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AN ASSESSMENT OF PHYTOPLANKTON DIVERSITY IN RELATION TO ENVIRONMENTAL VARIABLES IN THE INDUS RIVER ESTUARY, SINDH, PAKISTAN

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Abstract

Phytoplankton diversity, community structure and seasonal oscillation were appraised from the Indus River Estuary (IRE) in 2017~2018. Due to high turbidity in the IRE overall diversity was low whereas diatoms were higher than that of the dinoflagelates. A total of twenty two species include sixteen diatom and six dinoflage were recorded. Among diatoms *Pseudonitzschia* (1960 cells/ml) was abundantly netted followed by *Thalassionema* (1412 cells/ml) and *Thalassiosira* (570 cells/ml). Genus *Protoperidinium* of the dinoflagelates amounting 570 cells/ml was abundantly found in October. Species distribution, richness and eveness calculated to determine diversity peofile. The highest value (2.38, 0.88) of Shannon and Simpsons' diversity indices calculated for diatoms in September appears to be suitable season due low turbidity in the IRE. A cluster analysis was established to understand similarity among season/species is graphically represented by a dandrogram distinguishes three clades. A multivariate approach of canonical correspondence analysis (CCA) was established to determine impact of environmental variables on the seasonal distribution and abundance plankton communities. Overall 73.43% variability (eigenvalue 0.451, P value 0.324) deduced from first component PCI and 19.85 PCII (eigenvalue 0.0127, P value 0.996) from loaded data sets. It is summarized that meagre density of plankton is more likely controlled by the abiotic factor rather than biotic mechanism (Heterotrophs). Thus plankton distribution is triggered by the turbidity, temperature and salinity rather than conductivity or dissolved oxygen. The outputs of this study can be an input to understand food~web dynamics in the IRE.

Key words: Indus river estuary, Microplankton, Diversity, CCA, Turbidity.

Introduction

Phytoplankton distribution determines ocean productivity (Cullen *et al*., 1993), bloom events frequently occur in estuarine and coastal regions, where the waters are influenced by the input of terrigenous materials (Anderson *et al*., 2002). Indus River carries huge silt from northern mountainous region toward southern region that supports Indus Delta and numerous organisms at estuarine area. However, due to low precipitation and most of the water used at upper riparian areas caused serious threats to lower regions and consequently damaged delta and estuarine environment, diversity by grasping agriculture land and increased salinities in IRE (Berger *et al*., 2019; Hintz & Relyea, 2019). Due to overexploitation, reduction in the Indus River discharge of water and sedimentary flow, pollution, urban expansion, and salinity the mangroves of Indus delta are under stress (Mairaj *et al*., 2021; Farooqui and Valeem, 2009). In subtropical and tropical regions, the consequence of nutrients and organic matter take place by mangrove ecosystems. Coastal swamps serve as barrier and have the capability to enhance the quality of runoff prior being released into the ocean (MacDonnell *et al*., 2017). The spatial and temporal variations of the major nutrients are important for assessing the general well-being of an aquatic ecosystem (Mantyka-Pringle *et al*., 2016). The hydro morphology and biological equilibrium of any riverine structure is regulated by biological and anthropogenic influences (Ijaz *et al*., 2017; Qamar et al. 2020). Hypoxia and the occurrence of harmful algal blooms (HABs) are recorded quite frequently from the Indus delta shelf area (Chaghtai & Saifullah, 2006) and almost resulted in enormous fish kills. The water quality of the Indus River is also influenced by sediment imbalance, diverse vegetation, fertilizer use in the dam's catchment area, and upstream barrages, industrial expansion, and land consumption for human settlements (Irfan *et al*., 2019).

Generally speaking, different taxonomic groups of phytoplankton contribute to primary production and the interaction between trophic levels (Roy *et al*., 2006). In natural environment planktonic taxa are recognized by the range of resistance to abiotic components such as temperature, pH and dissolve oxygen. Phytoplankton blooms are often caused by differences in salinity or by the resulting stratification of the water column (Sew, 2020). As indicated in other areas and in several different habitat types, the influence of freshwater is known to have a profound effect on the biomass, productivity and social composition of phytoplankton (Harnstrom *et al*., 2009). The research on phytoplankton composition in relation to habitat characteristics is essential for constant monitoring of ecological conditions (Dembowska *et al*., 2018). The species biomasses, relative composition, abundance, temporal and spatial distribution of phytoplankton are an expression of the environmental health or biological integrity of a water body (Inyang *et al*., 2020). The earlier studies from the Indus delta and adjacent offshore regions are limited to (Rabbani & Khan, 1986; Harrison *et al*., 1994; Saifullah & Taj, 1995; Saifullah, 1997; Harrison *et al*., 1997). Therefore, this study was aimed to ascertain recent trends, seasonal oscillations and habitat characteristics using multivariate approaches to understand factors involved in the phytoplankton distribution and community structure in the IRE.

Material Methods

Sampling protocol: Phytoplankton samples were collected using a plankton-net of 60µm mesh size from September 2017 ~ April 2018 from the Indus River Estuary (Fig. 1). The net was toed horizontally with a medium size fishing boat and operated for ten minutes. The samples were immediately fixed in 4% formalin and transported to the laboratory for quantitative and qualitative analysis. Species identification was made up–to maximum possible (see Faust & Gulledge, 2002; Tomas, 1996). Identification and enumeration of cells was analysed under the Nikon ECLIPSE Ni optical microscope and Leica DMi8 inverted microscope available at Zhejiang University Zhoushan. The photomicrographs were taken at 20µm or 40µm magnifications. The environmental variables including temperature, salinity, dissolved oxygen and conductivity recorded using Hydrolab, HL-4 made by USA.

Data analysis: Statistical analysis was performed using Excel spread sheets. Various ecological approaches were used to quantify species richness not solely to measure basic comparisons among sites but would also address saturation of local communities colonized in IRE.

Phytoplankton diversity data was used to estimate Shannon-Weiner diversity indices.

Shannon diversity index (H′): $H = -\sum p_i \, ln p_i$ Wilson & Bossier, (1971).

Simpson's diversity index *(1-λ')*: Similar phytoplankton data set were also applied on the Simpson diversity index

$$1 - \lambda = 1 - (\sum n\,(n-1) / N\,(N-1)$$

[where $P_i = n_i/N$, n_i= No. of individuals of a species, N=No. of individuals of all species]

Species evenness (J'): This index was used to assess species evenness J' = H' / (Log_s),

$$J' = H' / (\log S) \text{ (Pielou, 1969)}$$

where S= number of spp.

Bray-Curtis similarity index: The index was applied to test the similarities in the species composition sampled in different months and locations.

Multivariate approach: Four environmental variables such as temperature, salinity, dissolved oxygen and conductivity were used to determine canonical correspondence analysis (CCA).

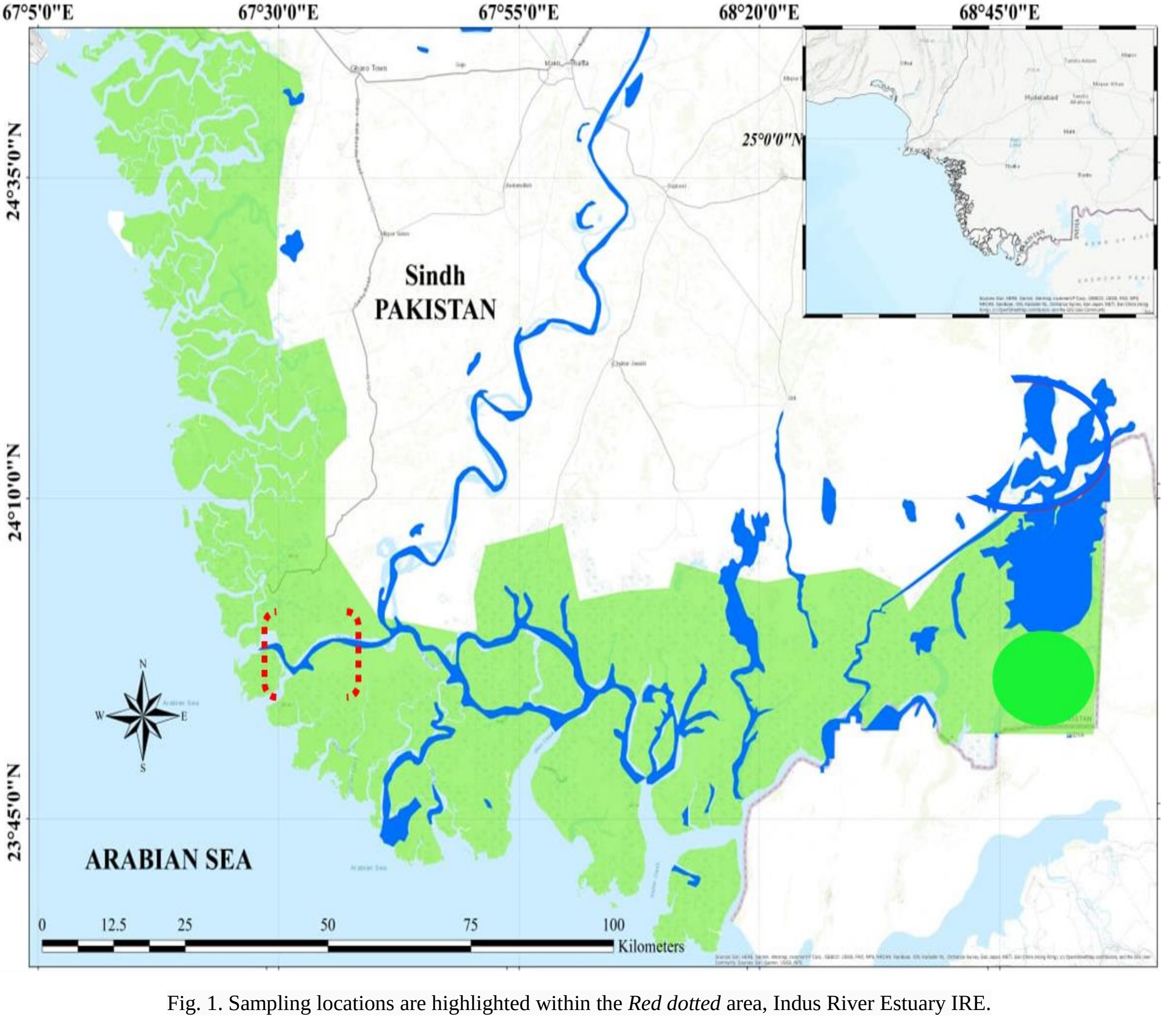

Fig. 1. Sampling locations are highlighted within the *Red dotted* area, Indus River Estuary IRE.

Table 1. List of commonly occurring phytoplankton species and amount of cells in various months with mean and standard deviation values (nos./litre).

Genera	2017				2018					
	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	mean	STDEV
Pseudonitzschia	976	4	1960	0	5	107	0	0	381.5	721.241
Thalassionema	810	5	340	50	138	1412	160	38	369.125	496.276
Thalassiosira	570	77	400	100	110	234	103	74	208.5	183.611
Protoperidinium	613	67	570	140	0	151	7	4	194	252.568
Unknown diatom	256	5	40	8	0	1072	95	16	186.5	367.895
Prorocentrum	36	685	70	130	0	18	10	183	141.5	228.678
Rhizosolenia	274	3	700	0	12	58	2	0	131.125	248.149
Coscinodiscus	243	26	300	97	28	90	50	56	111.25	103.262
Noctiluca	163	12	266	4	0	1	1	66	64.125	99.2449
Skeletonema	340	0	0	0	0	29	0	0	46.125	119.176
Ceratium	82	7	118	22	0	24	26	57	42	40.6553
Cerataulina	80	0	26	7	0	19	0	0	16.5	27.5369
Chaetoceros	114	1	0	0	0	9	0	0	15.5	39.9213
Pleurosigma	23	0	50	0	8	10	8	3	12.75	16.7566
Unknown dinoflagelate	16	8	22	10	9	20	6	9	12.5	6
Bacillaria	32	0	56	0	0	0	0	3	11.375	21.158
Nitzschia	20	0	55	0	0		0	0	10.71429	20.902
Odontella	22	0	10	0	3	28	0	0	7.875	11.217
Navicula	13	4	7	0	0	22	5	10	7.625	7.347
Asterionellopsis	23	0	0	0	0	27	0	0	6.25	11.622
Dinophysis	6	5	12	0	0	2	1	0	3.25	4.234
Planktonella	1	0	1	0	2	4	2	8	2.25	2.659

Results and Discussion

A total of sixteen diatom and six dinoflagelates species were identified and encountered during entire sampling period (Fig. 2). Among them genus *Nitzschia* was dominant in the Indus River Estuary (IRE). High phytoplankton diversity was recorded in September, November (2017) and February (2018) whereas rest of the month were less diverse owing to riverian flow or turbidity (Table 1). Among diatoms *Pseudonitzschia* (1960 cells/ml) was abundantly netted followed by *Thalassionema* (1412 cells/ml) and *Thalassiosira* (570 cells/ml). Genus *Protoperidinium* of the dinoflagelates amounting 570 cells/ml was abundantly found in October. Moroever, genues *Dinophysis* and *Planktonella* were rarely observed thorough out study period. Estimates of the percentage composition reveals that *Pseudonitzschia* was the species found in highest numbers during autumn and early winter seasons (September & November) whereas rarely observed in rest of the sampling months while *Thalassionema* comprises of (18%) in spring and an unidentified species was over 9%. In the autumn *Prorocentrum* appeared to be soar quantities. Nevertheless, fourteen species were <5% with scarcity of *Planktonella* which was merely appeared in April (Fig. 3). In the estuarine ecosystem water quality, light penetration, suspended load and salinity play a crucial role in the distribution and abundance of planktons Mairaj et al. (2021).

Among six dinoflagelates *Protoperidinium* spp., dominated entire population with 42.5% and the limited cells counted for *Dinophysis caudata* (Fig. 4). A monthly diversity profile was statistically tested to understand species richness, eveness and abundance reveals that the highest dominance (0.58) was in autumn corroborates suitable season for diatoms when turbidity was lowered. Similarly, Shannon and Simpsons' diversity (2.38, 0.88) were the highest in September and the lowest in October and evenness can be deduced from the pooled species data (Fig. 5, Table 2). Similarity index among species is graphically represented by a dandrogram that show three distinct clusters (Fig. 6). A multivariate approach of canonical correspondence analysis (CCA) was established to determine impact of environmental variable e.g. temperature, dissolved oxygen, turbidly, pH, salinity and conductivity (Fig. 7) on the distribution and abundance of the phytoplankton. First component PCI revealed 73.43% variability and PCII 19.85 deduced from loaded data sets (Fig. 8). Further it is interpretable that dominant species in relation to the four environmental variables may have potential influence on phytoplankton distribution. Moreover, eight species showed positive co-relation with temperature which may have prominent effect on their distribution. The close association of salinity revealed the effect of tidal influence in estuarine area, whereas four species show negative relation with salinity, conductivity and dissolve oxygen that defines their adaptableness to large range of difference in physicochemical properties. The salinity has negative co-relation with four plankton species (Ud, Na, Pi, Ni), those are distributed at the left side of axis-I and axis-II positively co related with conductivity and temperature (species abbreviations are given in table 3). The length of the ecological gradients lines of the IRE denotes each variable in relation to individual position with the biplot (Fig. 8). Overall outputs of the CCA revealed that phytoplankton abundance and diversity is mainly regulated by abiotic factors than that of the biotic factors (heterotrophs). CCA suggests that their distribution was influenced by an interaction between physico-chemical factors and phytoplankton consequently it is acceptable that physico-chemical factors influence the distribution along with common factor e.g., turbidity in IRE.

Table 2. Summary of different diversity indices calculated from pooled data of twenty-two diatom species (Bold value indicates highest).

Diversity indices	Sep-17	Oct	Nov	Dec	Jan-18	Feb	Mar	Apr
Dominance (D)	0.121	**0.581**	0.205	0.182	0.323	0.292	0.213	0.185
Simpson (1-D)	**0.879**	0.419	0.796	0.818	0.677	0.709	0.787	0.815
Shannon (H)	**2.377**	1.005	2.019	1.842	1.393	1.656	1.788	1.970
Evenness (e^H/S)	0.490	0.195	0.396	**0.631**	0.447	0.262	0.427	0.552
Margalef	**2.483**	1.908	2.113	1.419	1.391	2.342	2.109	1.915
Equitability (J)	**0.769**	0.381	0.686	0.800	0.634	0.553	0.678	**0.768**

Table 3. Abbreviations of sixteen cases (Fig. 5) used in CCA analysis.

Species name	Abbreviation
Asterionellopsis glacialis	Ag
Bacillaria paxillifer	Bp
Cerataulina pelagic	Cp
Chaetoceros spp.	Ch
Coscinodiscus spp.	Co
Navicula spp.	Na
Nitzschia longissima	Nl
Odontella sinensis	Os
Planktoniella sol	Ps
Pleurosigma angulatum	Pa
Psudonitzschia seriata	Pss
Rhizosolenia setigera	Rs
Skeletonema costatum	Sc
Thalassionema frauenfeldii	TF
Thalassiosira spp.	Th
Unknown diatom spp.	Ud

Global oceans contribute about 46% in planetary photosynthesis from a series of complex biochemical process in the ocean and reduce carbon and other elements produced from the atmosphere and important candidates for gauging the health in the ocean (Falkowski, 1994). Primary productivity strengthen almost all the food webs and built the link between physical environment and fish, birds and mammals whereas understanding ecological phenomenon in aquatic ecosystem requires learning about diversity and relationship with ocean productivity (Vallina *et al*., 2014). Plankton as an ideal indicator for sustainable managing global oceans but sensitive to the environment and changes in plankton communities reveal environmental changes that complicates fish stock assessment (McQuatters, 2019). IRE carries turbid water during flood season that does not allow light penetration and consequently act as a limiting factor involved in reducing plankton diversity. Therefore, high diversity in autumn and winter season was due to the low turbidity. In general, phytoplankton biomass is typically regulated by the concentration of the suspended load (turbidity) carrying river water to estuaries (Kocum *et al*., 2002). Phytoplankton accumulates at the surface because of availability of nutrients with thermal stratification and in certain situations they create hypoxic conditions below surface. Whereas Tang *et al*., 2004 observed that a high value of chlorophyll usually coincided with a low ingestion rate in the Pearl River Estuary. Therefore, plankton abundance was more likely controlled by abiotic factors rather than biotic mechanisms. Diatoms from Parana, Brazil studied (Fernandes and Brandini, 2004) found that decrease in diatoms between autumn and winter is associated with meagre availability of silicate and low precipitation. A low phytoplankton density reveals that their abundance is more likely controlled by the abiotic factor rather than biotic mechanism. Moreover, plankton distribution is triggered by the temperature and salinity rather than conductivity and dissolved oxygen. The estuary has a strong tidal impact that retains the structure well integrated with no clear stratification. The planktons were influenced by the fluctuating geomorphology and discrete dry and wet seasons during the study period. The creek has a high impact by the monsoon and anthropogenic activity on the planktonic growth which is further influence by the increase nutrient supply. Results represent an incorporated approach for management and examining the process of the Indus delta to make certain efficient environment conservation and management.

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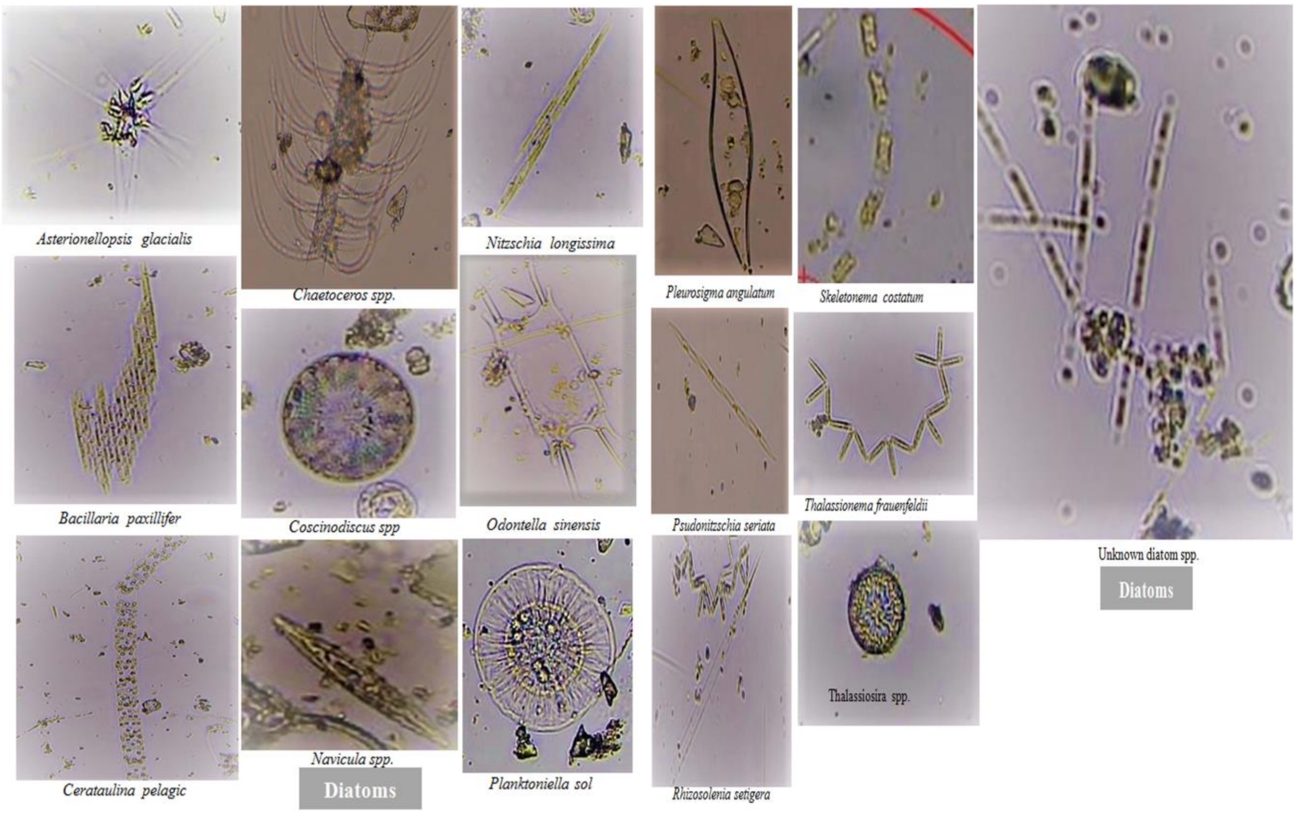

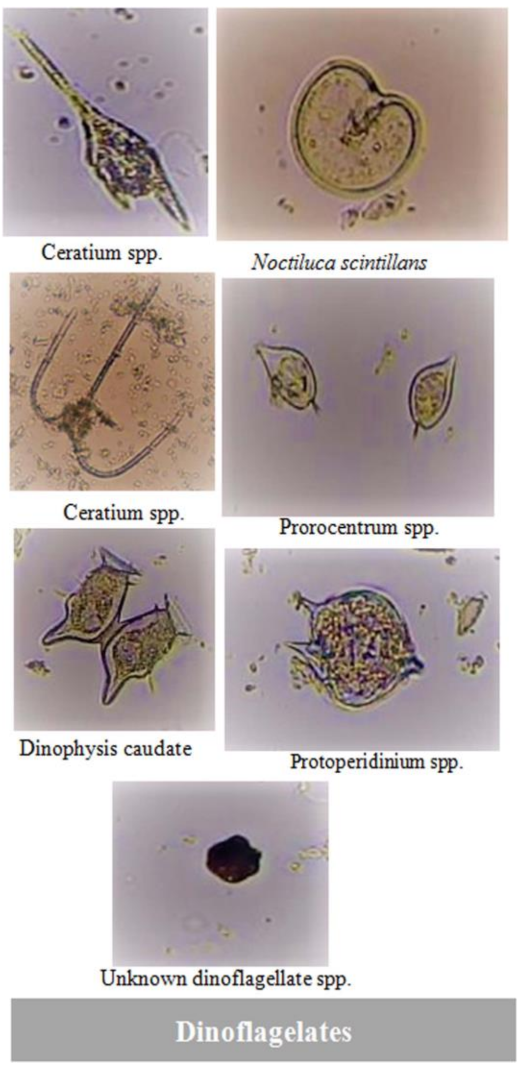

Fig. 2. Phytoplankton diversity: An image of each species provided.

Asterionellopsis glacialis (10-30 um, central disc diameter); *Bacillaria paxillifer* (50-150 um, chain length); *Cerataulina pelagic* [50-200um N(length), 5-75um (width); *Chaetoceros* spp. (60-120 um, length); *Coscinodiscus* spp. (40-60 um, diameter); *Navicula* spp. (60-100 um, length); *Nitzschia longissima* (50-100um, chain length); *Odontella sinensis* (80-440 µm, apical axis); *Planktoniella sol* (60-100 um, diameter); *Pleurosigma angulatum* (65-75 um); *Psudonitzschia seriata* (2-8um, width 40-175um, length); *Rhizosolenia setigera* (4-20 µm, width, 116-450 µm, length); *Skeletonema costatum* (2-61 um, pervalar axis); *Thalassionema frauenfeldii* (10-110 um, apical axis, 2-6um, width); *Thalassiosira* spp. (14-56, diameter); Unknown diatom spp.; *Ceratium* spp. (100-800um length); *Dinophysis caudate* (70-110 µm, length, 37-50 µm, dorso-ventral width); *Noctiluca scintillans* (60-100 µm, diameter); *Prorocentrum* spp. (35-70 µm long, 20-50 µm wide; *Protoperidinium* spp. 60-80 um length, 50-60 width; Unknown dinoflagelate spp.

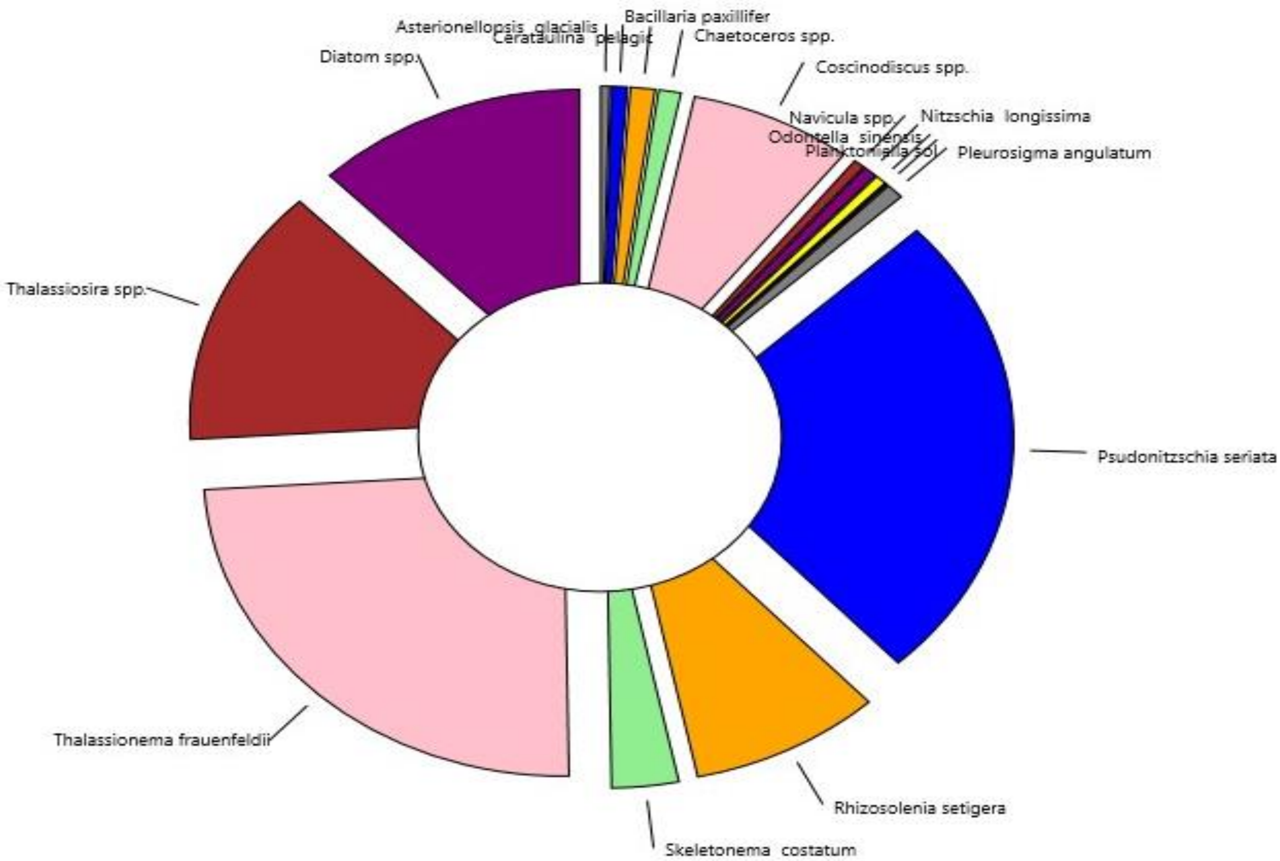

Fig. 3. Percentage composition of sixteen diatoms netted from mouth of the Indus River Estuary (IRE), Sindh, Pakistan, (Nil indicates unknown species).

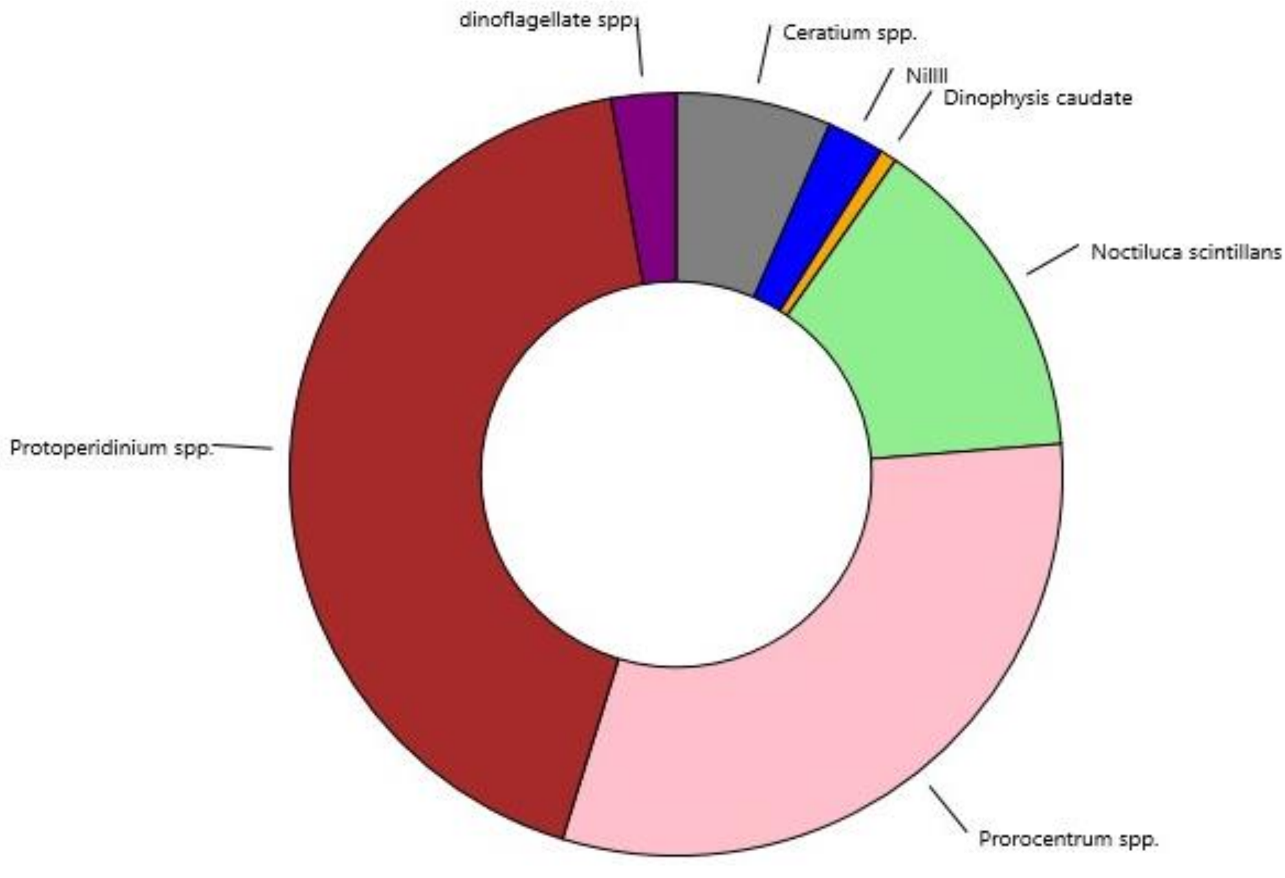

Fig. 4. Percentage composition of six dinoflagelates netted during present study (Nil indicates unknown species).

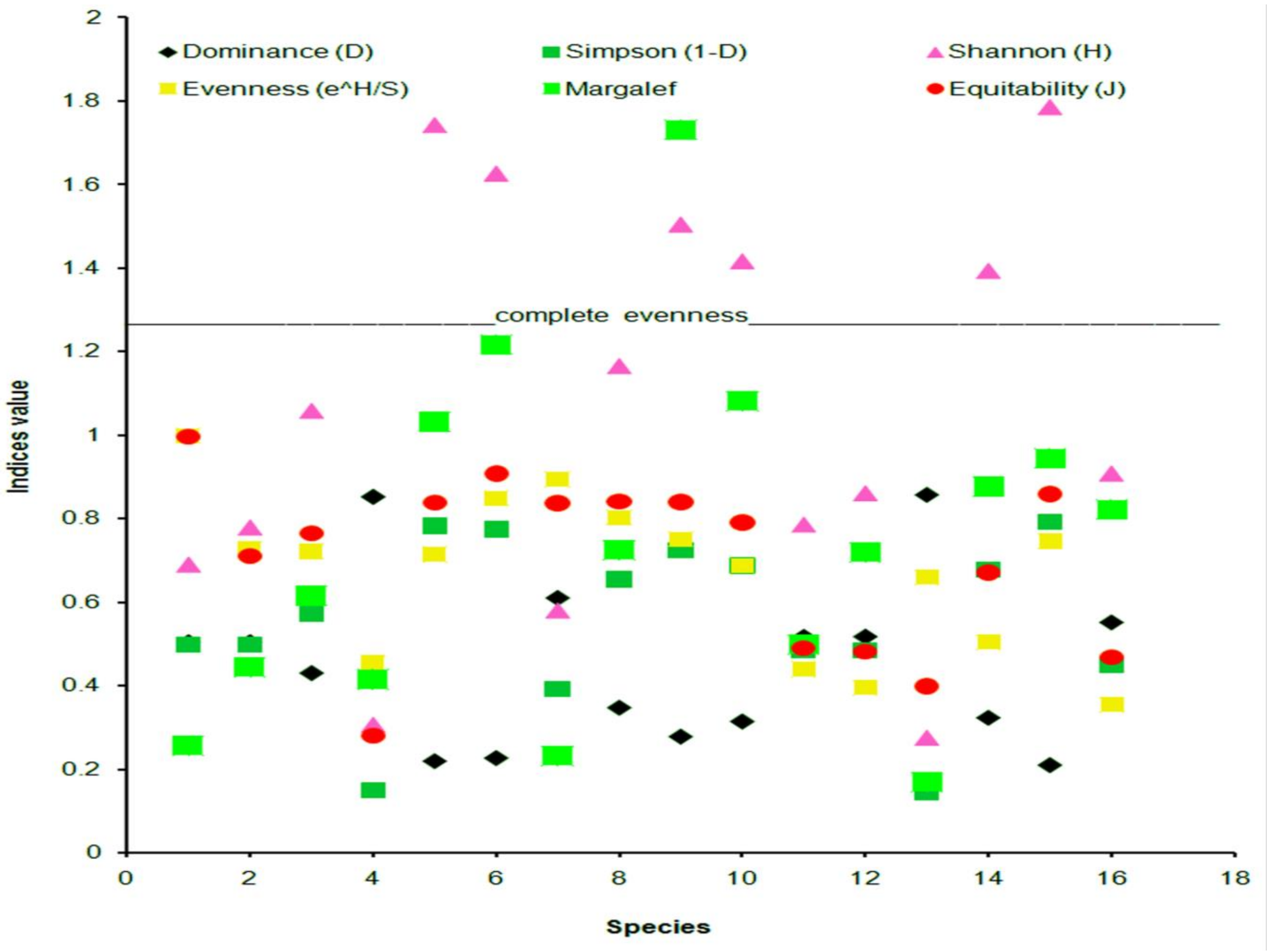

Fig. 5. Summary of the diversity indices calcluated to undestand evenness of the speices in a uniqe ecosystem (IRE).

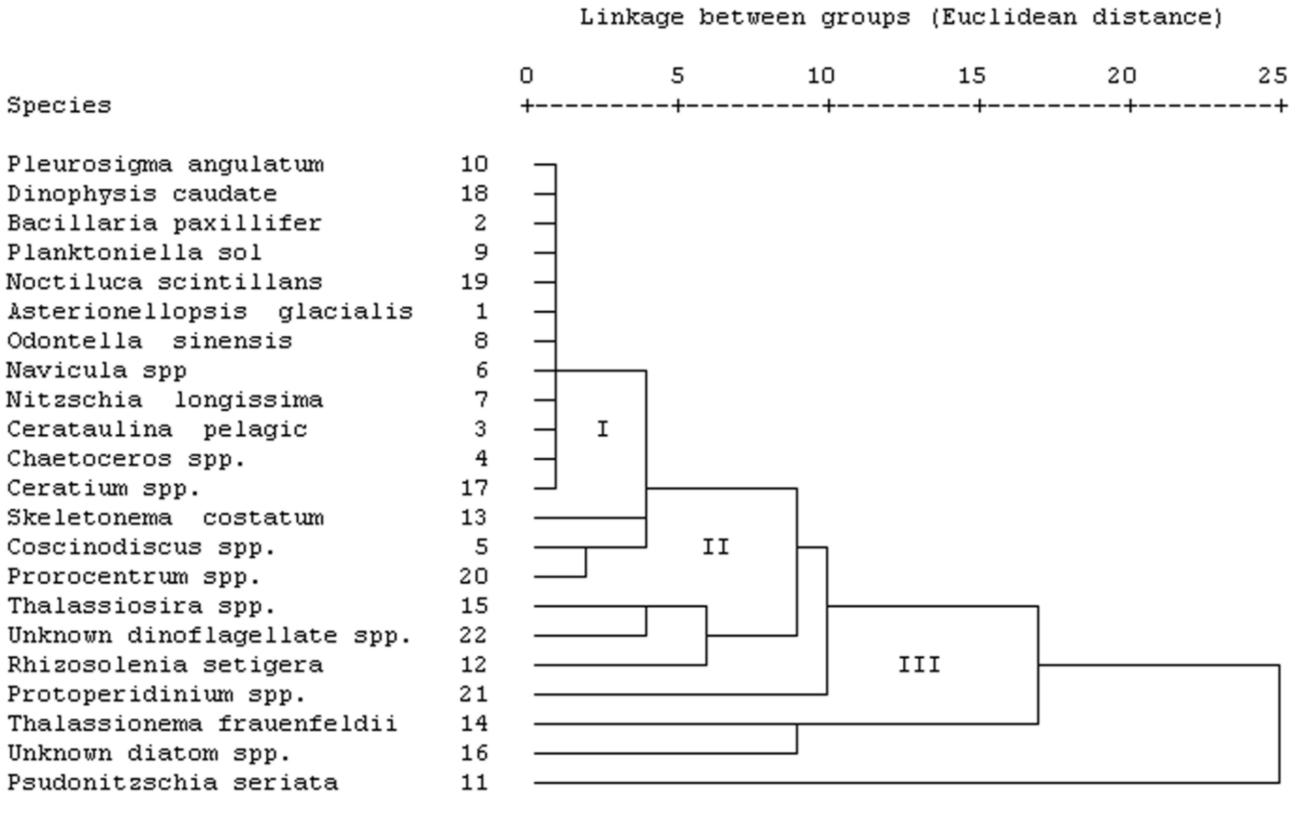

Fig. 6. Dandrogram is established from pooled data of twenty two plankton species based on the Euclidean distance.

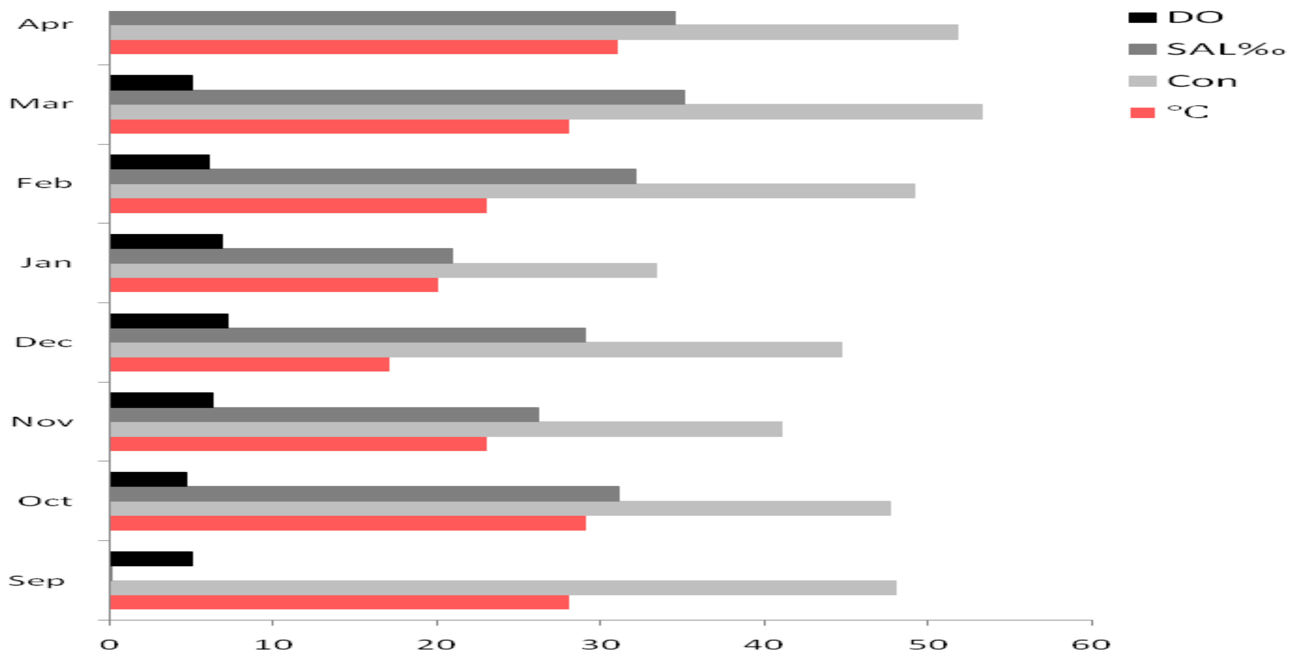

Fig. 7. Water quality parameters recorded during onboard sampling of phytoplankton in the IRE.

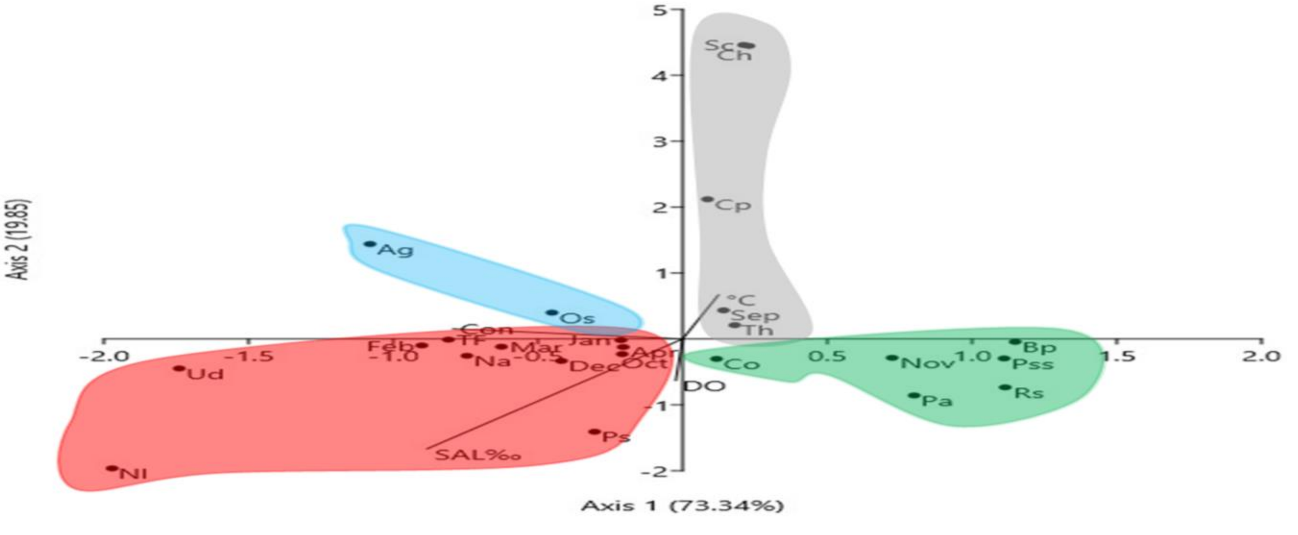

Fig. 8. Canonical correspondence analysis (CCA) of physicochemical properties (Temperature, salinity, dissolved oxygen and conductivity) and plankton distribution with biplot showing the pattern of established to test the relationship among plankton species and environmental variables.

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