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Substrata Wise Benthic Diatom Community Structure of Aganashini Estuary, Uttara Kannada District

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***Abstract*— The Estuarine ecosystem is generally considered as the ideal cradle grounds of many freshwater as well as marine phytoplankton/benthic diatom communities. Some of the critical factors influencing their growth and abundance in the estuarine regions are: salinity gradient (induced by the dilution of salt water with fresh water), low turbulence compared to that of the open sea, temporal and spatial influx of nutrients into the estuaries due to monsoons and pollutions caused by anthropogenic activities. Estuaries, thus possessing a highly dynamic characteristics are important ecological biotopes that embrace a significant and diverse biota within them. Among the aquatic biotas, the most important ones are the diatom communities. They form the basis of the tropical food web with more than 40% of primary productivity. The community structure of diatoms are highly sensitive to the changes in the environmental factors and hence their presence and absence in a particular area are usually considered as biological indicators/bio-monitoring tools of fluctuations in the environmental parameters. This communication reports the variation in species composition of benthic diatoms along the shoreline of Aganashini river estuary at six different sampling stations. The sampling stations are chosen based on the difference in their salinity gradients. The benthic diatom community based on substratum is correlated with that of the physico-chemical/environmental parameters of the estuary.**

Keywords*— *Benthic diatom, substrata wise diatom community structure, salinity, Aganashini river estuary, physico-chemical parameters, Canonical Correspondence Analysis, Species diversity and Multivariate cluster analysis.

INTRODUCTION

Estuaries are ranked among the highest productive ecosystems of the earth (Ramachandra et.al, 2012). They are also considered as dynamic water bodies because of their temporal and spatial variation in nutrients levels received from the river and other land based discharges. Estuaries are also considered as biologically active zones (Khlebovich, 1974; Kibirige and Perissinotto, 2003), because of their intense biological production at low salinities which is favored by a variety of phytoplanktons (marine, brackish, and fresh water). Among nutrients, nitrates, phosphates and silicates are the major limiting nutrients, which when present in surplus supports the growth and establishment of various phytoplankton communities in these regions. Besides the availability of nutrients, other physical variables such as flushing rate, salinity and turbidity also influence the phytoplankton community structure, growth and abundance (McLusky, 1971; Cleorn, 1987; Ferreira et al., 2005, N.V.Madhu et.al, 2006).

The Aganashini River in the Uttara Kannada district of Central Western Ghats, originates at Manjguni near Sirsi and flows westwards towards the Arabian Sea. The Estuarine region of the river extends from 14.4649 ºN, 74.4908 ºE near uppinpattana (upstream) to 14.50707 ºN, 74.34600 ºE near Kirubele (downstream) with a permanent opening to the Arabian Sea at Tadri. The total length of the river is 121 km of which the estuary covers only the final 27 km stretch till the river mouth at Tadri. The Aganashini River estuary is a highly productive and biologically rich waterscape of the coastal Karnataka region because of its high organic nutrient loadings and its deposition in the estuary from the forests of the Central Western Ghats during monsoons and the rich mangrove vegetation that serves as a micro habitat of diverse faunas, birds and fishes which indirectly plays a significant role in nutrient supply (Subhaschandran et.al, 2012).

The major primary production of any estuarine waters are thought to be from diatoms, which forms the basis of the food web. Diatoms are ubiquitous, unicellular micro-organisms and a major group of microalgae, which possesses distinct golden brown pigments and are mainly characterized by their unique, ornate cell wall

made up of hydrated silica. They are popularly known as "Algae in glass houses". They belong to the division Bacillariophyta and account for a substantial portion (upto 40%) of ocean's primary productivity. Diatoms majorly store their energy in the form of lipids or as chrysolaminarin (a β-1, 3 and β-1, 6 linked glucose units in the ration of 11:1), a soluble and complex polysaccharide. They are classified as centric and pennate species that are commonly referred to as Centrales and Pennales. Centric diatoms are predominately phytoplanktons that dominates majority of the oceans and pennate diatoms are usually benthic and mostly confined to freshwater and marine benthic zones. Benthic diatoms attach themselves onto a substratum and forms a biofilm which usually helps them in forming colonies as well as protects them from getting washed off. It also helps them in ease of locomotion within the biofilm. They secrete a mucilaginous substance which comprises of Extracellular polysaccharides (EPS) called as Chrysolaminarin. This EPS are highly specific and a typical characteristic feature of the class Bacillariophyceae. Based on the substratum on which the diatom attaches, they are named as Epipelic – sediments, Episammic – sand, Epilithic – rocks and Epiphytic – macrophytes/plants. The present study investigates the diatom community structure of six different stations in the Aganashini river estuary, categorized based on their salinity ranges. The diatom community structure with respect different substratum across all the six stations were analyzed, compared and correlated with the physico-chemical parameters of the water collected from the each station.

MATERIALS AND METHODS

1. **Study Area:** The study area comprises of six different sampling stations of the Aganashini river estuary. Stations 1 (Divgi) and 2 (Hegde) are low saline regions, while stations 3 (Kagal) and 4 (Bargi) are mid saline and stations 5 (Kirubele) and 6 (Belekan) are high saline regions. The stations and their GPS locations are given in table 1.

Table 1. Sampling stations and its location

Station id	Station name	Location
1	Divgi	14.444188° N, 74.435981° E
2	Hegde	14.475050° N, 74.427980° E
3	Kagal	14.495050° N, 74.379725° E
4	Bargi	14.518810° N, 74.387250° E
5	Kirubele	14.512353° N, 74.358647° E
6	Belekan	14. 520360° N, 74.344170° E

2. **Sampling strategy:** The six sampling stations were chosen based on the preliminary reconnaissance survey of the region and also considering the distance from the river mouth of the Aganashini river estuary. Tidal action of Aganashini estuary is of semi-diurnal type, wherein the estuary experiences two nearly equal high and low tides each day. Stations 1 and 2, which are at the upper reaches (head) of the estuary experiences nearly zero salinity during monsoon season and a very low salinity (~5 – 8 ppt) during non-monsoon months are categorized as Oligohaline (low salinity range) stations. Stations 3 and 4, which are approximately in the middle portion of the estuary experiences moderate salinity (~5 – 10 ppt) during monsoons and high salinity (~ 18 to 24 ppt) during non-monsoons are categorized as mesohaline (mid salinity range) regions. Stations 5 and 6, which are almost near the river mouth and experiences high

salinity (~ 18 – 28 ppt) throughout the year are categorized as Polyhaline (high salinity) regions. Benthic diatom samples were collected from the shorelines of all six stations. All possible substrata like stones, cobbles, boulders, macrophytes, sedges, shells, halophytes (mangrove plantations), sediments and sand were gathered and the diatom biofilms were collected in 50 ml capacity plastic vials. The diatoms were collected as per the standard protocols given by Taylor et.al, (2007). The samples were collected for a period of 5 months from July - November 2016. Table 2 gives the details of the diatom samples collected from the different substrata that are present and found accessible at each stations.

Stations	Different Substrata considered for diatom collection	Common Diatom names
1	laterite (Cobble) Stones	Epilithic
2	Sediments, laterite (Boulders) and Sedges	Epipelic, Epilithic and Epiphytic
3	Shells, Mangrove plants, sediments	Epilithic, Epiphytic
4	Sediments, Mangrove plants, laterite (Boulders)	Epipelic, Epiphytic and Epilithic
5	Rocks, Sand	Epilithic
6	Rocks, Sand	Epilithic and Episammic

3. **Water Sample Collection:** water samples were collected in a disinfected 500 ml capacity non-reactive plastic sampling bottles from all the six stations. The samples were collected mainly during low tide. Because the land mass, mud-flats, sediments and stones will be exposed during low tide as the water recedes towards the downstream, low tide time of the day was preferred for benthic diatom sample collection. Air temperature, Water temperature, pH, Electrical conductivity, DO and salinity were measured onsite. Nutrient (Nitrates, Phosphates and silicates) analysis were done in the laboratory. The nutrient analysis were done following the standard protocols of APHA (1998).

4. **Diatom Sample preparation, Treatment and Identification:** The diatom samples were pre-treated and prepared for analysis by $KmNO_4$ and Hot HCl method as prescribed by Round et.al (1990), Taylor et.al (2007). The pre-treated and acid digested samples were repeatedly centrifuged with distilled water till the sample becomes circumneutral. The supernatant was discarded and the pellet was diluted until it becomes slightly turbid. The treated and prepared samples were observed in an optical microscope (Model: Olympus BX51 integrated with Olympus camera: TV1X-2) under 40× resolution. The images of the diatom frustules were captured by adjusting the dilution of the sample to maximum 2 -5 valves per view using the digital camera connected with the microscope. The images captured were compared with diatom identification keys by Henry Van Heurck [3] and Karthik et.al (2013) [4] and identified to the lowest taxon possible for some species, while for few species it is identified to the genus level based on its key features. The identified species are also enumerated as per the protocol given in DARES enumeration protocol.

5. **Data Analysis:** Species diversity and species richness index of all the five months was calculated using Shannon wiener's diversity index and Pielou's Species Evenness index. Diatom assemblages and community structure found on each substrata were considered separately with respect to stations for the data analysis. The relationship between biological (Diatom population) and physico (- temperature, salinity, turbidity), chemical (-pH, EC, DO, Nitrates, Phosphates and Silicates) on each stations across all 5 months were analyzed using Canonical Correspondence analysis. Multi criteria hierarchical cluster analysis was also performed on stations to determine the similarity between the stations based on abiotic and biotic factors. All these analysis were done using statistical software PAST v3.

RESULTS AND DISCUSSIONS

1. **Water Chemistry:** As the sample collection was carried out during monsoon and post monsoon seasons (July - November 2016), the ranges of physical parameters like temperature, salinity were comparatively less than that of the ranges that usually prevails in the sampling region. The minimum and maximum average air temperature across all the six stations ranged between 29.9 – 32.14°C. The water temperature was lesser than air temperature by almost 1°C with minimum and

maximum range of 27.44 – 29.98°C. Continuous and intermittent precipitation that happened during the sampling period could have cooled the water, resulting in a lower temperature. The salinity ranges of upstream (station 1, 2) and middle region stations (3, 4) were very low (ranged between 2 – 6 ppt) due to the dilution effects of monsoonal run-offs from the river. Another reason for this low salinity ranges in the sampling stations could be attributed to the time of sampling. As the samples were collected during low tide, the fresh water discharge from the river upstream could also have a significant effect in reducing the salinity. The salinity of stations 5, 6 were 10 and 12 ppt respectively as these stations are near the river mouth because of which the salt (sea) water mixing rate is high. The pH of all the stations remained alkaline with an average value of 8.04. The variation in pH across the stations were only marginal. According to Perkins (1976), the pH value of estuarine waters under normal conditions would range from 6.7 to 9.25. The DO values of all the six stations were high (with an average of 8.468 mg/L) due to the turbulence and wave action of flushing waters into the estuary from the hill-tops and forests during monsoons. Nair (1985) reported that the DO level can be increased through freshwater input. Nutrient analysis of the water samples collected from the stations did not show any drastic fluctuations in its levels. The average Nitrate levels of the sampling stations was 0.343 mg/L. Kumar et al. (2009), pointed out a higher nitrate concentration in his experiments on estuarine waters during monsoon due to increased freshwater influx. Bazlur Rahaman et.al (2013) who captured the nutrient dynamics of the Sundarbans mangrove estuarine system reported a nitrate concentration of 0.08–0.46 mg/L during monsoon seasons. Phosphate values of the stations were comparatively less than that of the nitrate concentrations with an average of 0.146 mg/L. The phosphate values obtained in the present study are comparable with the studies conducted by Bazlur Rahaman et.al (2013) (0.10–0.16 mg/L) and Wahid et.al (2007). The silicate values showed an increasing trend from the river mouth towards upstream with a least value in station 6 (5.2 µg/L) and highest in station 2 (9.86 µg/L). The reason behind this fluctuations in silica level from upstream to downstream could be due to the higher deposition of silt particles in riverine regions during monsoon season. Banerjee et.al, 2004 associates this lower level of silica in coastal bay (downstream region) with the abundance of siliceous diatoms which incorporates the silicates available in the sea water in their cell structures as biogenic silica, thus leaving the downstream regions with low levels of silica.

2. **Benthic diatom community structure:** The community structure of diatoms is highly dependent on various physical and chemical factors and hence the species composition and density generally varied across seasons. Among the substrata, Epipelons were found to hold a diverse species composition and assemblage with 19 different taxa of diatoms, followed by Epilithons with 12 different taxa. Epiphytons had an assemblage of 10 different taxa. The Episammic diatom community (in station 5 and 6) was the least with only four different taxa. The reason for this poor diatom community structure on sand could be because of abrasion of moving grains of sand due to higher wave action which is unfavorable for the diatoms to establish their assemblages. Townsend et.al (2005) noticed and reported a similar trend of diatom assemblage on Episammons than Epilithons and Epidendrons. Table 2 gives the list of substrata wise benthic diatoms of all the six stations of Aganashini river estuary. Common diatoms were present in all substrata, but their relative abundances varied to a considerable extent.
3. **Diversity Indices:** Month wise variation in the species diversity and species evenness is given in table The station Kagal had the highest index of diversity with 1.994 all through the 5 months of sampling with new species occurrence in each month's sampling. Bargi station was recorded with the second maximum diversity index (1.982). Kirubele and Hegde had diversity indices of 1.72 and 1.71 respectively. The station Hegde was recorded with a maximum number of species with a higher relative abundance. Divgi and Belekan showed a least diversity with indices 1.64 and 1.32 respectively.
4. **Canonical Correspondence Analysis:** Canonical Correspondence Analysis (CCA) was aimed to find the relationship between environmental variables and benthic diatom community distribution. In the station Divgi, a total of 20 diatoms of different genera were present which was considered with the 8 environmental parameters for the CCA analysis. Eigen value of axis 1 (λ=0.664), axis 2 (λ=0.437) and axis 3 (λ=0.269) explained 92.36% relationship between species and environmental variables. In Divgi,

species of Synedra, Melosira, Coconeis, Coscinodiscus, Achnanthes, Eunotia, Nitzschia and Cyclotella showed a positive correlation with axis 1 which indicates the effect of AT, WT and Silicates on its distribution. In station Hegde, 19 different diatoms were identified and CCA analysis on these species with environmental parameters showed an Eigen value of axis 1 (λ=0.159), axis 2 (λ=0.051) and axis 3 (λ=0.044) which explained 95.3% of relationship between species and environmental variables. Gomphonema, Synedra, Amphora, Raphoneis, Melosira, Coscinodiscus, Pleurosigma, Coconeis, Pinnularia, Nitzschia and Achnanthes showed a positive correlation with axis 1 and the environmental parameters found to be responsible for their distribution was only air temperature and. In Kagal, Phosphates, Silicates and Salinity had a significant effect on the species presence and its abundance, with dominant species being Nitzschia, Navicula, Bacillaria, Gomphonema, Pleurosigma and Pinnularia. Eigen values of axis 1 (λ=0.226), axis 2 (λ=0.185) and axis 3 (λ=0.102) showed a 88.71% correlation. In Bargi, the species presence is more correlated towards axis 2 with water temperature, pH and silicates. The dominant species in this station were Amphora, Navicula, Cymbella, Pinnularia, Surirella, and Gomphonema. In Kirubele, air temperature, water temperature, pH, salinity and DO had a significant effect on the species presence. In this station, the presence of 10 different diatom species were observed. Eigen values of axis 1, 2 and 3 for the station were λ=0.327, 0.138 and 0.026 respectively which together described 97.68% correlation. In Belekan, pH and nitrates on axis 1 had a significant effect on the presence of the diatom species. Eigen values of axes 1, 2 and 3 0.749, 0.332 and 0.139 with 97.09% correlation. Achnanthes was found to be the only dominant species in this region which is driven by salinity, nitrates and water temperature.

5. **Multi-criteria Hierarchical Cluster Analysis:** The multi-criteria hierarchical cluster analysis done on stations using the set of environmental parameters and the presence or the absence of the species in that particular stations gave the clear separation of stations based on their physical and biological parameters. Wards method was used to derive the hierarchical clusters. The results of the analyses is depicted in figure 2. Hegde and Divgi were categorized into one cluster which is upstream of the river, followed by Kagal and Bargi as another cluster and Kirubele and Belekan in yet another cluster with less distance between the clade which shows that the clusters are highly similar in their physical and biological parameters.

CONCLUSION

Substrata wise diatom community structure analysis showed a significant difference in the community structure with maximum diversity and species composition in Epipelons (Diatoms on sediments). Results also show that the Stations 2 (Hegde) and 4 (Bargi) are the hotspots of benthic diatom communities with higher species richness and diversity are the favorable spots for identification of diverse diatom communities. As these regions are easily accessible and also having a mix of centric as well as pennate species, these could be used as spots of diatom collection for isolation and culturing in order to explore the biofuel prospects.

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Table 1: Simple Linear Correlation of environmental variables with the CCA (p<0.05)

Variables	Divgi		Hegde		Kagal	
	Axis 1 (λ=0.664)	Axis 2 (λ=0.437)	Axis 1 (λ=0.159)	Axis 2 (λ=0.051)	Axis 1 (λ=0.226)	Axis 2 (λ=0.185)
AT	**-0.535**	**-0.627**	**-0.505**	-0.017	-0.126	0.05
WT	**-0.643**	-0.392	0.348	-0.044	0.326	0.030
pH	-0.0006	0.459	0.335	-0.431	-0.005	-0.397
Salinity	-0.114	0.491	-0.288	0.338	-0.489	**0.604**
DO	0.265	**0.626**	-0.112	**-0.617**	-0.278	-0.344
Nitrates	-0.095	0.420	-0.251	-0.272	-0.413	-0.261
Phosphates	0.221	0.011	-0.164	0.466	-0.219	**0.589**
Silicates	**-0.875**	-0.230	0.128	-0.258	0.145	**-0.503**

Table 2: Simple Linear Correlation of environmental variables with the CCA (p<0.05)

Variables	Bargi		Kirubele		Belekan	
	Axis 1 (λ=0.185)	Axis 2 (λ=0.175)	Axis 1 (λ=0.327)	Axis 2 (λ=0.138)	Axis 1 (λ=0.749)	Axis 2 (λ=0.332)
AT	-0.00038	-0.485	0.004	**0.616**	-0.007	**-0.812**
WT	-0.256	**-0.580**	0.241	**0.654**	0.229	**-0.705**
pH	0.462	**0.575**	**-0.521**	-0.245	**0.507**	0.385
Salinity	-0.083	-0.182	**-0.574**	0.246	0.447	-0.177
DO	0.444	-0.021	0.335	**-0.509**	-0.035	0.145
Nitrates	0.118	0.056	-0.299	0.207	**0.555**	-0.406
Phosphates	-0.432	0.334	-0.430	**-0.707**	0.119	0.487
Silicates	0.175	**0.864**	0.277	-0.107	0.471	0.399

Table 3. Diatom distribution across the stations with respect to substrate

Substrata	Diatom	Stations					
		1	2	3	4	5	6
	Class: Bacillariophyceae						
Sediments (Epipelons)	*Gomphonema sp.*		+				
	Diploneis subovalis		+				
	Encyonema prostratum		+				
	Pinnularia saprophila		+				
	Amphora sp.		+		+		
	Nitzschia apiculata		+				
	Navicula johnsonii		+				
	Nitzschia archibaldii		+				
	Stauroneis acuta		+		+		
	Nitzschia paradoxa		+				
	Navicula confervacea		+				
	Aulacoseira granulata		+				
	Aulacoseira ambigua				+		
	Cyclotella meghiniana		+		+		
	Navicula nobilis		+				
	Navicula cesatii						
	Navicula ventricosa		+				
	Navicula forcipata		+				
	Frustululia crassinervia		+				
	Pleurosigma sp.		+		+		
	Coscinodiscus subtilis				+		
	Navicula sp.				+		
	Melosira sp.				+		
	Achnanthes longipes		+		+		
	Cymbella sp.				+		
	Synedra ulna		+				
	Stephanodiscus hantzchianus				+		
	Nitzschia circularis		+				
	Surirella striatula				+		
	Surirella tenera		+				
Plants (Epiphytons)	*Diploneis subovalis*		+				
	Nitzschia obtusa		+				
	Melosira sp.		+				
	Nitzschia sp.		+				
	Cyclotella meghiniana		+		+		
	Amphora sp.		+				
	Navicula sp.		+				
	Cosinodiscus radiatus			+			
	Achnanthes tumescens				+		
	Pleurosigma sp.				+		
	Melosira distans				+		
	Melosira jurgensii				+		
	Nitzschia sp.	+	+		+		
	Fragilaria brevistriata					+	

Cobbles/ Boulders/shells (Epilithons)	*Achnanthes longipes*		+				
	Amphora sp.		+		+		
	Nitzschia constricta		+				
	Melosira sp.		+	+	+		
	Cyclotella meghiniana		+	+	+		
	Coscinodiscus subtilis		+	+			
	Synedra sp.	+			+		
	Nitzschia apiculata				+		
	Navicula spp.			+	+	+	
	Cymbella sp.			+	+		
	Surirella tenera			+			
	Synedra acus			+			
	Surirella striatula				+		
Sand (Episammons)	*Coscinodiscus radiatus*						+
	Diploneis subovalis						+
	Cyclotella sp.						+
	Navicula sp.						+

Table 4. Diversity indices of stations across all five months

Month	Stations	Species diversity (H')	Species Evenness (E)
July	1	0.565	0.815
	2	2.242	0.874
	3	2.069	0.898
	4	2.055	0.771
	5	1.853	0.843
	6	1.359	0.844
August	1	1.537	0.641
	2	1.360	0.480
	3	2.189	0.789
	4	2.201	0.794
	5	1.535	0.788
	6	1.774	0.853
September	1	2.052	0.891
	2	2.492	0.898
	3	1.439	0.600
	4	1.386	0.666
	5	1.917	0.872
	6	1.294	0.804
October	1	1.967	0.709
	2	1.476	0.510
	3	2.268	0.800
	4	2.300	0.811
	5	1.673	0.933
	6	1.928	0.877
November	1	2.126	0.828
	2	1.004	0.370
	3	2.007	0.837
	4	1.989	0.800
	5	1.656	0.796
	6	0.253	0.157

Fig 1: Canonical Correspondence Analysis Results of all six stations

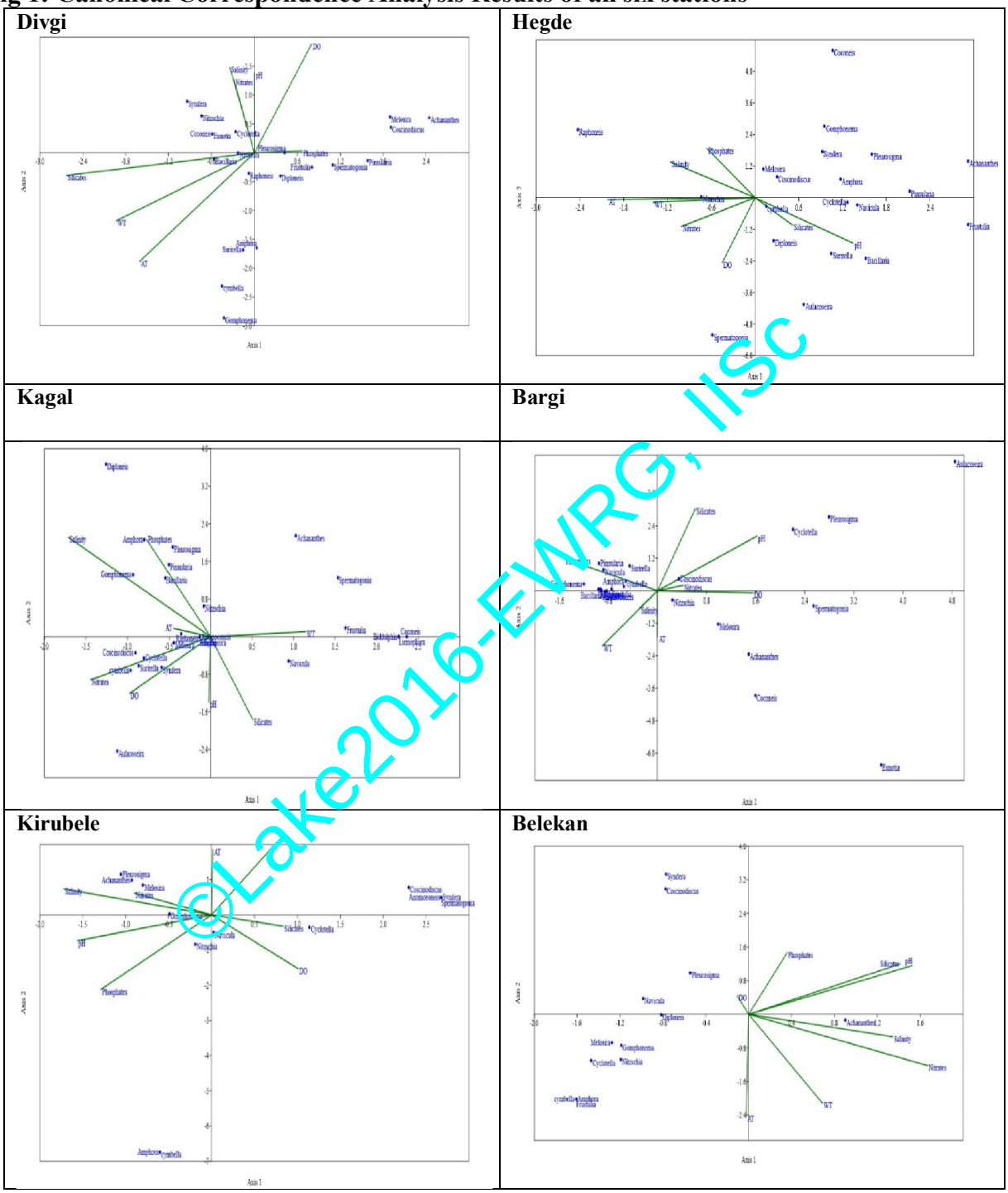

Fig 2. Multivariate Cluster Analysis