L during non-bloom conditions[60]. However, some non-toxic species *e.g.*, *H.* cf. *circularisquama,* is recently found in Pakistan waters which is type of shellfish killing species, common in Japanese water and impacted damage pearl oyster[61] and caused 4 billion yen loss in Hiroshima Bay, Japan[62].

Harmful algal blooms are reportedly increasing in the northern Arabian Sea associated with climate change. As a result of change in land-sea temperature gradient a shift in the speed and direction of winds occurs resulting in strong upwelling of dissolved nutrients. A regular monitoring program must be started for recording of harmful algal blooms in Pakistani waters. The Government of Pakistan and other relevant departments must take an action to save the health of the seas as well as human population depending on fish and fisheries.

Conflict of interest statement

We declare that we have no conflict of interest.

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