ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2016; 4(1): 361-369

www.fisheriesjournal.com
Received: 29-01-2016
Accepted: 02-03-2016

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Effect of substrate type on the response of diatoms to environmental gradients: Implication for bioassessment and biomonitoring programs

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Abstract
The distribution of epipelic, epilithic and epiphytic diatom communities in relation to the environmental variables was studied in River Nile, Rosetta and Damietta branches to assess the best substrate type most correlate with these measured environmental variables. Chemical and biological characters were studied at 40 locations during autumn-2013 and spring-2014. Eleven environmental variables; temperature, pH, electrical conductivity, total dissolved salts, Nitrogen-Nitrate, Nitrogen-Ammonium, Nitrogen-Nitrite, soluble reactive phosphorus, total phosphorus, reactive silicate and biochemical oxygen demand were measured and used for assessing the diatom-environmental relationship. The sites especially those of Rosetta were characterized by high nutrient concentrations as well as total dissolved salts (TDS) and electrical conductivity (EC) compared to the main stem of the river. A total of 224 diatom species belonging to 26 genera were recorded. Out of them, 61 species were considered as the most frequent species. Common diatom species relatively varied among different substrates and were not exclusively found upon a specific substrate. Diversity and evenness had higher values at the southern sites with partial and non-significant variations, respectively, among substrates which slightly decreased northward. However, the diversity average was slightly higher in epilithic than epiphytic and epipelic communities. Three Canonical Correspondence Analysis (CCA) related epipelic, epilithic and epiphytic diatom community structures to simultaneous effect of predictor environmental variables. CCA analyses explained 51%, 43% and 39% of the diatom species variance in epipelic, epilithic and epiphytic communities, respectively, the mantel tests enforced these results. These relationships mirrored that epipelic diatom communities in River Nile and its branches were the most related communities to the environmental variables and are the best fit for the monitoring program.

Keywords: River Nile; Rosetta Branch; Damietta Branch; diatom communities; environmental variables; Canonical Correspondence Analysis.

1. Introduction
The periphyton community, especially diatoms, has been recognized as powerful indicators of stream health/biotic integrity [1]. This could be established through their variation in composition and relative abundance [2, 3]. On the large spatial scale, community structure of the attached diatoms in freshwater systems is controlled by human derived factors especially those resulted in eutrophication. The effect of nutrient on the limitation of periphyton was previously discussed in other studies and the phosphorus, nitrogen and silicon were the most influential factors [1]. At the small spatial scale of the sample site, substrate is another potential source of diatom assemblage heterogeneity. Substrate type has an impact on nutrient content or biomass of benthic algae. Thus, differences in substrates can potentially confound responses of diatom assemblages to stress associated with human activities [4]. Benthic diatoms are present on almost all stable substrata; for example, rocks (epilithon), sand (epipsammic), woody debris (epidendrum), sediment (epipelon), aquatic vegetation (epiphyton) and dead leaves [5]. However, the effect of substrate on diatom-based water quality assessment in River Nile is not fully estimated. Even though streams may provide more than one substrate type, monitoring programs that provide information about river health often standardize benthic diatom collection to one substrate to minimize substrate influence on diatom heterogeneity [5]. Epilithic diatoms are the favored community for monitoring water quality [5]. And almost all methods based on diatom indices concentrate on this community [6]. Epilithic diatoms are least dependent on the substrate as a nutrient source and this type of substrate often supports a diverse diatom flora [7].

Epilithic diatoms are not recommended in other studies due to many difficulties in their sampling [8]. On the other side, epiphytic diatom assemblages or artificial substrates was objected, while the use of epipelic community was the most recommended [8]. Other investigations found no apparent benefit to sampling discrete habitats for water quality monitoring using diatoms where no clear evidence of substrate specific diatoms [9, 10]. However, the best assessment program was recommended using diatom assemblages from different habitats [9]. The main purpose of this study was to identify the major environmental correlates of diatom distribution patterns in the Nile and which type of attached diatom communities was the most correlate to environmental gradient and are recommended as the best contributor for monitoring and water quality assessment program.

2. Materials and Methods

2.1. Area of study

The geographical and hydrological features of River Nile have been exhaustively presented by many authors [11-13]. The total area of Nile basin is about 2.9 million km^2 and covers a wide range of climatic zones. The river extends from latitude 4°S to latitude 31°N [12]. Our study covered the stretch of River Nile beginning at Upper Egypt from Aswan Old Dam northward to Cairo and its two main branches, Rosetta (the western) and Damietta (the eastern) branches (Fig. 1). From Aswan High Dam, a distance of about 910km, Nile flows northward in a relatively narrow flat-bottomed groove until reaching Cairo. This region belongs to the arid region of North Africa which is generally characterized by hot summer and fresher cold winter with low rainfall [14]. North to Cairo, River Nile enters triangular lowland delta. This triangular cultivated delta region is enclosed by Rosetta and Damietta branches. Land use categories are varied along the River Nile including agriculture, settlement, urbanization, recreation, fishing, tourism, and transportation. Settlement activities made a great impact on river stability due to disturbance on river system such as building dams, gravel bars, fishing, navigation facilities, and revetment of the river banks.

2.2 Sampling

A total of 40 sites distributed along River Nile and its branches (Table 1 and Fig. 1) were sampled during autumn-2013 and spring-2014; these seasons are favoring diatom growth as has previously been defined [13]. Samples for chemical and biological measurements were collected and the measurements of water temperature, pH, electric conductivity, dissolved oxygen and total dissolved salts were recorded in situ. Attached diatoms upon three different substrates (epipelic, epiphytic and epilithic) were collected at the selected sites by sampling of sediment, submerged plants and boulders or cobbles. Sampling of epiphytic and epilithic communities was depending on presence of submerged macrophytes and hard substrates, boulders or cobbles and also water level where the sampling of these substrates were not available in some cases specially during autumn due to low water level. Surface sediment was collected using Ekman grab; sampling was repeated until undisturbed samples were collected. Epiphytic diatom communities were collected by cutting three undisturbed bundles of submerged macrophytes using scissors into a bucket filled with filtered river

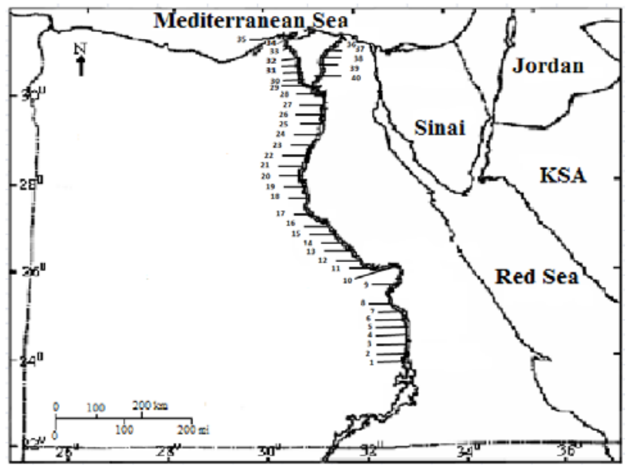

Fig 1: River Nile map showing the sampling sites

Water and then macrophytes were shacked vigorously in a plastic bottle until all attached communities were de-attached. For epilithic diatoms, exposed surfaces of five boulders or cobbles were sampled by vigorous brush with a toothbrush (thoroughly washed between samples) to remove all obvious surface films of algae. As far as possible, macrophytes and hard substrates free of filamentous algae and obvious siltation were selected. A known area of surface sediment was put into a small glass bottle as well as sub-samples of collected epiphytic and epilithic communities, followed by the addition of a few drops of 4% formaldehyde.

Table 1: The forty sampled sites of River Nile and Rosetta and Damietta branches with their distance (Km) from Aswan Old Dam (AOD) and the potential pollution source

St. No.	Distance	Pollution Source, if present	St. No.	Distance	Pollution Source, if present
1*	0.5		21	740	
2	3.8	Kema Factory for Chemical Industries	22	786	
3	28		23	810	
4	52	Egyptian Sugar Company and Integrated Industries, Kom Ombo, Aswan	24	845	
5	78	Agriculture Drain	25	878	Ghamaza Drain of Industrial wastes
6	116	1- Edfu Ferrosilicon Factory 2- Egyptian Sugar Company and Integrated Industries, Edfu, Aswan	26	892	
7	152		27*	935	
8	200	Luxor Tourist Port	28	942	
9	230	1-Qena Company Newsprint Paper 2-Egyptian Sugar Company and Integrated Industries, Qus, Qena	29	964	El Rahawy Drain of Sewage wastes
10	258		30	982	
11	310	Egyptian Sugar Company and Integrated Industries, Dishna, Qena	31	1010	
12	377		32	1035	
13	410		33*	1060	Salt and soda Egyptian company and Alexandria Company for oils and soaps

14	465		34	1105	
15	510		35	1143	
16	550		36	1138	
17*	590		37*	1097	Kafr Saad Electric Power Station
18	625		38	1052	Talkha Electric Power Station
19	660		39	1015	
20	700	El-Minia Drain of Swage and Industrial wastes	40	975	

*; stations selected for heavy metals measurement

2.3 Chemical analysis

Water samples were collected in 2 L polyethylene bottles and kept in ice box till analyzed. Dissolved oxygen (DO, mg L^{-1}) was measured by using the modified Winkler method [15]. Biochemical oxygen demand (BOD, mg L^{-1}) was determined by using the 5 day method. Concentrations of nitrite, nitrate, ammonium, orthophosphate (µg L^{-1}), reactive silicate (mg L^{-1}) were determined using colorimetric techniques with the formation of reddish purple azo-dye, copper-hydrazine sulfate reduction, phenate, ascorbic acid molybdate and molybdosilicate methods, respectively. Nitrite, nitrate, ammonium, orthophosphate were measured few hours after collection. Total phosphorous (TP, µg L^{-1}) was measured as reactive phosphate after persulfate digestion. Concentrations of Fe, Cu, Co, Ni and Pb (mg L^{-1}) were measured after digestion with a purified nitric acid using atomic absorption model Savantaa ASS with GF 5000 graphite furnace [15]. Heavy metals were measured in spring at five selected sites from Nile main stem, Rosetta and Damietta branches as shown in Table (1).

2.4 Diatom laboratory processing

Cleaning and digestion of the diatom samples were occurred using concentrated sulfuric and nitric acids [16]. Preparation of permanent slides using a high refractive index medium (Naphrax) was performed [16]. Diatom identification and counting were achieved along random transect using a light microscope (Zeiss, Model Axiovert 25C) at a 40× magnification and 100x if needed. From each permanent slide, at least 400 valves were enumerated and all valves had taken into account. Identifications were made at the species and variety level [17-19].

2.5 Statistical analysis

Variance in chemical parameters between different regions of Nile main stem and its branches as well as between seasons were tested with one-way ANOVA and carried out using SPSS 20.0 package. Diversity and evenness were calculated using primer software version 5 [20]. Species and samples were grouped using TWINSPAN (Two-Way Indicator Species Analysis), [21] using the relative abundance matrix for the abundant and dominant species and the classified categories matrix according to TWINSPAN analysis of the indicator species was carried out. Canonical Correspondence Analysis (CCA), a direct gradient multivariate analysis, is widely used. Taxa and samples can be directly related to measured environmental variables. CCA was carried out with Canoco for Windows version 4.5 [22] for 61 taxa (Table 5) with relative abundance ≥ 0.5%. CCA biplots were drawn with Cano Draw for Windows. The environmental variables were submitted to a stepwise forward selection procedure in which the statistical significance of each variable was tested by the Monte Carlo permutation test (499 permutations) at a cut-off point of P = 0.05. Mantel test was used to assess the significance of overall "community-environment" relations among each diatom assemblage (epipelic, epilithic and epiphytic) and all measured environmental variables, the test was carried out by xlstate 2014 v 5.

3. Results

3.1. Chemical properties of selected sites

Most chemical variables showed significant variation between seasons (P<0.01), except for NH_4, TP, SiO_4 and pH (Table 2). pH values in the sampling seasons were decreased along branches especially Rosetta branch compared to the main stem. pH values were always slightly alkaline; ranged between 7.15 at stations 2 to 8.5 at station 17 during autumn. The highest temperature (31.5 °C) was recorded at station 35 "the northern station of Rosetta branch" during spring while the least (15.1°C) was measured during autumn at station 29 "the southern station of Rosetta branch". TDS and EC showed gradual increase northward till the most northern site of Rosetta compared with a slight decrease in Damietta Branch (Table 2). Both TDS and EC showed high significant difference between Nile regions and its branches, and the variation was more significant during autumn (P<0.0001).

Most sites of Nile Branches, especially those of Rosetta, were characterized by high nutrients (NO_2, NO_3, NH_4, PO_4 and TP) than other sites of the main stem of Nile with values indicating to eutrophic state (Table 2). Nitrogenous salts concentration showed significant variations (P<0.0001) between Nile main stem and River Branches especially in spring. The highest values of different nitrogen ions were recorded in Rosetta branch during spring (Table 2). TP values increased northward until reached its maximum (1586.3 µgL^{-1}) at station 33 during autumn in Rosetta branch. The highest silicate concentrations (2.02 mgL^{-1}) were detected at the most southern sites of the Nile then gradually decreased northward. Biological Oxygen Demand (BOD) values were highest in the southern region of Nile main stem and Damietta Branch but least in Rosetta Branch in both seasons. Variations in BOD were more significant in autumn (P<0.0005) than in spring (P<0.005).

Table 2. Seasonal distribution of chemical characters at the selected stations in River Nile and its branches

St.	Distance	NO_2 (µgL^{-1})		NO_3 (µgL^{-1})		NH_4 (µgL^{-1})		PO_4 (µgL^{-1})		TP (µgL^{-1})		SiO_4 (mgL^{-1})		BOD (mgL^{-1})		TDS (mgL^{-1})	EC (µScm^{-1})			pH	
		Aut	**Sp**	**Aut**	**Sp**	**Aut**	**Sp**	**Aut**	**Sp**	**Aut**	**Sp**	**Aut**	**Sp**	**Aut**	**Sp**	**Aut**	**Sp**	**Aut**	**Sp**	**Aut**	**Sp**
1	0.5	1.4	9.5	0.0	134.0	299.2	13.3	553.1	57.1	0.0	174.5	0.0	1.7	4.1	1.2	123.4	117.2	199.1	189.1	7.5	7.7
2	3.8	1.4	15.7	13.3	119.4	1194.4	443.4	330.0	114.1	463.1	293.5	1.8	1.6	0.0	0.8	194.0	132.1	315.0	213.0	7.2	7.7
3	28	4.1	11.0	9.3	144.6	168.0	17.7	241.9	60.3	324.4	244.6	1.6	1.6	3.3	2.8	126.1	124.8	203.0	201.0	7.6	8.0
4	52	0.0	24.8	0.0	200.3	0.0	15.9	0.0	60.3	0.0	216.8	0.0	2.0	0.0	2.9	0.0	132.7	0.0	214.0	0.0	7.8
5	78	0.5	12.4	17.2	131.3	32.0	9.7	101.3	58.7	157.5	202.2	1.6	1.9	4.6	3.1	142.1	130.6	229.0	211.0	7.8	7.9
6	116	0.0	6.2	34.5	615.5	39.2	46.9	135.0	141.8	219.4	415.8	1.3	1.7	3.3	2.6	141.0	131.8	227.0	213.0	7.8	7.9
7	152	2.3	5.7	17.2	563.8	26.4	16.8	86.3	44.0	157.5	0.0	1.5	1.6	4.1	2.8	142.1	130.6	229.0	211.0	7.6	7.7
8	200	1.8	6.2	14.6	665.9	138.4	37.2	232.5	62.0	230.6	0.0	1.4	1.6	6.0	3.0	144.3	132.2	233.0	213.0	7.9	8.0
9	230	5.0	10.0	13.3	557.1	24.8	53.1	73.1	32.6	155.6	0.0	1.5	1.6	5.4	3.5	146.4	135.7	236.0	219.0	8.2	8.0
10	258	8.2	7.6	27.9	602.2	160.8	43.4	288.8	24.5	375.0	375.0	1.4	1.3	4.4	3.1	153.1	140.7	247.0	227.0	7.6	7.5
11	310	7.3	8.1	15.9	585.0	29.6	34.5	93.8	538.0	189.4	285.3	1.1	1.6	4.9	2.2	154.8	136.4	250.0	220.0	7.8	7.8
12	377	12.7	8.6	8.0	436.4	26.4	13.3	73.1	45.7	161.3	484.2	1.2	1.5	6.7	3.2	155.7	140.4	251.0	226.0	8.2	7.9
13	410	7.7	11.4	13.3	401.9	26.4	26.5	88.1	48.9	140.6	882.1	1.3	1.4	7.0	1.6	154.0	145	248.0	234.0	8.4	8.1
14	465	0.0	9.0	0.0	376.7	0.0	22.1	0.0	50.5	0.0	0.0	0.0	1.4	0.0	0.0	0.0	138.7	0.0	224.0	0.0	8.1
15	510	7.3	11.9	15.9	358.2	131.2	46.9	360.0	114.1	530.6	539.7	1.3	1.4	5.9	1.5	152.4	143.3	246.0	231.0	8.4	8.4
16	550	5.5	10.0	0.0	156.5	13.6	47.8	67.5	29.3	0.0	406.0	0.0	1.4	4.9	0.0	149.1	145.5	240.0	235.0	8.5	8.3
17	590	19.5	9.0	9.3	132.7	81.6	23.9	451.9	60.3	525.0	577.2	1.1	1.4	4.9	2.3	148.4	144.8	239.0	234.0	8.5	8.2
18	625	5.0	5.2	13.3	74.3	21.6	27.4	138.8	52.2	309.4	365.2	1.2	1.0	6.6	0.0	154.9	144	250.0	232.0	8.4	8.2
19	660	2.7	2.4	6.6	49.1	75.2	26.5	163.1	66.8	375.0	472.8	1.0	1.0	4.5	2.3	154.1	144.4	249.0	233.0	8.4	8.0
20	700	5.0	2.9	9.3	63.7	274.4	28.3	217.5	92.9	866.3	673.4	1.0	0.9	5.1	3.0	155.8	145.6	251.0	235.0	8.5	8.1
21	740	5.9	4.3	10.6	41.1	102.4	78.8	256.9	62.0	1070.6	331.0	1.0	1.0	4.8	2.9	160.7	146.1	259.0	236.0	8.4	8.2
22	786	5.9	4.3	13.3	46.4	284.8	43.4	251.3	70.1	873.8	441.8	1.0	1.0	4.3	0.9	169.0	152.7	273.0	246.0	8.3	8.3
23	810	13.2	22.9	18.6	340.9	20.0	85.8	97.5	78.3	125.6	438.6	1.4	0.9	4.9	0.4	181.1	153.4	292.0	247.0	8.3	8.3
24	845	1.4	23.3	29.2	58.4	316.8	101.8	131.3	88.0	172.5	433.7	1.1	0.9	3.1	1.0	197.9	157.2	319.0	254.0	8.1	8.3
25	878	10.9	16.7	42.4	141.9	96.8	28.3	97.5	65.2	650.6	450.0	0.7	1.0	3.3	1.6	342.0	167	552.0	269.0	8.1	8.2
26	892	4.1	0.0	18.6	27.9	182.4	27.4	131.3	70.1	476.3	474.5	0.5	0.9	1.0	0.7	231.0	267	373.0	430.0	8.1	7.7
27	935	0.0	1.9	17.2	67.7	286.4	46.9	1102.5	83.2	1293.8	331.0	0.6	0.7	3.2	2.2	227.0	239	367.0	386.0	8.0	8.1
28	942	0.9	1.4	15.9	29.2	48.8	87.6	183.8	334.2	343.1	477.7	0.6	0.6	0.0	1.5	225.0	167.1	363.0	269.0	8.2	8.1
29	964	5.9	15.2	23.9	57.0	227.2	1965.5	378.8	409.2	1494.4	1581.5	0.6	1.5	3.2	1.0	228.0	157.8	368.0	254.0	7.9	6.6
30	982	6.4	73.8	27.9	80.9	1669.6	988.5	658.1	117.4	1464.4	891.8	1.1	0.6	0.3	2.1	409.0	149	660.0	240.0	7.8	7.4
31	1010	6.8	229.0	21.2	1008.2	1496.0	325.7	658.1	153.3	928.1	639.1	0.7	0.4	0.6	0.0	363.0	305	586.0	500.0	7.5	7.1
32	1035	23.2	270.0	27.9	1374.3	1161.6	575.2	305.6	140.2	753.8	823.4	1.0	0.3	0.5	0.0	366.0	195.4	591.0	315.0	7.6	7.1
33	1060	25.5	283.8	19.9	795.9	1561.6	478.8	665.6	120.7	1586.3	1069.6	1.3	0.3	0.5	0.8	430.0	263	694.0	425.0	7.6	6.8
34	1105	11.4	332.4	58.4	748.2	1667.2	406.2	1035.0	195.7	1147.5	714.1	1.0	0.4	0.6	1.5	427.0	208	689.0	336.0	7.5	6.9
35	1143	64.5	3.3	47.8	34.5	1515.2	51.3	1005.0	75.0	1267.5	679.9	1.3	0.4	1.7	0.3	410.0	259	661.0	418.0	7.7	7.3
36	1138	13.6	14.3	306.4	50.4	173.6	35.4	120.0	92.9	187.5	513.6	0.8	0.3	3.6	2.4	235.0	216	380.0	348.0	7.5	7.4
37	1097	26.4	17.1	31.8	153.9	191.2	77.9	345.0	91.3	549.4	562.5	0.6	0.5	5.0	3.3	236.0	179.8	381.0	290.0	7.4	8.1
38	1052	15.9	1.4	39.8	25.2	286.4	43.4	435.0	101.1	680.6	472.8	0.6	0.7	4.0	2.2	273.0	171	440.0	276.0	7.7	7.8
39	1015	4.1	1.4	18.6	27.9	12.8	59.3	67.5	34.2	204.4	489.1	0.6	0.7	6.2	2.2	221.0	175.4	356.0	283.0	8.0	7.6
40	975	5.0	0.0	22.6	0.0	9.6	6501.7	61.9	0.0	97.5	0.0	0.7	0.0	5.4	1.6	222.0	151.1	358.0	244.0	8.3	7.6

Aut; Autumn, Sp; Spring

3.2 Heavy metal measurements

The values obtained from the measuring of the heavy metals and numbers of selected sites were represented in Table (3). Cu was under the detected level throughout the area of study, whereas Fe had highest values in Damietta Branch (5.19 mgL^{-1}) and middle region of the main stem (3.391 mgL^{-1}). Metals of Ni, Co and Pb ranged between undetected values to highest values of 0.079, 0.022 and 0.16 at north of the main stem, middle of the main stem and Damietta Branch, respectively.

Table 3: the five measured heavy metals concentrations (mgL^{-1}) at the selected five sites during spring

Sample No.	Stations	Fe	Ni	Co	Cu	Pb
1	st 1	1.28	0.017	<0.005	<0.002	0.148
2	st 17	3.391	<0.005	0.022	<0.002	0.04
3	st 27	2.309	0.079	<0.005	<0.002	<0.005
4	st 33	1.611	<0.005	0.008	<0.002	<0.005
5	st 37	5.192	<0.005	0.016	<0.002	0.16

3.3 Diatom community structure

A total of 224 diatom species belonging to 26 genera were recorded. Out of them, 61 species were the most frequent in the study area (≥0.5% occurrence). Common diatom species were not exclusively found upon a specific substrate, although their relative abundances varied between substrates and sites. Among the cosmopolitan species present in these habitats, *Achnanthes lanceolata, A. minutissma, Cocconeis placentula, Cyclotella meneghiniana, C. ocellata, C. operculata, Cymbella microcephala, Fragilaria construens, Gomphonema minutum, Nitzschia amphibia, N. leistikowi* and, *N. palea* were the most present. The epipelic habitats supported species of both *Cyclotella* and *Fragilaria*.

The epipelic community was dominated by *C. ocellata* followed by *F. construens* at all sampling sites. *A. minutissma* and *F. construens* were considerably present except in Rosetta branch. C. *operculata* was abundant in the northern region of the Nile and its branches but it was replaced by *C. placentula* in the southern region of the main stem of the Nile. The dominant species of the epilithic community was clearly differentiated. *A. minutissma*, *C. affinis*, *C. ocellata* then *C. microcephala* dominated the southern sites. The middle and north region of Nile showed the dominance *C. ocellata*, *F. construens* and *A. minutissma* followed by *G. minutum* in the middle and *C. microcephala* in the northern region. Epilithic communities of Rosetta branch was highly dominated by *C. meneghiniana* followed by *N. amphibia* and *N. palea,* while Damietta branch was dominated by *F. construens* followed by *N. amphibia*. The epiphytic communities were dominated by *A. minutissma, C. ocellata, C. placentula* then *C. placentula var. euglypta* at the southern and middle sites of Nile, whereas *C. ocellata* and *F. construens* dominated the northern sites. Rosetta branch was dominated by *Cyclotella* spp while Damietta branch was dominated by *C. ocellata* followed by *F. construens*.

3.4 Diatom diversity and evenness

There is no such a clear pattern in the diversity distribution along the area of study. Even though, a partial significant difference between diversity on different substrates (P<0.06) was observed, diversity average was slightly higher in epilithic than epiphytic and epipelic communities. During spring, the epipelic samples showed species diversity with a minimum of 2.37 at station 38 and a maximum of 3.77 at station 28. The epilithic diversity ranged between 1.96 at station 2 and 4.08 at station 9. The epiphytic diversity ranged from 1.76 at station 34 to 4.4 at station 36. Damietta branch harbored the highest diversity values between sites with an exception at station 40.

Difference between sites was not significant in both Nile main stem and its branches (mean P = 0.14). The most southern sites characterized by high evenness values which slightly decreased upward till the north of the main stem. Evenness of epipelic diatoms ranged from 0.54 at station 23 to 0.74 at station 28. Evenness of epilithic diatoms ranged from 0.43 at station 2 to 0.78 at station 37. The epiphytic evenness ranged from 0.38 at station 34 to 0.86 at station 35.

3.5 Twinspan (Two-Way Indicator Species Analysis)

The results of the TWINSPAN analysis of the sample dataset incorporated twelve levels of classification with 66 groups (Table 4 and Fig. 2). TWINSPAN analyses resulted in 32 main groups assigned (A)-(Af), and 34 final groups assigned with number from 1 to 34. Among the final groups, 16 groups contain diatoms samples from exclusively one substrate type and 18 groups contain diatoms samples of more than one substrate type. Some of the final groups were distinguished depending on regions where close sites were clustered together. At the fourth level, fourteen groups were distinguished. Groups P, R, W and Y (with indicator species: *A. lanceolata*, *C. turgidula*, *G. tackei* and *C. meneghiniana,* respectively) were further separated. Group (P) was the biggest one (comprised 40 sites) with *C. operculata*, *C. meneghiniana*, *F. leptosturon* var. *obliqua* and *A. minutissma* as indicator species. Group (P) was distinguished to sixteen final groups through eight levels.

Table 4: Twinspan final groups of River Nile, Rosetta and Damietta branches sites

groups	Sites included	indicator species
1	St 35 Phyt, St 36 Lith, St 36 Phyt	*Gomphonema truncatum*
2	St 35 Lith	*Amphora fogediana*
3	St 6 Phyt	*Achnanthes suspecta*
4	St 2 Lith	*Achnanthes minutissma*
5	St 1 Pel, St 1 Lith, St 17 Pel, St 19 Phyt	*Cymbella caespitosa*
6	St 20 Lith, St 23/ Phyt, St 26 Phyt	*Cymbella caespitosa*
7	St 4 Phyt, St 6 Lith, St 6 Lith	*Cymbella microcephala*
8	St 3 Phyt, St 15 Phyt	*Cocconeis placentula var. lineata*
9	St 1 Phyt, St 3 Lith, St 4 Lith	*Cocconeis placentula var. euglypta*
10	St 12 Lith, St 17 Lith	*Cocconeis placentula var. euglypta*
11	St 32 Lith, St 32 Phyt, St 33 Phyt, St 34 Lith	*Achnanthes lanceolata*
12	St 34 Phyt	*Achnanthes lanceolata*
13	St 8 Pel, St 28 Pel	*Cymbella turgidula*
14	St 2 Pel, St 11 Pel, St 12 Phyt, St 16 Pel, St 37 Pel	*Achnanthes lanceolata*

15	St 10 Pel, St 15 Lith, St 16 Lith	*Gomphonema tackei*
16	St 9 Lith, St 14 Lith, St 21 Lith	*Gomphonema tackei*
17	St 8 Phyt, St 13 Phyt	*Cyclotella meneghiniana*
18	St 5 Lith, St 5 Phyt, St 10 Lith, St 11 Phyt	*Cyclotella meneghiniana*
19	St 3 pel, St 6 Pel, St 12 Pel, St 15 Pel, St 15/ Pel, St 21 Pel	*Nitzschia inconspicua, Amphora inariensis, Achnanthes lanceolata var. rostrate*
20	St 2 phyt	*Cocconeis placentula var. lineata*
21	St 18 Pel, St 30 Pel, St 34 Pel, St 40 Phyt	*Fragilaria construens var. pusilla*
22	St 37 Lith	*Amphora venta*
23	St 29 Phyt, St 31 Phyt, St 32 Pel	*Cymbella lepoceros*
24	St 22 Pel, St 29 Pel, St 39 Phyt	*Cymbella leptoceros*
25	St 22 Lith, St 37 Phyt	*Navicula viridula*
26	St 28 Lith, St 28 Phyt	*Nitzschia obtusa*
27	St 14 Pel, St 18 Lith, St 19 Lith	*Diploneis oblengella*
28	St 19/ Lith, St 39 Lith	*Diploneis oblengella*
29	St 23 Phyt	*Bacillaria paradoxa*
30	St 25 Lith, St 27 Pel, St 27 Phyt	*Gomphonema gracile*
31	St 25 Phyt, St 39 Pel	*Gomphonema gracile*
32	St 23/ Pel	*Achnanthes lanceolata*
33	St 19 Pel, St 20 Phyt, St 25 Pel, St 38 Pel	*Navicula kriegerii*
34	St 19/ Pel, St 23 Pel	*Navicula kriegerii*

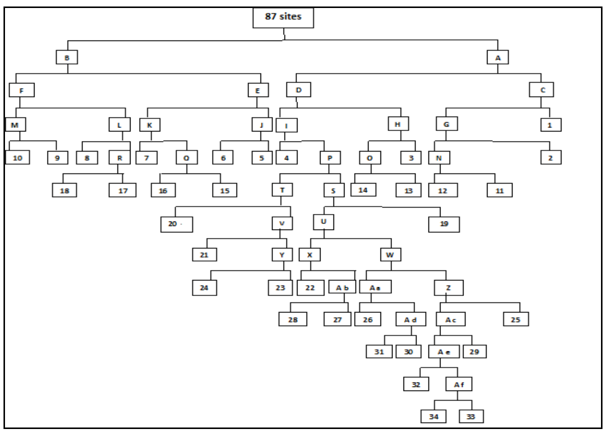

Fig 2: Twinspan analysis groups of River Nile, Rosetta and Damietta branches sites

Twinpan analysis gathered the epipelic communities in 13, 19, 32 and 34 final groups with *C. turgidula, N. inconspicua, Amphora inariensis, A. lanceolata var. rostrata, A. lanceolata and Navicula kriegerii,* as indicator species. The epilithic samples were clustered in 2, 4, 10, 16, 22 and 28 final groups with *Amphora fogediana*, *A. minutissma*, *C. placentula* var. *euglypta*, *G. tackei*, *A. veneta*, *Diploneis oblengella* as indicator species. The epiphytic samples were separated in groups 3, 8, 12, 17, 20 and 29 with *Achnanthes suspecta*, *C. placentula* var. *lineata*, *A. lanceolata*, *Cyclotella meneghiniana*, *Bacillaria paradoxa* as indicator species.

3.6 Canonical Correspondence Analyses (CCA) and Mantel test

In these CCAs, sampling sites including species were associated to different environmental variables. Three CCAs were performed to relate different diatom communities' structure, upon different substrates, to simultaneous effects of predictor environmental variables. These environmental variables through the CCAs explained 51%, 43% and 39% of the spatial and temporal variation in epipelic, epilithic and epiphytic communities, respectively, with total variation in the data set of 2.31, 2.65 and 3.28, respectively.

Based on CCA, epipelic diatom communities (Fig.3) were more associated with ammonium, silicate and orthophosphate (P<0.05), which together accounted for 81.8% of their total variations. Epilithic diatom communities (Fig.4) were highly associated with ammonium, silicate and nitrite, which together accounted for 60.8% of total epilithic variations. On the other side, epiphytic communities (Fig.5) were more associated with nitrite, nitrate and silicate, which together accounted for 60% of total epiphytic variations. Mantel test results indicated that epipelic diatom assemblages were significantly related to eutrophication (r_m= 0.48; P<0.05). The correlation amongst epilithic and eutrophication was weaker but highly significant (r_m = 0.26; P<0.005) whilst for epiphytic and eutrophication was very weak and partially significant (r_m= 0.18; P<0.07).

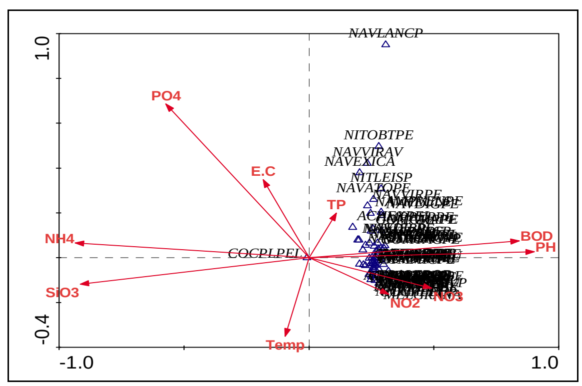

Fig 3: CCA of epipelic diatom tax and different environmental variables

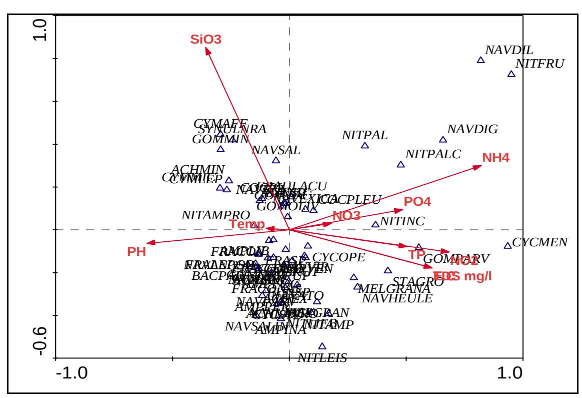

Fig 4: CCA of epilithic diatom taxa and different environmental variables

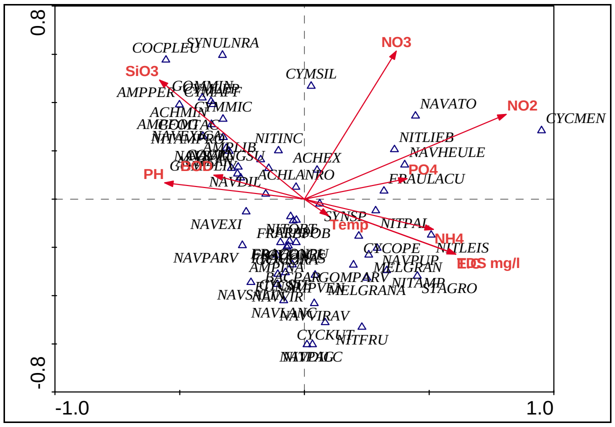

Fig 5: CCA of epiphytic diatom taxa and different environmental variables

Table 5: Sixty one taxa with relative abundance ≥ 0.5% identified in samples from Nile main basin and Rosetta and Damietta branches with their coded name used in CCA.

No.	Coded name	species name
1	Achexpel	Achnanthes exigua Grunow
2	Achlanpel	Achnanthes lanceolata (Brébisson) Grunow
3	Achlanrospel	Achnanthes lanceolata var. rostrata (Oestr.) Lange-Bertalot
4	Achminpel	Achnanthes minutissma Kutz.
5	Ampfogpel	Amphora fogediana Krammer
6	Ampinapel	Amphora inariensis Krammer
7	Amplibpel	Amphora libyca Ehrenberg
8	Ampperpel	Amphora perpusilla Grunow
9	Ampvenpel	Amphora venta Kutz.
10	Bacparpel	Bacillaria paradoxa Gmelin
11	Cocplpel	Cocconeis placentula Ehrenberg
12	Cocpleupel	Cocconeis placentula var. euglypta Ehrenberg
13	Cyckutpel	Cyclotella kutzingiana Thwaites
14	Cycmenpel	Cyclotella meneghiniana Kutz.
15	Cycocepel	Cyclotella ocellata Pantocsek
16	Cycopepel	Cyclotella operculata (Ag.) Kutz. Hust. Kies
17	Cycstepel	Cyclotella stelligera Cleve & Grunow
18	Cymaffpel	Cymbella affinis Kutz.
19	Cymleppel	Cymbella lepoceros (Ehrenberg) Kutz.
20	Cymmicpel	Cymbella microcephala Grunow
21	Cymsilpel	Cymbella silesiaca Bleisch
22	Eunsppel	Eunotia sp
23	Fraconpel	Fragilaria construens (Ehrenberg) Grunow
24	Fraconaspel	Fragilaria construens var. asymetrica A. Cl.
25	Fraconpuspel	Fragilaria construens var. pusilla Grunow
26	Fralepoblpel	Fragilaria leptosturon var. obliqua May.
27	Frasppel	Fragilaria sp
28	Fraulacupel	Fragilaria ulna var. acus (Kutz.) Lange-Bertalot
29	Gomgrapel	Gomphonema gracile Ehrenberg
30	Gomminpel	Gomphonema minutum (C. Agardh)
31	Gomolivpel	Gomphonema olivaceum (Hornem.) Brébisson
32	Gomparvpel	Gomphonema parvulum Kutz.
33	Gomtacpel	Gomphonema tackei Hustedt
34	Melgranpel	Aulacoseira granulata (Ehrenberg) Simonsen
35	Melgranangpel	Aulacoseira granulata var. angustissima (O. Muller)
36	Navangsubpel	Navicula anglica var. subsalsa Grunow
37	Navatopel	Navicula atomus (Kutz.) Grunow
38	Navdigpel	Navicula digitoradiata (Gregory) Ralfs
39	Navdilpel	Navicula diluviana Krasske
40	Navexipel	Navicula exigua (Gregory)
41	Navexicappel	Navicula exigua var. capitata Patrick
42	Navheuleppel	Navicula heufleri var. leptocephala (Brebisson) Peragallo
43	Navlancpel	Navicula lanceolata Ehrenberg
44	Navparvpel	Navicula parva Ehrenberg
45	Navpuppel	Navicula pupula Kutz.
46	Navsalpel	Navicula salinarum Grunow
47	Navsalintpel	Navicula salinarum var. intermedia Grunow
48	Navvirpel	Navicula viridula Ehrenberg
49	Navviravepel	Navicula viridula var. avenacea Brébisson
50	Nitamppel	Nitzschia amphibia Grunow
51	Nitamprospel	Nitzschia amphibia var. rostrata Hustedt
52	Nitfrupel	Nitzschia frustulum Kutz.
53	Nitincpel	Nitzschia inconspicua Grunow
54	Nitleispel	Nitzschia leistikowii Lange-Bertalot
55	Nitliebpel	Nitzschia liebetruthii Rabenhorst
56	Nitobtpel	Nitzschia obtusa W. Smith
57	Nitpalpel	Nitzschia palea (Kutz.) W. Smith
58	Nitpalcpel	Nitzschia paleacea Grunow
59	Stagropel	Stauroneis groenlandica var. subquadrat
60	Synsppel	Synedra sp

4. Discussion

When comparing diatom, macrophyte, macroinvertebrate and fish metrics as indicators of environmental gradients, diatom metrics most strongly correlated with measures of eutrophication [23] and are more powerful indicators in accessing ecological stream/river quality and have potential for application in routine monitoring programs [24]. Unlikely, greet debate concerning effect of substrate type on response of diatoms to eutrophication gradients has been evolved.

Several environmental variables explained most of the variation of diatom taxa distribution in the Nile. Increasing in nutrient concentrations is most pronounced in northern of the main stem, Rosetta and Damietta branches due to discharging of municipal and industrial effluents in combination with agricultural drainage and decrease flow as the water arrives the Nile estuary. The results of this study showed that ammonium is the strongest environmental gradient underlying diatom distribution in River Nile waters, followed by nitrite, which together were closely related to community patterns. Nitrogen and phosphorus are the most commonly investigated nutrients of the benthic habitats, these nutrients are the most likely to be growth limiting [25]. Nutrients availability becomes the key limiting factor that determines the dominance of one algal group over another, not the ratios between these nutrients [26]. Another study revealed the positive effect of inorganic nitrogen on diatom assemblages indicating that they were nitrogen limited [27, 28].

Many of diatom species common in this study were cosmopolitan and were not exclusively colonize a single substrate type but have been frequently found as common taxa upon different substrate types. These include *Achnanthes minutissma, A. lanceolata, Cocconeis placentula, C. placentula var. euglypta Cyclotella meneghiniana, C. ocellata, C. operculata, Cymbella microcephala, C. affinis, Fragilaria construens, Gomphonema minutum, Nitzschia amphibia, N. leistikowi, N. palea*, and *C. microcephala*. This frequently distribution of many species may be due to the long period of low flows that preceding sample collection, rapid reproduction, and ongoing immigration of species onto substrates which favored the dominance with commonly occurring, presumably better adapted diatoms [10]. In addition, the development of a mature, complex periphyton matrix with its own biological, chemical and physical characteristics may have mediated or even negated any substrate influence on the diatom assemblage [10]. In this regard, the abundance of common species on different substrates may be varied but no clear evidence of specific substrate-diatoms relations were established [10]. In contrast, there was a difference between living substrates and inert ones on attached diatoms where the living (macrophytes) substrates were an important nutrient source for benthic algae and the importance of this nutrient supply did not decrease with increasing water trophy [29]. The findings of common occurrence of most species upon different substrates were enforced by the non-significant (P = 0.06) variations of the species diversity among these substrates.

According to our data, the diatom communities, especially epipelic assemblages, highlighted significant high diversity values in the southern region. This may reflect the negative effect of ammonium and nitrite concentrations at the northern sites of the main stem and branches sites, as well as the positive effects of the intermediate disturbance hypothesis at the southern regions. As water bodies characterized by intermediate nutrient concentrations were often associated with high diatom diversity whereas the sites dominated by diatoms indicative of organic pollution or higher disturbance had lower species diversity [30, 31]. Decreases in diversity of diatom assemblage under stress have been reported [32]. Furthermore, distribution pattern of diatom species diversity, measured by Shannon's index in lotic systems, was consistent with the intermediate disturbance hypothesis, and indicates that highest diversity is maintained under intermediate disturbance by nutrients pollution [33]. Although it has to be considered that diversity indices for the benthic diatom assemblages are very variable, no authors have described direct cause-effect relationship between chemical pollution and diversity [34].

According to CCAs, the summation of all canonical eigen values of the epipelic was the highest compared with the epilithic and epiphytic samples. This value pointed to how the diatom community was affected by the environmental gradients, which mirrored that epipelic community was the most sensitive and related to the change in the environmental variables and consequently is considered as the best fit to assessment and monitoring program in River Nile Basin. Environmental variables through three CCA explained 51%, 43% and 39% of the diatom species variance in epipelic, epilithic and epiphytic communities, respectively, which re-strengthen by mantel correlation matrix between the studied assemblages, as a first group, and the eutrophication variables, as a second group. Indeed, the low river flow velocity which is the case of River Nile can decrease the scouring of epilithic and epiphytic communities from their substrates [35]. This agrees with other studies, where the use of the epipelon is the most appropriate community for monitoring studies in Pampean plain rivers [36-38]. Pampean plain rivers are characterized by low velocity and high nutrients pollution, a case similar to River Nile Basin. Stones are however not always present along all river sections, and when present, they may not be the predominant kind of substrate for colonization by diatoms [31]. Autogenic sloughing and grazing probably removed epiphytic diatoms, and at the same time exposed new algal substrate for diatom colonization. This loss of epiphytic diatoms may account for the observed lowest correlates of epiphytic diatom assemblages to the changes in environmental variables [10]. On the other hand, insignificant differences in the determination of water quality based on different natural substrates (epilithic, epiphytic, epipsammic and epipelic) were reported and the choice of a specific substrate may not affect the accuracy of water quality assessment [4, 39]. Anyway, the collection of samples from multiple substrata at a single site for monitoring programs was sometimes preferred that aim to assess the full complement of diatom species typically, based on the assumption that some species are specific to a substrate [40-42].

5. Conclusion

Sites of Rosetta Branch were characterized by high nutrients (NO_2, NO_3, NH_4, PO_4 and TP) than other sites of the main stem of Nile and Damietta Branch. Most chemical variables showed significant variation between seasons ($P<0.01$) with pronounce increase during spring. TDS and EC showed gradual increase northward till the most northern site of Rosetta compared with a slight decrease in Damietta Branch. Leading diatom species were common upon the three tested substrates, with no specification in the distribution of common diatom taxa. Diversity and evenness had higher values at the southern sites with partial and non-significant variations, respectively, among substrates. TWINSPAN analyses resulted in 34 final groups, 16 groups contain exclusively one substrate type and 18 groups contain diatom samples from more than one substrate type. Some of these final groups were distinguished depending on regions where close sites were clustered together. Environmental variables through three CCA explained 51%, 43% and 39% of the diatom species variance in epipelic, epilithic and epiphytic communities, respectively. CCA analyses indicated that epipelic diatom communities were the most related communities to the environmental variables and the best fit used for eutrophication assessment and monitoring program in our study system.

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