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TAXONOMY AND SEASONAL DISTRIBUTION OF PSEUDO-NITZSCHIA SPECIES (BACILLARIOPHYCEAE) FROM THE COASTAL WATERS OF PAKISTAN

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Abstract

This is the first detailed record of *Pseudo-nitzschia* species including their seasonal abundance and taxonomic identification from coastal waters of Pakistan bordering northern Arabian Sea. As reported from other coastal waters, considerably lower cell abundance was encountered. The seasonal abundance was found highest as 2.3×10^3cells l^{-1} in the month of July 2003 at station A, inside of the Manora Channel which is facing the problem of eutrophication. It shows the possible relation of *Pseudo-nitzschia* species concentrations with nutrient enrichment. Their abundance coincided with the chlorophyll a values at station A as compared to station B where it showed an inverse correlation, suggesting that other factors like autotrophic species contributed the chlorophyll a concentrations. These studies are based on light microscopy (LM) and scanning electron microscopy (SEM) related with the morphological structure of the *Pseudo-nitzschia fraudulenta* and *Pseudo-nitzschia sub-fraudulenta* from the coastal waters of Karachi, Pakistan.

Introduction

During the last two decades harmful bloom forming species of phytoplankton are increasing and have been a subject of worldwide interest for the presence of some toxic species (Cochlan *et al*., 2008; Wange *et al*., 2008). Toxin production is directly implicated with human and environmental health even some less abundant species become important as they can produce significantly potent toxins. High species diversity of bloom forming diatoms including toxic species occurs in marine environment. Several species of the pennate diatom genus *Pseudo-nitzschia* (Peragallo) recently received recognition because of the production of the neurotoxin domoic acid (Bates *et al.*, 1989; Trainer *et al.,* 2000; Bates, 2000; Cho *et al.,* 2001) which causes major economic problems for fisheries industry in many part of the world (Scholin *et al.*, 2000; Parsons *et al*., 2002; Aifeng *et al.*, 2005; Schnetzer *et al.*, 2007). Domoic acid (DA) enters the food chain through filter feeders and grazers and accumulates in shellfish or fin-fish at the higher trophic level. Contaminated shellfish results in amnesic shellfish poisoning (ASP) in humans and found induced great mortality rates to bird, fish and mammals (Hasle, 1993; Ramsdell., 2010).

Pseudo-nitzschia (Peragallo) is a small genus contains approximately two dozen species confirmed for the production of domoic acid (Hasle, 1993 and Hasle & Syvertsen, 1997; Bates *et al.*, 2000). *Pseudo-nitzschia fraudulenta* (Cleve) Hasle and *Pseudo-nitzschia subfraudulenta* (Hasle) Hasle are distributed in Atlantic, Pacific and Indian Oceans (Hasle *et al.*, 1996; Vrieling, 1996; Rhodes *et al.*, 1998; Hasle, 2002) but has not been reported from Pakistan (northern Arabian Sea). Previous available reports (Saifullah & Moazzum, 1978; Shameel &Tanaka, 1992) on diatom species have not indicated any sign of the presence of *Pseudo-nitzschia* species from the coast of Pakistan. Recently *P. delicatissima, P. seriata* and *P.longissima* have been reported from Sindh coast (Ghazala *et al.*, 2006). These species were identified only under the light microscope showing unconfirmed results. Furthermore, identification of *P.longissima* is not valid because it does not exist. Another species *Pseudo-nitzschia Pungens* (c.f.) was observed from Churna Island, Balochistan coast (paper submitted). It was confirmly reported through SEM for the first time from Karachi coast (Naz *et al*., 2011). The *P. delicatissima* and *P. pseudodelicatissima* were also reported from regional areas in the central Arabian Sea (Schiebel *et al.*, 2004).

The present research therefore reports the occurrence of *Pseudo-nitzschia fraudulenta* and *Pseudo-nitzschia subfraudulenta* for the first time from coastal waters of Karachi, Pakistan and also elucidates their correlation with the ecological condition existing in the region. The presence of toxic *Pseudo-nitzschia* species in the coastal waters of Karachi may have an implication on fisheries industry and therefore a regular monitoring of their abundance and seasonality is required.

Materials and Methods

Water samples were collected bimonthly from 1m depth during May 2002 to July 2003 using 1.7L Niskin bottle from Manora Channel, coastal waters of Karachi. A total of 180 samples were retrieved from two stations established for regular sampling. Station A (24˚49.77'N 66˚57.85'E) was a polluted area with impact from Layari River and mangrove ecosystem and station B (24˚47.93'N 66˚58.87'E) was at the mouth of the channel in open water, a non-polluted station with more oceanic ecosystem influence. Samples were fixed in Lugol's solution and used for analysis of seasonal abundance by sedimentation technique (Utermohl, 1958) using an inverted microscope (Olympus, BX-51, Japan). Samples for scanning electron microscopy (SEM) were collected in July 2007 and November 2008 from inshore waters of Manora Channel, Karachi coast (24˚49.77'N 66˚57.85'E) was cleaned by $KMNO_4$ oxidation of the organic material (Sournia, 1978). Specimen was prepared for SEM by air drying material on clean cover slips. Material was picked up onto a double sticking tape which was then mounted on a stub. Stubbs were gold coated and viewed on a SEM (JSM6380A) Identification of *Pseudo-nitzschia* species was based on LM and SEM characteristics described by Tomas, 1997 & Skove *et al.*, 1999.

Water parameters: Temperature, salinity (refrectometer), dissolved oxygen (DO; Winkler's method: Hanna C100), pH (Hanna HI9023, Italy) and chlorophyll *a* (Chl a; Strickland & Parsons, 1972) were measured and correlated with *Pseudo-nitzschia* abundance.

Results

Environmental conditions and seasonal abundance of *Pseudo-nitzschia* species at station A: The water parameters (Chl a, temperature, salinity, pH, DO, transparency) were correlated with the abundance of *Pseudo-nitzschia*. Chl a concentration ranged from 2.7-25.2µg/L with an average of 7.9±12.3µg/L (Table 1). Maximum values for Chl a concentration was observed in December, 2002 whereas minimum values were recorded in May, 2002. Salinity values fluctuated between 34-40 psu (mean±S.D; 2.0±36.9). The surface water temperature ranged from 20-32◦C (mean±S.D; 3.2±26.7) (Table 1). High salinities were recorded in June, December, 2002 and January, 2003 and maximum temperature was observed in the month of July 2003. Dissolved oxygen values ranged from 2-5 mg/L (mean±S.D; 1.1±3.2) with highest concentration recorded in the month of July 2003. Transparency ranged between 22-81cm (mean±S.D; 23.0±45.9), maximum transparency was in March, 2003 and minimum in May, 2003 (Table 1). Total phytoplankton abundance ranged from 2.06-12.35×10^3 cells l^{-1}, (mean±S.D; 6123±39270) with high values of 16.82×10^3 cells l^{-1} were recorded in the month of October, 2002. Total diatoms ranged from 1.27-11.72×10^3 cells l^{-1}, (mean±S.D; 3791±5293). Diatoms contributed 5-97% in the months of October, 2002 and February, 2003 respectively. Total abundance of diatoms constitutes 64-97% to total phytoplankton abundance and total abundance of *Pseudo-nitzschia* species constitute between 0.41-46.26% to total diatoms with cell densities ranged between 0.02-2.30×10^3 cells l^{-1}. It is interesting to note that these species were more frequently observed at St. A as compared to station B. Highest cell numbers of *Pseudo-nitzschia* species were observed in the months of July as 2.30×10^3 cells l^{-1} and in April, 2003 as 1.58×10^3 cells l^{-1} (Fig. 1 & 2).

Table 1. Range and Average (Mean ± SDev) of total cell abundance (cells-l) of phytoplankton, diatom, *Pseudo-nitzschia* and Salinity (psu), Temperature (^oC), Dissolved oxygen (DO, mg/L), Chlorophyll a (µg/L), pH and Transparency (Trans, cm) within the study month from station A and B.

St	Total phytoplankton	Diatom	*Pseudo-nitzschia*	Salinity	Temp	DO	Chlorophyll*a*	Trans	pH
	(10^3 cells l^{-1})	(10^3cells-l)	(10^3cells-l)	(psu)	(°C)	(mg/L)	(µg/L)	(cm)	
A	2.06 - 12.35	1.27 - 11.72	0.02 - 2.30	34 - 40	20 - 32	5-6	2.7 - 25.2	22 - 81	7-8
	6123 ± 39270	3791 ± 5293	570 ± 784	2.0 ± 36.9	3.2 ± 26.7	1.1 ± 3.2	7.9 ± 12.3	23.0 ± 45.9	0 ± 7.6
B	4.92 - 16.32	2.78 - 14.04	0.007 - 0.32	35 - 40	27 - 31	3.1 - 5.4	1.2 - 13.5	55 - 169	7.7 - 8.1
	5019 ± 16327	4850 ± 9723	108 ± 144	2.4 ± 36.8	2.37 ± 28	1.1 ± 4.7	5.2 ± 6.4	56 ± 103	0.1 ± 7.8

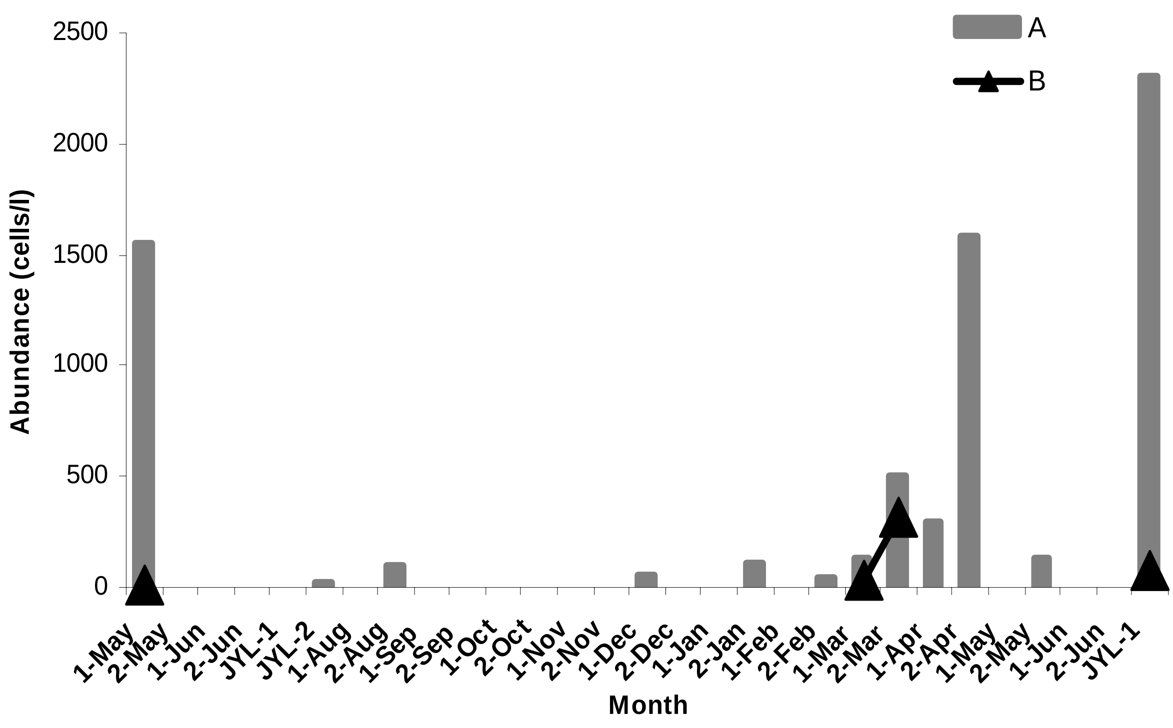

Fig. 1 *Pseudo-nitzschia* abundance (cells l^{-1}) at stations A and B.

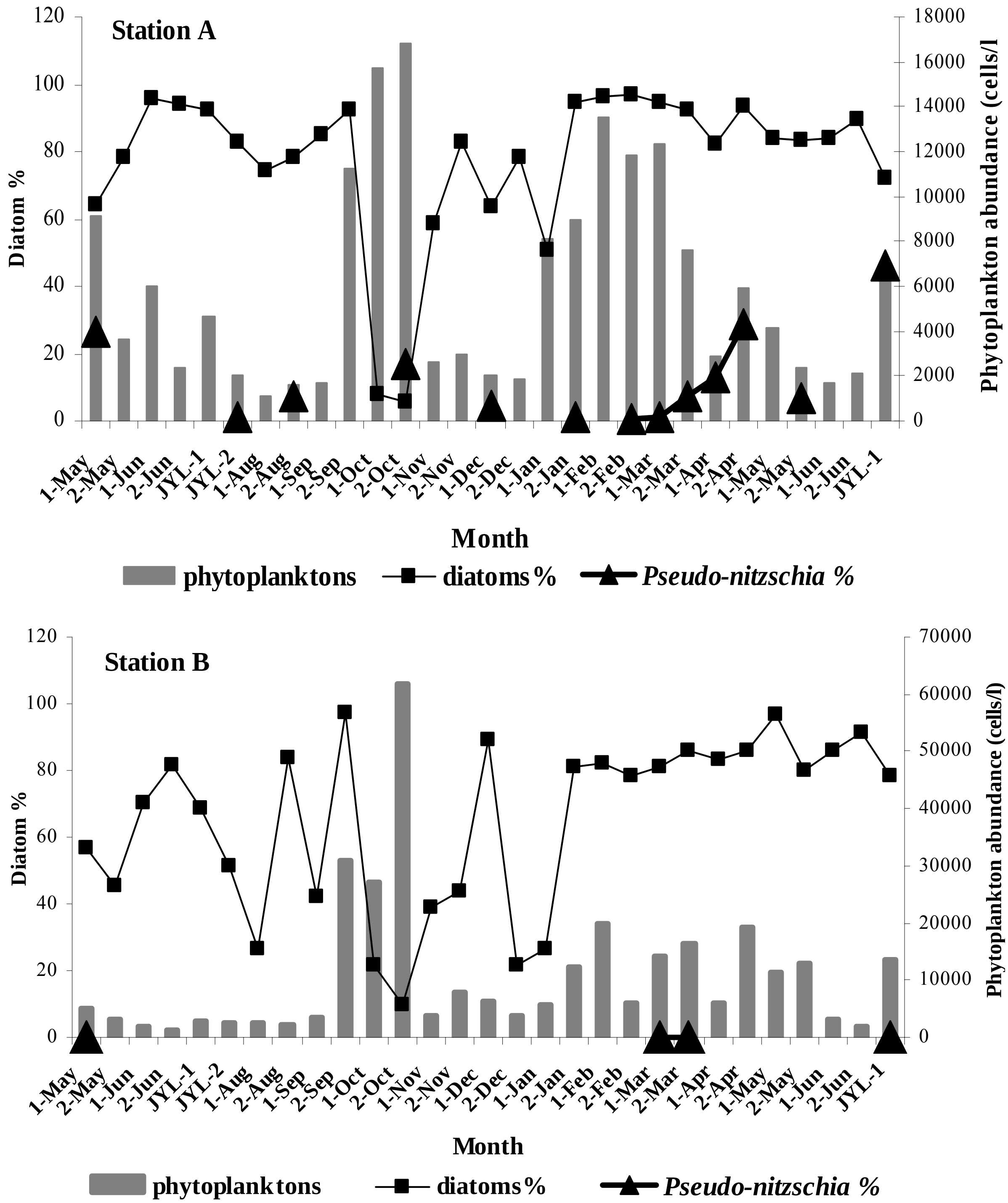

Fig. 2.Total phytoplankton abundance (cells l^{-1}) and percentage % contributions of diatoms and *Pseudo-nitzschia* at stations A and B.

Environmental conditions and seasonal abundance of *Pseudo-nitzschia* species at station B: Chl a concentration ranged from 1.2-13.5µg/L with an average of 5.2.±6.4µg/L (Table 1). Maximum values for Chl a concentration was observed in May, 2002 whereas minimum values were recorded in March, 2002. Salinity values fluctuated between 35-40 psu (mean±S.D; 2.4±36.8). The surface water temperature ranged from 27-31°C (mean± S. D; 2.37±28). High salinity was recorded in March, 2003 and maximum temperature was observed in the month of July, 2003. Dissolved oxygen values ranged from 3.1-5.4 mg/L (mean± S.D; 1.1±4.7) with highest concentration recorded in the month of March, 2003. Transparency ranged between 55-169 cm (mean± S.D; 56±103), maximum transparency was in March, 2003 and minimum in July, 2003 (Table 1). Total phytoplankton abundance ranged from $4.92\text{-}16.32\times10^3$ cells l^{-1}, (mean±S.D; 5019±16327) with high values of 61.66×10^3 cells l^{-1} recorded in the month of October, 2002. Total diatoms ranged between $2.78\text{-}14.04\times10^3$ cells l^{-1}, (mean±S.D; 4850±9723). Diatoms contributed 10-97% in the months of October 2002, September 2002 and May 2003 respectively.

Total abundance of diatoms constitutes 57-86% to total phytoplankton abundance (Fig. 2) and total abundance of *Pseudo-nitzschia* species constitute 0.25-2.27% to total diatoms with cell densities ranged between $0.007\text{-}0.32\times10^3$ cells l^{-1}. *Pseudo-nitzschia* was found only at four occasions at station B. Highest cell numbers of *Pseudo-nitzschia* species were observed in the month of March, 2003 with cell abundance of 2.27×10^3 cells l^{-1} (Fig. 2).

Identification of *Pseudo-nitzschia fraudulenta* (Cleve) Hasle: It is similar to another species ***P.*** *subfraudulenta* and cannot be easily distinguished through light microscopic observations. This species forms chains by overlapping valve ends of individual cells. Overlapping of cells is short as compare to other *Pseudo-nitzschia* species (Fig. 3A). The shape of valve is linear in the middle part and gradually fusiform towards the apices (Fig. 3B). The SEM observations further revealed valve morphology and other characteristic features required for identification. In *P*. *fraudulenta*, apical and transapical axis measures 78µm and 5.7µm, respectively in 10µm (Fig. 3B). Central larger inter space and central nodule are present (Fig. 3C). Striae are biseriate consisting of two rows of poroids (Fig. 3C). Fibulae and striae are 14-15 and 24 in 10µm, respectively (Fig. 3B). There are 5-6 poroids in 1µm (Fig. 3C & D).

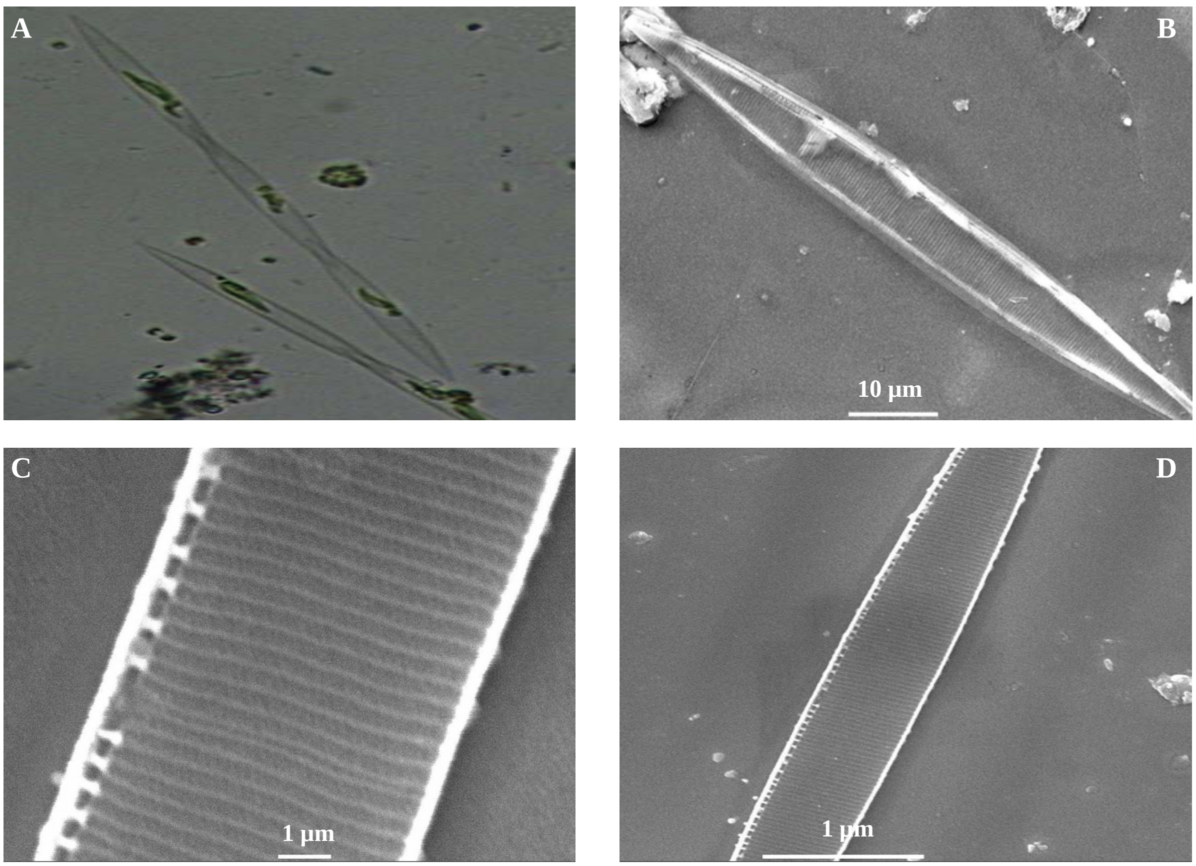

Fig. 3. *Pseudo-nitzschia fraudulenta* (A) light microscopic (LM) micrograph of (Scale bar: 50 µm). (B) scanning electron micrograph whole valve shows valve tips, (C, D) showing central interspace and nodule (C) poroids per 1 µm.

Identification of *Pseudo-nitzschia subfraudulenta* (Hasle) Hasle: The major difference between this species and *P*. *fraudulenta* is the presence of more linear valve in *P*. *subfraudulenta* especially in the middle part of the cell. In light microscopy its cells are found in chains with short overlapping at the valve ends (Fig. 4E). The apical axis is 78.5 µm and transapical axis 5.4µm (Fig. 4F). Striae are biseriate consist of two rows of poroids (Fig. 4G). Fibulae and striae are not discernible in light microscope (LM) but visible in SEM, scanning electron microscopy. Striae are 27-28 and 14 fibulae per 10µm (Fig. 3F). There are 5-6 poroids per 1µm (Fig. 4H).

Statistical analysis: Pearson s correlations applied to investigate relationship between *Pseudo-nitzschia* species abundance and water parameters. *Pseudo-nitzschia* abundance showed a positive relationship with chlorophyll a, dissolved oxygen and temperature. Salinity, transparency and pH have negative correlation with the *Pseudo-nitzschia* abundance at station A (Table 2). At station B *Pseudo-nitzschia* abundance showed negative correlation with chlorophyll a, salinity and temperature but positively related with dissolved oxygen, transparency and pH (Table 3).

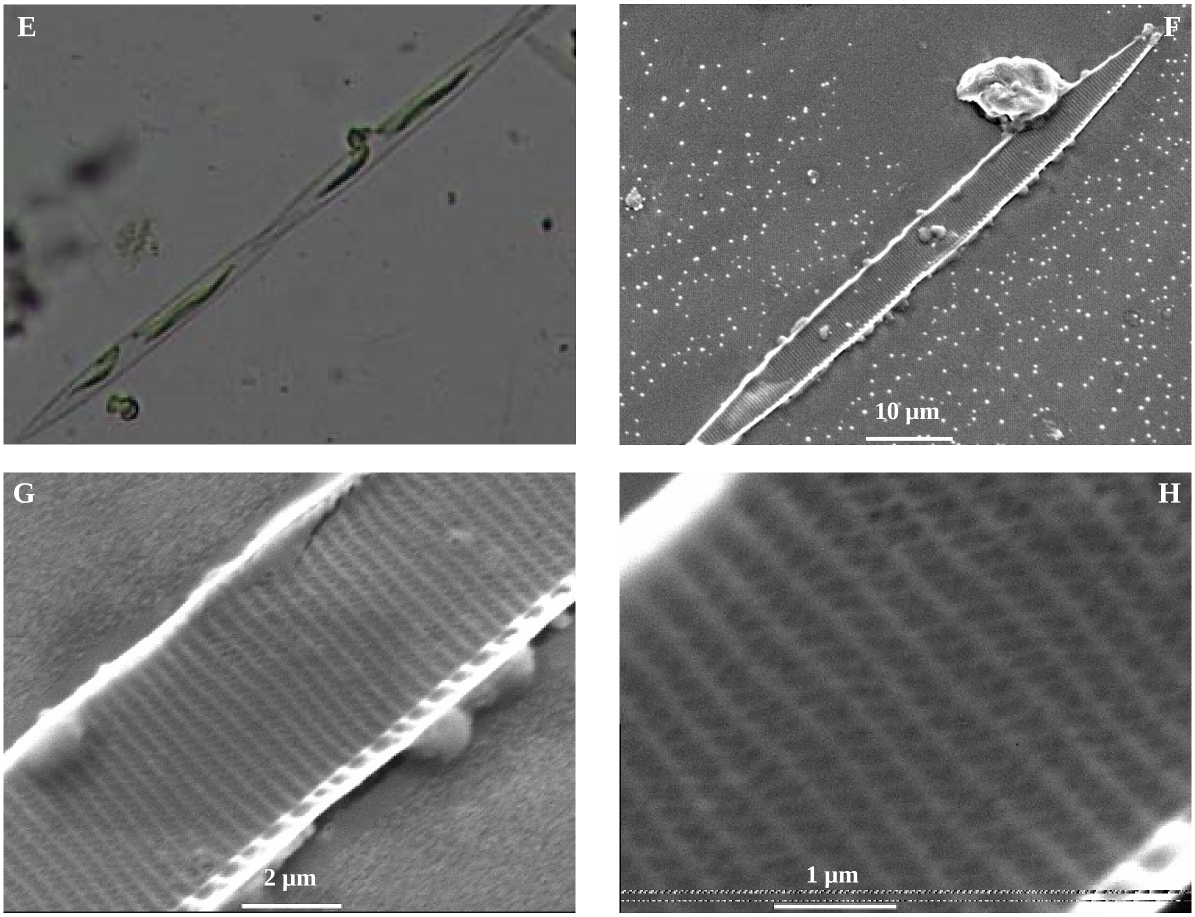

Fig. 4. *Pseudo-nitzschia subfraudulenta* (E) LM, (light microscopy) two cells showing short overlapping, (F) SEM (scanning electron microscopy) whole valve showing valve tips and central portion with more linear shape, (G) showing striae and fibulae, (H) showing contiguous poroids per 1 µm.

Table 2. Correlations (Pearson) of *Pseudo-nitzschia* abundance with water parameters at station A.

	Abundance	Chl a	Do	Temp	Salinity	Trans
Chl a	0.027	-	-	-	-	-
DO	0.234	-0.115	-	-	-	-
Temp	0.700*	0.147	0.344	-	-	-
salinity	-0.263	-0.289	0.215	-0.179	-	-
trans	-0.033	-0.084	0.012	-0.403	0.062	-
pH	-0.399	0.079	0.194	-0.122	-0.068	0.310

Chl a, DO, Temp, Trans, refer to chlorophyll a, dissolved oxygen, temperature, transparency respectively
*=Significant at probability 0.05

Table 3. Correlations (Pearson) of *Pseudo-nitzschia* abundance with water parameters at station B.

	Abundance	Chl a	Do	Temp	Salinity	Trans
Chl a	-0.783					
DO	0.470	-0.437				
Temp	-0.281	0.348	-0.005			
salinity	-0.568	-0.188	0.091	-0.510		
trans	0.274	-0.264	0.432	-0.586	0.312	
pH	0.964*	-0.127	0.458	0.043	-0.130	0.424

Chl a, DO, Temp, Trans, refer to chlorophyll a, dissolved oxygen, temperature transparency respectively
*=Significant at probability 0.05

Discussion

The well known toxin producing genus *Pseudo-nitzschia* is a major concern of the worldwide investigations during last two decades. This is the first attempt to describe their identification and seasonal distribution correlated with the environmental factors from coastal waters of Pakistan bordering northern Arabian Sea. Phytoplankton abundance showed great variability and clear seasonality along the study area which is highly influenced by Asian monsoon system. A clear contrast of seasonal pattern of phytoplankton abundance was seen between eutrophic station A and open waters with more oceanic influence at station B. High phytoplankton abundance encountered from station B which is experiencing pollution and stress free environment as compared to station A showing that phytoplankton species proliferating and surviving better in a pollution free environment. Similar results were reported from eastern Arabian Sea (Parab *et al.*, 2006). Diatoms were the most prominent group contributing high percentage 97% in different months at both stations.

As compared to other coastal waters (Dortch *et al.*, 1997; Bates *et al.*, 1998; Kaczmarska *et al.*, 2005; Almandoz *et al.*, 2008) *Pseudo-nitzschia* cell concentrations encountered from this region were considerably low, and 1.58×10^3 cell-^{1} density was observed from February to April, 2002. Similar lower cell densities were observed in the same months from Spanish water by Quijano-Scheggia *et al.,* (2008). Thessen & Stoecker**, (**2008) from the Chesapeake Bay, Mid Atlantic Ocean and Schnetzer *et al.,* (2007) from Southern California recorded highest cell abundance from February to May and March to April respectively. Our findings also showed presence of *Pseudo-nitzschia* species cells in the same months but with lower cell density (1.58×10^3 cells l^{-1}). Seasonal pattern showed higher values at station A but overall densities were consistently lower all the year at both stations.

This investigation shows that *Pseudo-nitzschia* species have comparatively high abundance at station A located inside Manora Channel and this could be attributed generally to the influence of domestic and industrial effluents being regularly pumped in through Layari River (Beg *et al.*, 1984). The increasing trend of eutrophication in the region due to these river inputs benefits the harmful species to proliferate and form blooms (Dortch *et al.*, 1997). The sampling area is also influenced by the upwelling phenomenon. These processes of anthropogenic factor and natural upwelling caused by monsoon system are enough to alter the diatom community and encourage the development of toxic species. According to Bates *et al.,* (1998) increase in cell density corresponded with the trigger of nitrates from rivers input. Highest cell abundance 2.3×10^3 cells l^{-1} was seen in the month of July, 2003. This period is well known for the SWM (Southwest monsoon) in the Arabian Sea. During this period euphotic zone has well-mixed water column which is thought to be the requirement for the survival of *Pseudo-nitzschia* species (Horner & Postel, 1993). Horner & Postel, (1993) reported highest cell abundance for *Pseudo-nitzschia* species in the same month.

Pseudo-nitzschia species abundance coincided with the chlorophyll a values at station A as compared to station B where it showed an inverse correlation suggesting that other factors like autotrophic species, picoplanktons or detritus contribute the chlorophyll a concentrations. *Pseudo-nitzschia* species tend to appear entire range of salinity and temperature at both stations, suggesting that there are more species because individual species prefer more narrow ranges of temperature and salinity.

Although recorded abundance from this region was low but presence of these species with increasing rate of eutrophication suggests initiation of a regular monitoring program including advance research on domoic acid analysis and the molecular recognition of species depends on correct species circumscription.

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