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Occurrence of HAB / toxic Dinoflagellates species from the coast of Karachi, Pakistan (Northern Arabian Sea)

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Morphological identification of dinoflagellate species were investigated from the coastal waters of Manora Island and Mubarak village Karachi, Pakistan throughout the two years of study period from (Apr 2008 – Mar 2010). A total number of seventy two dinoflagellate species were identified among them, 42 toxic and 30 HAB species were morphologically differentiated. Dominant species included *Alexandrium catenella, Dinophysis caudata, Gymnodinium* spp., *Prorocentrum micans, Noctiluca scintillans, Prorocentrum gracile, Tripos furca* and *Gonyaulax* spp. these species were previously reported. The sampling area of Mubarak village site was discovered for the first time for dinoflagellates community analysis. This site shows interesting results as some species were observed in high abundance. The high cell concentration of these toxic/ HAB species suggests that our findings add substantially to the HAB dynamics in area and on this basis predicts future event.

[Keywords: Dinoflagellates, Dominant species, HAB and toxic species, Northern Arabian Sea].

Introduction

Arabian Sea is considered as one of the most productive zones of the Indian Ocean[1].The ecology of phytoplankton is highly influenced by dynamic features of Arabian Sea[2].The circulation, wind pattern, upwelling, and precipitation during SWM and NEM dramatically alters the phytoplankton community structure especially in the coastal areas[3, 4, 5, 6]. About 7% (~300 taxa) of known phytoplankton species are responsible for the red tide formation[7] of which, only 2% species cause harmful or toxic blooms (HAB). Dinoflagellate is particularly important as they contribute 75% of all toxic/harmful taxa[8]. Dinoflagellates have both heterotrophic and autotrophic mode of nutrition and plays significant role in the energy transfer in aquatic food web[9] .The toxins produced by these organisms would bioaccumulate in organisms at higher trophic levels including human beings[10] and contaminate fishery products[11]. The harmful algal bloom HABs phenomenon stimulate via physicochemical and temporal variations[12]. The HAB events are being reported more frequently from the wider geographical locations including Arabian Sea[13-21] and are responsible for mass mortality of fishes and causing enormous economic losses in the fishery sector[22, 23, 17]. A red, brown and green discoloration of water have been reported due to the extensive growth of dinoflagellates, these blooms could be non-toxic but still obnoxious and can degrade water quality and physically clog gills of fish, shellfish and suffocation[24] decreased dissolved oxygen concentrations in the water column[12,16, 22] and attenuate light penetration that adversely effect on the life of benthic organisms[15] On the other hand, toxin producing species can cause a wide number of diseases, such as, PSP (Paralytic shellfish poisoning), DSP (Diarrhoeic shellfish poisoning), ASP (amnesic shellfish poisoning), CFP (Ciguatera fish poisoning)[12, 16, 22] under bloom conditions or even at low cell densities. Potentially toxic and harmful blooms have been reported from various parts of the Arabian Sea, for example, from the west coast of India[25, 26, 27] Oman, Iran regions[28, 29, 30, 31] from Arabian Gulf region[21] and Arabian Sea[19, 20] . A few putative bloom forming species have been previously reported from the Pakistan coastal water, such as, *Prorocentrum micans, Ceratium* shurunk[32] *Gonyaulax* Diesing[33] and *Noctiluca scintillans*[34]. A recent study on distribution and abundance of dinoflagellates from Manora channel reports sixty six bloom forming (non-toxic) and 28 toxic species [35, 36]. Reports on occurrence of blooms in Pakistan waters are few, for example, on *Noctiluca scintillans* from various coastal locations along Pakistan coast[34] and from Gawadar Bay[37]. The later has been implicated with the fish mass mortality. *Kerania* spp. bloom (author's personal observation). In this study the investigations were conducted at four sites of Manora Island and Mubarak village coastal and near shore waters.

Study area

Materials and Methods

The study was conducted at four stations for two years (Apr 2008 – Mar 2010) along the Karachi coast (Fig. 1). The locations of these stations were: Manora Island (MI-1; 24°45'4.75"N, 66°59'9.29"E), Mubarak Village (MV-1; 24°52'6.18"N, 66°37'21.86"E) coastal stations (10m depth) whereas, off Mubarak Village (MV-2; 24°45'39.12"N, 66°26'13.38"E) and off Manora Island (MI-2; 24°35'5.91"N, 66°46'26.34"E) are near-shore stations (50m depth) (Fig. 1).

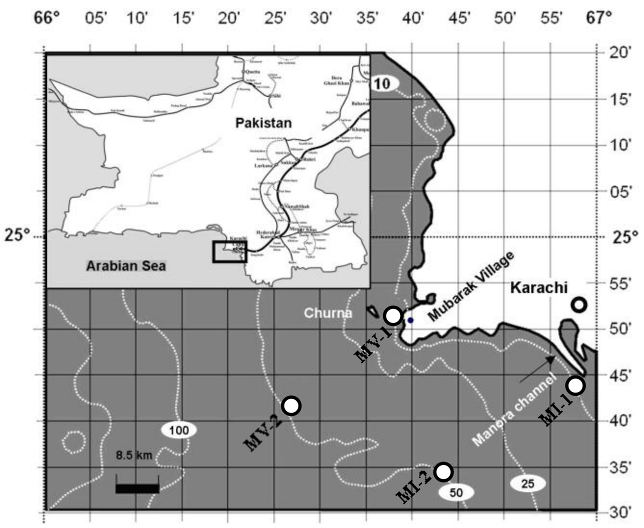


Fig.1. Map of Karachi coast showing stations at Manora Island and off Mubarak Village) MI-1 and MV-1 coastal waters (10m contour line) and MI-2 and MV-2 nearshore waters (50m contour line).

Sample Collection and Analysis

Monthly triplicate samples were taken from the surface water (1m depth) through Niskin bottle (1.7L). Samples were fixed in Lugol's solution (1%), store at 4°C in amber bottles. Dinoflagellate species were identified and counted in samples settled in settling chamber (50 ml; Hydro-Bios, Germany) for 24 hours according to previously described method[38] and dinoflagellate cells were observed and counted using an inverted microscope (*Olympus*, IX-51, Japan). Dinoflagellates species identified through literature available[39]. Current species names were confirmed through data bases[40, 41, 42] and check list [43, 44]. Water parameter data was taken for air and water temperatures from (mercury thermometer), salinity (refractometer; Otago), transparency (Secchi disc), and pH from (Hanna HI 9023).

Humidity data was obtained from National Weather Forecasting Centre, Meteorological Department Pakistan[45]. Dissolved oxygen (DO) was determined using kit employing Wrinkler's method (DO; HANNA-C100). Total chlorophyll (Chl *a*) was extracted samples (250-1000 ml) filtered through GF/F (0.7 µm; Whatman) stored in 90% acetone and values recorded on UV-visible spectrophotometer (Simadzu) as described by Strickland and Parsons[46].

Water parameters

Water parameters recorded in this study are shown in Table 1. Manora Island (MI) waters had higher average Chlorophyll *a* (Chl *a*) values compared to the Mubarak village waters (MV).

Its values ranged between 0.01 µg L^{-1} (MV) to 107.5 µg L^{-1} (MI). The average concentration of dissolved oxygen (DO) varied within a narrow range 7.7 – 7.8 mgL^{-1}; values fluctuated between 3.4 and 10.2 mg L^{-1}. Water temperature varied between 19 and 30 ºC and salinity fluctuated between 37 – 42 PSU. Transparency (sacchi depth) recorded here showed low turbidity values (7.6 – 14; 10.75 ± 1.58) and low were (0.9 – 4.5; 3.13 ± 0.9) at MI-2 and MI-1 respectively. The pH values had narrow range and remained between 7 and 8 during the study period (Table 1).

Dinoflagellates assemblage

Mean cell abundance of dinoflagellates, shown in Fig.2. was high at MI (6573 Cells/L) as oppose to MV (2453 Cells/L). A total of 72 HAB species were identified from the shallow waters of Karachi including 42 toxic species (Table 2). Mean cell densities of all species was recorded depicting 12 species had high percent contribution (~ or >1%) in the total dinoflagellate population. The most abundant species was *Tripos furca* (49.93% MI; 31.16% MV), *Alexandrium catenella* (5.82% MI; 19.11% MV), *Prorocentrum micans* (4.19MI; 3.97 MV), *P. triiestinum* (2.64 MI; 2.42 MV), *Tripos linetus* (2.34 MI; 0.16 MV), *Gonyaulax ceratocoroides*

(2.16MV; 2.40 MV), *Gymnodinum* sp1 (1.87 MI; 2.67 MV), *Alexandrium minutum* (1.37 MI; 2.69 MV), *Alexandrium tamarense* (1.36 MI; 3.02 MV), *Tripos fusus* (1.18 MI; 0.62 MV), *Prorocentrum gracile* (1.10 MI; 1.20 MV), *Dinophysis caudata* (0.94 MI; 0.82 MV). Table 2 also depicts type of toxins/poisoning these dinoflagellate species are reported to produce. Seasonal variation in the distribution and mean cell densities were also observed 23 abundant HAB species are shown in Fig.3. It appears that species grow in high numbers mostly during October to February with few exceptions and that the growth of these species varies from year to year. Moreover, it may be noted that many species that did not achieve high numbers in the year 2008 were abundant in 2009. (Fig.3). Micrographs of some of the dinoflagellate species are shown in Plate 1.

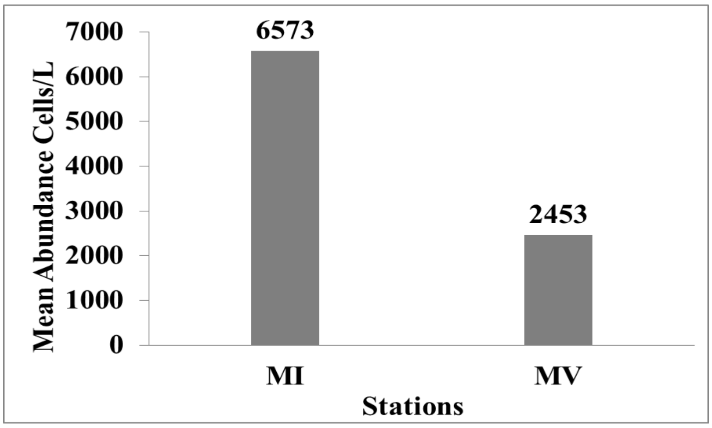


Fig. 2. Total abundance (Cells /L) of dinoflagellates at Manora Island and Mubarak Village Stations from Coastal areas of Karachi.

Table 1. Mean values and range (min - max) of temperature (ºC), (air and water), salinity (PSU), pH, humidity, transparency (m), dissolved oxygen (DO, mg L^{-1}) and chlorophyll *a* (µg L^{-1}) at stations.

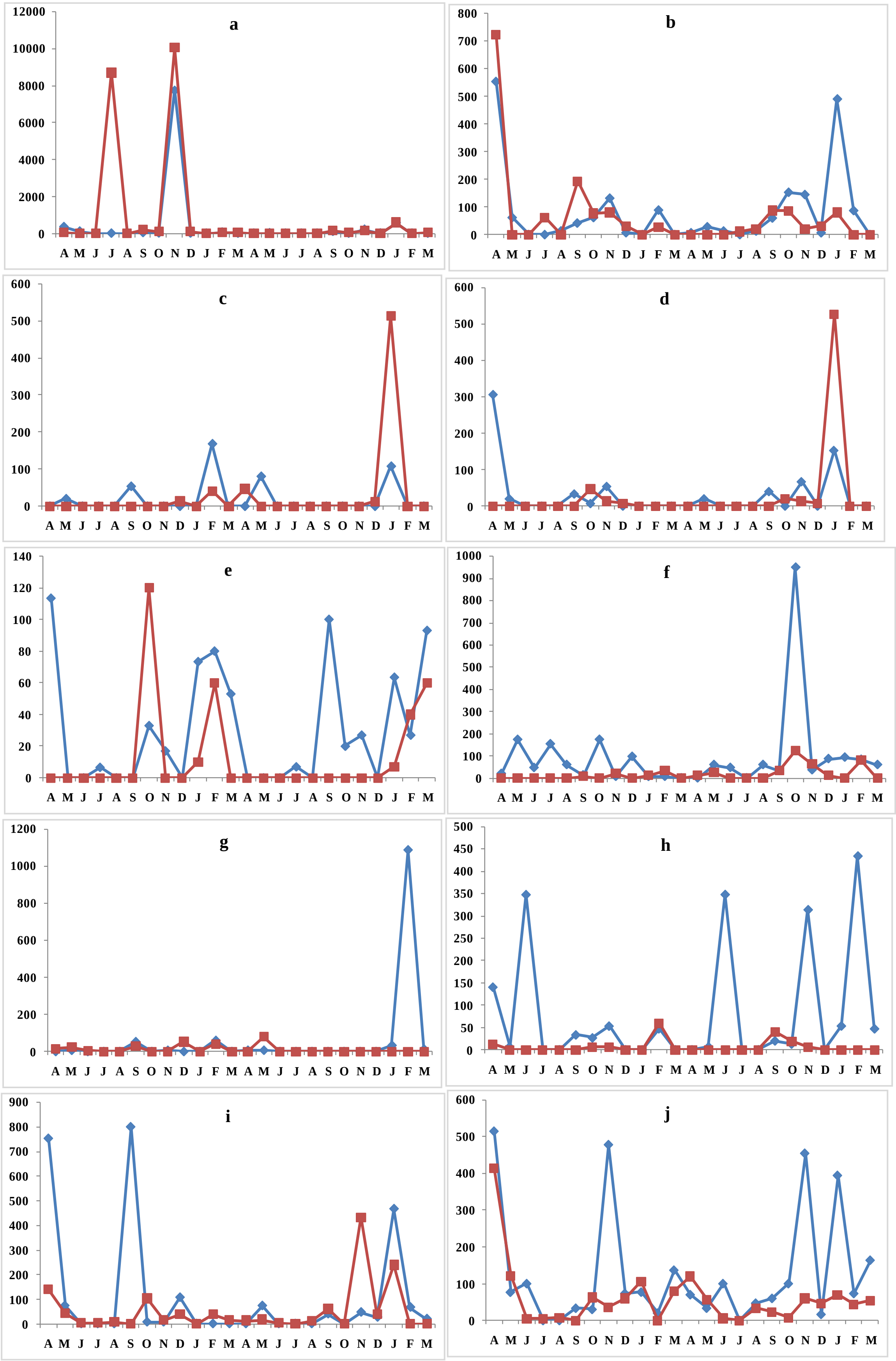
Stations	**Mean Values ± SD (Min-Max)**	
	MI	**MV**
Chlorophyll *a* (µg L^{-1})	23.62 ± 28.05 (1.35 – 107.5)	17.7 ± 27.4 (0.01 - 58)
DO (mg L^{-1})	7.7 ± 1.5 (4.5 - 10)	7.8 ± 1.8 (3.4 - 10.2)
Air Temp (ºC)	25.5 ± 2.8 (20 - 30)	24.75 ± 3.3 (19.1 - 31)
Water Temp (ºC)	24.5 ± 3 (19 - 29)	23.75 ± 3.35 (19 - 30)
Salinity (PSU)	38.9 ± 1 (37 - 42)	37.8 ± 0.8 (37 - 40)
Transparency (m)	5.9 ± 0.8 (0.9 - 14)	9.1 ± 1.05 (5.4 - 11)
pH	7.3 ± 0.29 (7 - 8)	7.3 ± 0.27 (7 - 8)
Humidity (%)	65.15 ± 6.2 (50 - 75.8)	69.15 ± 5.75 (55 - 77.8)

Table 2. Total cell mean abundance (Cells/L), percentage and toxic/ HAB dinoflagellates species from the coastal waters of Karachi.

Species	Manora		Mubarak		Effect	References
	Mean	spp%	Mean	spp%		
Akashiwo sanguinea	4.77	0.07	0.00	0.00	HAB	47
Alexandrium andersonii	13.14	0.20	0.00	0.00	PSP (STXs, GTXs)	48
Alexandrium catenella	382.46	5.82	468.74	19.11	PSP (STXs, GTXs)	49
Alexandrium minutum	89.97	1.37	66.09	2.69	PSP (STXs, GTXs)	49
Alexandrium ostenfeldii	13.76	0.21	18.25	0.74	PSP (STXs, GTXs)	50
Alexandrium pseudogonyaulax	8.75	0.13	3.89	0.16	PSP (STXs, GTXs)	51, 52
Alexandrium tamarense	89.15	1.36	74.03	3.02	PSP (STXs, GTXs)	53, 49
Alexandrium taylori	1.68	0.03	0.75	0.03	PSP (STXs, GTXs)	54
Alexandrium concavum	20.56	0.31	2.54	0.10	Unknown toxicity	55
Amphidinium carterae	1.25	0.02	1.85	0.08	PSP (STXs, GTXs)	56
Azadinium spinosum	0.00	0.00	2.96	0.12	lipophilic toxins	57
Cochlodinium polykrikoides	7.69	0.12	1.11	0.05	HAB	28
Dinophysis acuminata	7.50	0.11	13.53	0.55	DSP (Okadaic acid, DTXs,YTXs,PTXs)	28
Dinophysis acuta	4.91	0.07	1.57	0.06	DSP (Okadaic acid, DTXs,YTXs,PTXs)	58
Dinophysis caudata	61.82	0.94	20.15	0.82	DSP (Okadaic acid, DTXs,YTXs,PTXs)	59
Dinophysis fortii	3.57	0.05	0.98	0.04	DSP (Okadaic acid, DTXs,YTXs,PTXs)	60
Dinophysis infundibulum	2.56	0.04	0.00	0.00	DSP (Okadaic acid, DTXs,YTXs,PTXs)	58
Dinophysis miles	6.34	0.10	7.26	0.30	DSP (Okadaic acid, DTXs,YTXs,PTXs)	61
Dinophysis phalacromoides	0.00	0.00	1.11	0.05	DSP (Okadaic acid, DTXs,YTXs,PTXs)	49
Dinophysis Sp1.	0.00	0.00	1.86	0.08	DSP (Okadaic acid, DTXs,YTXs,PTXs)	49
Diplopsalis lenticula	3.75	0.06	0.00	0.00	DSP (Okadaic acid, DTXs,YTXs,PTXs)	59
Gambierdiscus toxicus	1.11	0.02	0.00	0.00	(CFP),(CTXs),(MTXs), Palytoxin, Gambierol	62
Gonyaulax polygramma	34.81	0.53	2.31	0.09	yessotoxin	47
Gonyaulax ceratocoroides	141.86	2.16	58.97	2.40	Homoyessotoxins	63
Gonyaulax verior	18.29	0.28	22.27	0.91	Yessotoxins	64
Gymnodinium catenatum	35.47	0.54	2.97	0.12	Saxitoxin	65
Gymnodinium filum	0.92	0.01	0.00	0.00	HAB	66, 49

Gymnodinium simplex	1.81	0.03	0.00	0.00	HAB	67
Gymnodinium Sp1.	123.01	1.87	65.42	2.67	Saxitoxin	49
Gyrodinium Sp1.	6.17	0.09	7.04	0.29	HAB	49
Gyrodinium varians	0.69	0.01	0.00	0.00	Ichthyotoxic	49
Heterocapsa circularisquama	1.06	0.02	18.29	0.75	Hemolytic	68
Heterocapsa triquetra	0.98	0.01	0.00	0.00	HAB	69
Karenia brevis	11.44	0.17	25.31	1.03	NSP (PbTxs)	70
Karenia mikimotoi	7.36	0.11	3.40	0.14	PSP	71
Lepidodinium chlorophorum	2.36	0.04	0.00	0.00	HAB	72
Lingulodinium polyedra	12.92	0.20	4.55	0.19	PSP (Homoyessotoxins, saxitoxin)	73
Noctiluca scintillans	43.95	0.67	24.81	1.01	HAB	74
Oblea rotunda	6.01	0.09	3.38	0.14	HAB	75
Ostreopsis ovata	2.14	0.03	1.11	0.05	(CFP),(CTXs),(MTXs), Palytoxin, Gambierol	59
Peridinium Sp1.	6.33	0.10	31.76	1.29	HAB	76
Phalacroma mitra	1.53	0.02	0.97	0.04	DSP (Dinophysistoxin),(okadiac acid)	77
Phalacroma rotundatum	16.57	0.25	0.19	0.01	DSP (Okadaic acid, DTXs,YTXs,PTXs)	77
Prorocentrum arcuatum	0.83	0.01	0.00	0.00	DSP	78
Prorocentrum balticum	1.25	0.02	0.56	0.02	Unidentified hepatotoxins	79
Prorocentrum dentatum	17.45	0.27	12.58	0.51	HAB	80
Prorocentrum donghaiense	1.10	0.02	0.00	0.00	HAB	81
Prorocentrum emarginatum	5.63	0.09	10.20	0.42	Unknown toxicity	82
Prorocentrum faustiae	0.83	0.01	0.00	0.00	Okadaic acid (DSP)	83
Prorocentrum gracile	72.53	1.10	29.53	1.20	HAB	84
Prorocentrum lima	2.14	0.03	0.93	0.04	DSP	85
Prorocentrum mexicanum	7.00	0.11	3.36	0.14	HAB	86
Prorocentrum micans	275.37	4.19	97.42	3.97	Palytoxin, Ovatoxin-a	49
Prorocentrum minimum	3.33	0.05	8.86	0.36	HAB	67
Prorocentrum redfieldii	8.43	0.13	3.56	0.15	HAB	87
Prorocentrum rhathymum	0.98	0.01	2.07	0.08	Haemolytic	88
Prorocentrum triestinum	173.73	2.64	59.62	2.43	HAB	87
Protoceratium reticulatum	7.18	0.11	3.11	0.13	DSP (Okadaic acid, DTXs,YTXs,PTXs)	89

Protoperidinium crassipes	1.25	0.02	0.00	0.00	CFP (Azaspiracid toxin)	90
Pyrocystis fusiformis	0.93	0.01	6.58	0.27	HAB	91
Pyrocystis lunula	3.02	0.05	0.46	0.02	HAB	92
Pyrocystis noctiluca	0.00	0.00	1.11	0.05	HAB	91
Pyrocystis Sp1.	0.56	0.01	0.27	0.01	HAB	92
Pyrophacus horologium	0.98	0.01	1.31	0.05	HAB	93
Pyrophacus steinii	7.92	0.12	0.00	0.00	HAB	27
Scrippsiella Sp1.	0.56	0.01	0.00	0.00	HAB	94
Scrippsiella spinifera	0.28	0.00	0.00	0.00	HAB	95
Scrippsiella acuminata	40.97	0.62	41.46	1.69	HAB	96
Tripos furca	3281.86	49.93	764.33	31.16	HAB	49
Tripos fusus	77.50	1.18	15.26	0.62	HAB	49
Tripos lineatus	153.70	2.34	3.81	0.16	HAB	49
Tripos muelleri	5.09	0.08	4.65	0.19	HAB	49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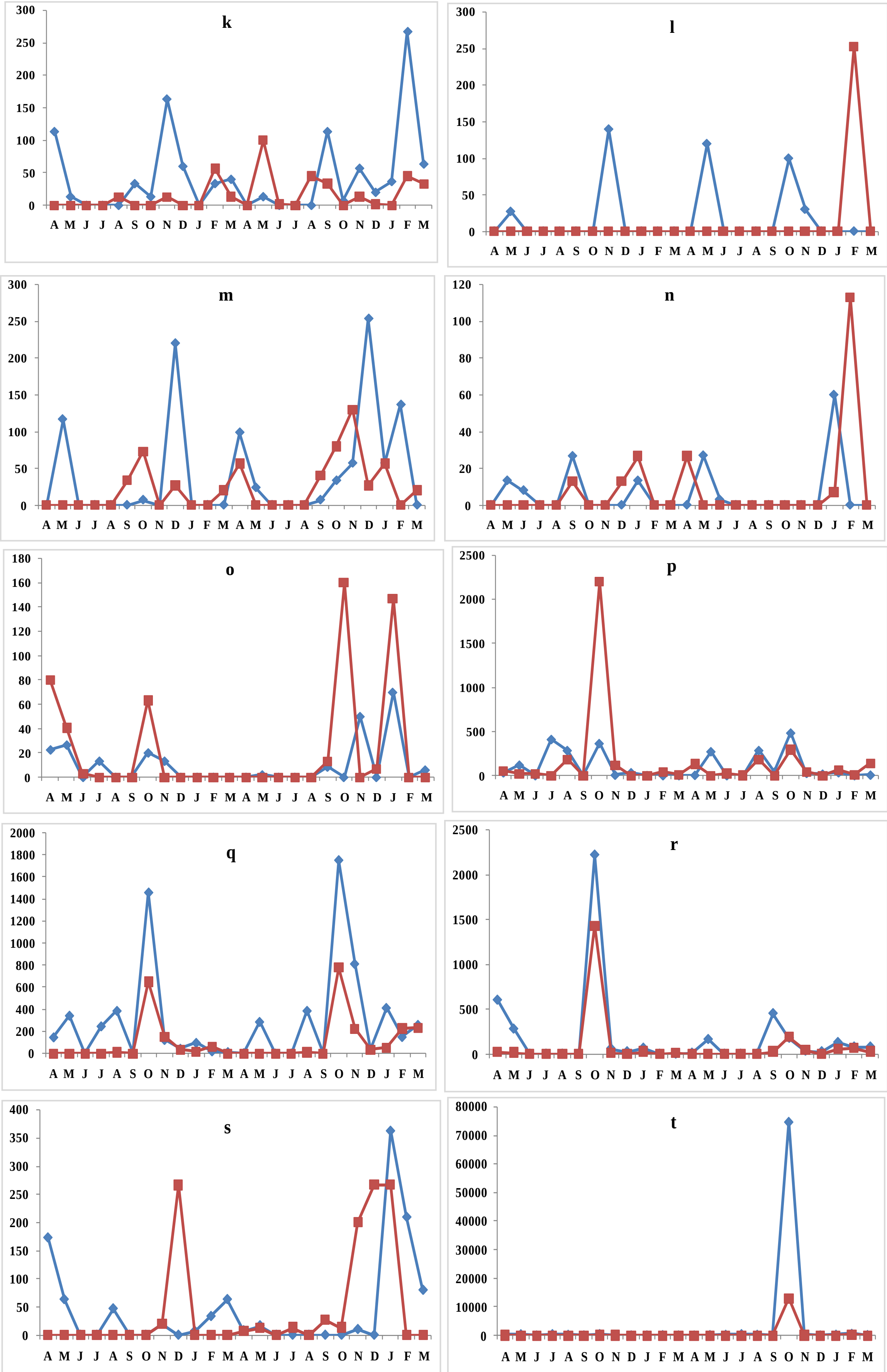
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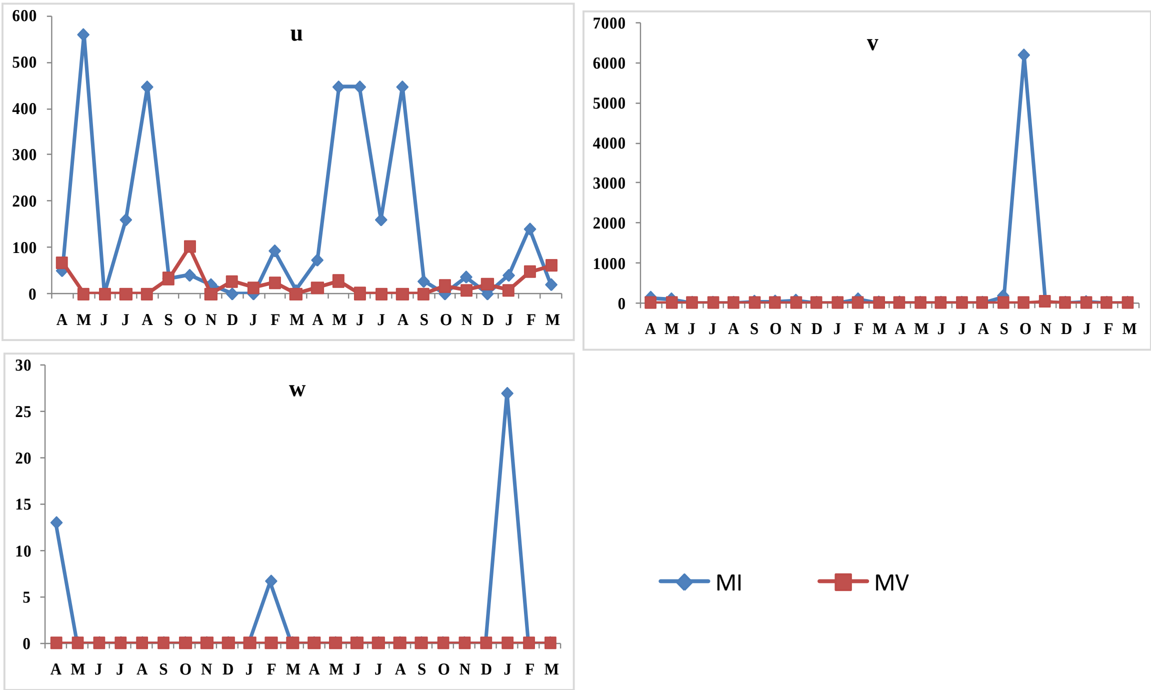

Fig. 3. Seasonal pattern and abundance (cells/L) of toxic/HAB species from Manora Island and Mubarak Village coastal waters. (a) *Alexandrium catenella,* (b) *A. minutum,* (c) *A. ostenfeldii,* (d) *A. tamarense* (e) *Dinophysis acuminate,* (f) *Dinophysis caudate,* (g) *Gonyaulax polygramma,* (h) *Gonyaulax ceratocoroides,* (i) *G. verior,*(j) *Gymnodinium* Sp1., (k) *G catenatum,* (l) *Karenia brevis,* (m) *Noctiluca scintillans,* (n) *Peridinium* Sp1., (o) *Prorocentrum dentatum,* (p) *P. gracile,* (q) *P. micans,* (s) *P. triestinum,* (t) *Scrippsiella acuminate,* (u) *Tripos furca,* (v) *T. fusus,* (w) *T. lineatus*

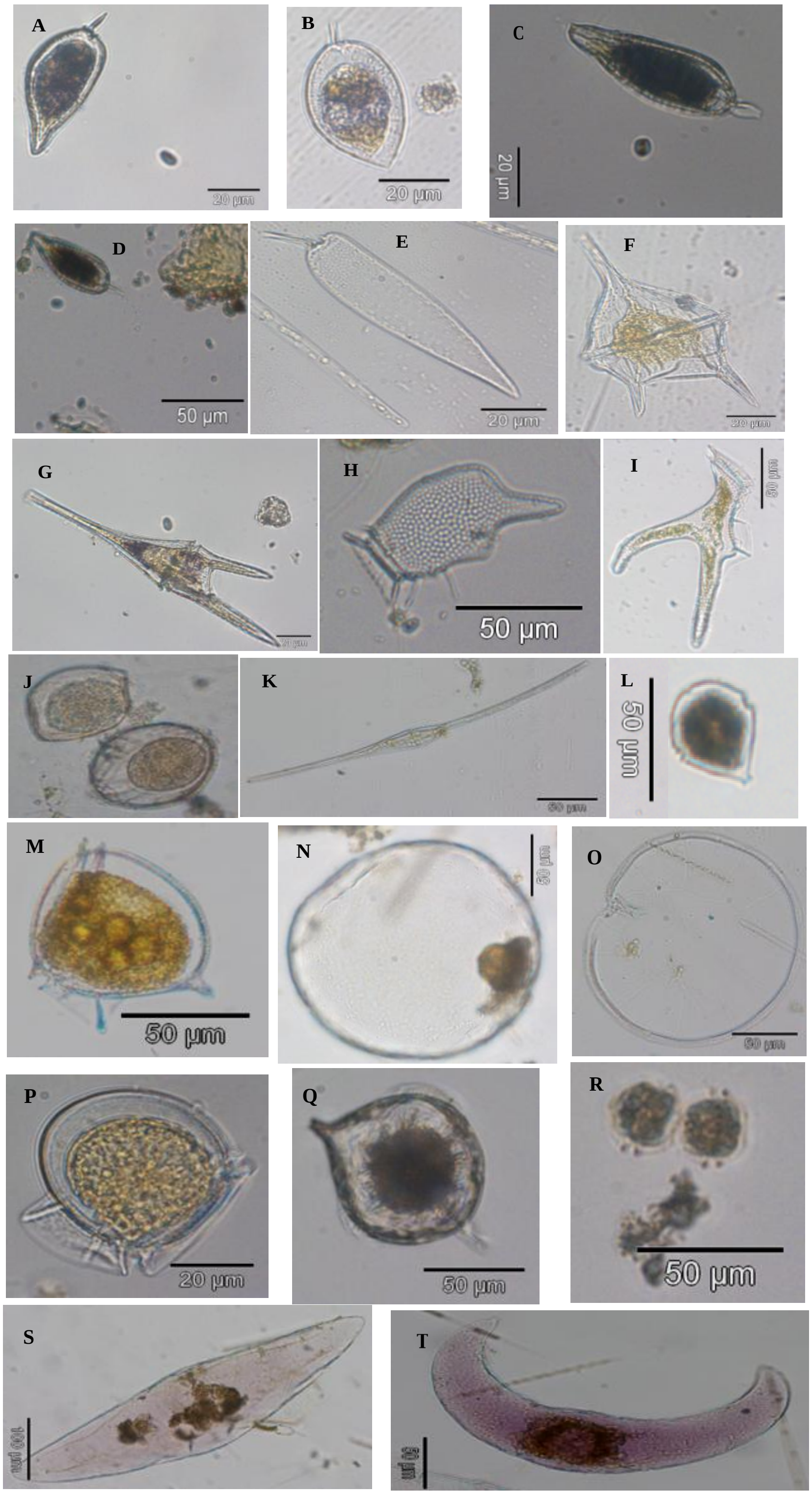

Plate 1. A- B, *Prorocentrum micans*, C- *Prorocentrum sigmoides*, D- *Prorocentrum arcuatum* , E- *Prorocentrum gracile*, F- *Tripos lineatus*, G *Tripos furca*, H- *Dinophysis caudata*, I- *Dinophysis miles*. J - *Protoperidinium* Cyst., K- *Tripos fusus*, L- *Scrippsiella trochoidea*, M- *Phalacroma circumsutum*, N- *Noctiluca scintillans*, O- *Pyrophacus horologicum*, P - *Dinophysis rotundata*, Q -*Protoperidinium* Sp., R - *Alexandrium catenella*, S- *Pyrocystis fusiformis*, T- *Pyrocystis lunula.*

Discussion

Marine dinoflagellates are the key players in marine ecosystem as they serve as primary producers and grazers. Many species were known to produce harmful blooms by increasing cell density and also produce toxins which even at low numbers can have negative impact on human and environmental health and are responsible for socio-economic losses. In addition to the unique monsoonal reversal from southwest (June to September) and northeast (December to March) monsoons (November to March), local manipulation in the coastal waters influences the microplanktonic community. Monsoonal reversal changes the hydrographical phenomenon causing upwelling in the Arabian Sea which brings nutrient rich water to the euphotic zone[97]. The regional and coastal influences appear to cause variations in the distribution and abundance observed at Manora Island (MI) and Mubarak Village (MV) stations. Manora waters are affected by influx of domestic and industrial waste via Malir and Liyari rivers[98, 99] depicting dinoflagellate abundance as oppose to Mubarak village waters; the latter is explored for the first time in present study. Earlier studies conducted in the Manora channel[36] show similar dinoflagellate fluctuation pattern but the numbers of HAB species reported were relatively lower. Taxonomic evaluation of some dinoflagellates have been the subject of few earlier studies from coast of Pakistan[33, 34, 100, 36, 101]. *Tripos furca* was recorded in high abundance in this study and by Munir and co-workers[36, 101] from Karachi coastal waters and also from other parts of Indian Ocean [102, 103, 104, 105, 106] is of concern with respect to human and environmental health. The presence of *Tripos furca* form red tides along many coasts is observed to be a potential harmful species[107]. Average cell abundance recorded in this study (1420 Cells/L) is higher

compared to numbers recorded by Munir and co-workers [36] from inside the Manora channel.

These blooms have been recorded from Oman and Saudi Arabian waters[29, 74] High abundance of *Dinophysis caudata* and *Prorocentrum micans* at MI is alarming as both of these species cause Diarrhetic shellfish poisoning (DSP) species[108] and occasionally kills fish[109]. Earlier studies also showed occurrence of these species inside Manora Channel waters[36]. *Dinophysis caudata* has also been observed in high cell density from other coastal waters including southeastern Arabian Sea[110, 111]. High cell abundance of *Dinophysis* species were linked with NH_4^+ in the stratified waters of southeastern Arabian Sea[112]. Occurrence in high numbers of this species and dinoflagellates in general in MI near-shore waters may be due to the influence of the influx of domestic and industrial wastes being drained in the area[99]. Another nontoxic species, *Noctiluca scintillans,* is a bloom forming bioluminescent species that occurs in significant numbers in Pakistan waters. Both green and red blooms were reported previously from Pakistan[34, 113]. Fish kill event were reported from various parts of the world[114, 115] and from regional waters of Pakistan, for example, from ormara bloom of *Karenia brevis* was observed during 2009-2010 (author communications), the *Pyrodinium bahamense* bloom was reported by Rabbani and co-warker[37] from Gawarder, the other reports are available from Oman[30] and India[27, 116] respectively. The frequency of their occurrence is apparently increasing widely[22, 117] . According to D'Silva and co-workers[117] total 101 harmful algal blooms (39 spp.) were recorded during 1908 to 2009, and Padmakumar[27] depicted 15% frequency of HAB increased from last twelve years in Indian Ocean. It is envisaged that atmospheric nitrogen deposition would be doubled in the Arabian Sea by 2030 compared to what it was in 2000 [118] . This condition will shift the N:P to positively affect the formation of harmful algal blooms, therefore, in future a regular system of monitoring plankton to understand the processes involved needs to be further investigated in the northern Arabian Sea.

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