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Environmental impacts of phenol pollution on phytoplankton biodiversity at Assiut region, Egypt

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ABSTRACT

Phenol is a common organic pollutant in the aquatic habitats. However, its adverse effects on the composition and diversity of phytoplankton are still poorly understood. Phenol can cause toxic effects to different living organisms even at low concentrations. The present study investigated the effect of phenol on phytoplankton diversity and community structure in samples collected from ten polluted sites. The concentration of phenol in the investigated sites were generally higher than 0.05 mg L^{-1} which is over the allowable limit. The spatial complexity of the microalgal community was investigated using different alpha (α) diversity measures for the largest microalgal groups (Cyanobacteria, Chlorophyta, Bacillariophyta). Distance-based redundancy analysis (dbRDA) indicated that phenol pollution had adverse effects on both phytoplankton diversity and taxonomic structure. Accordingly, the algal pollution index (API) was negatively correlated with richness and diversity of the main phytoplankton groups. The most tolerant species to phenol stress belong to Chlorophyta and Cyanobacteria. In addition, the total phytoplankton community was grouped into 19 functional groups (FGs) which associated with the preference of a certain environmental conditions. A laboratory toxicity experiment was also performed to identify the negative effects of short-term exposure to phenol on different microalgal species. Thus, the most sensitive taxa were disappeared in response to the phenol treatment. Overall, this study is valuable in indicating the adverse effects of phenol pollution to the natural phytoplankton community.

INTRODUCTION

The pollution of water resources is a common problem that is being faced today. There is a growing demand for wastewater treatment to reduce the pollution caused by

fast industrialization and urbanization as well as to compensate the exhaustion of freshwater resources. Phenol is a hazardous organic pollutant in both terrestrial and aquatic environments. Several industrial effluents such as petroleum processing plants, oil refineries, plastic, paper and pulp, pharmaceutical and agrochemical industries represent the main sources of phenol and its derivatives in the environment [1]. The concentration of phenol and its derivatives in wastewater is usually between 10 and 300 mg L^{-1}. However, its concentration can increase up to 4.5 g L^{-1} in highly polluted wastewater [2]. Additionally, in natural water, phenol concentrations are between 0.01 and 2 µg L^{-1} [3]. Moreover, the World Health Organization (WHO) indicated that the maximum allowable concentration of phenol in drinking water should be below 1 µg L^{-1} [4]. Thus, the removal of phenol and its derivatives from polluted water have become a necessity to preserve the environmental quality.

Microalgae are important taxa in the aquatic habitats which lies in the base of the food chain. Thus, the adverse effects of phenol on phytoplankton community are also reflected on other organisms in the food chain. Phytoplankton are generally characterized by fast response to various toxic contaminants such as phenol owing to their small and a relatively large surface area [5]. A remarkable decrease in the total carbon assimilation and growth rate of microalgae in response to phenol has been recorded in earlier studies. For instance, Megharaj et al. [6] showed a marked inhibition in growth, chlorophyll a and b concentrations, total protein, and carbohydrate in the Chlorophyceae algae *Chlorella vulgaris* and *Scenedesmus bijugatus* under phenol stress. Several microalgae can remove phenol by biodegradation and utilization as a carbon source, however, this mechanism varies between microalgal species and environmental conditions [5,6]. However, the influence of phenol pollution on the composition and different diversity parameters of freshwater phytoplankton is still poorly understood. Thus, the present study aimed to investigate the adverse effects of phenol pollution on phytoplankton diversity and community composition. Furthermore, an *in vitro* experiment was performed to further estimate the negative effects of phenol on phytoplankton community structure and diversity.

MATERIALS AND METHODS

Study region and sample collection

Freshwater samples were collected from the water surface in summer (2018) from ten different sites located at Assiut region, Egypt (Table 1). The studied water bodies were located at a densely populated area. Therefore, water pollution occurred owing to municipal wastewater, agriculture drainage and industrial pollution. A total of about 5 L of water samples were collected from each site in a plastic container. After settling for 24 hours at room temperature, the water was decanted, and the collected phytoplankton were fixed with 1% (v/v) formaldehyde solution.

Physico-chemical analysis of freshwater samples

1. Determination of water temperature, pH and electrical conductivity

The temperature of the water samples was determined simultaneously during collection. Water pH was estimated by pH meter (211Hanna instruments, USA). Electrical conductivity (EC, µS cm^{-1}) was estimated by EC meter (YSI Model 35 yellow spring, OH, USA).

2. Determination of sodium and potassium

Sodium and potassium ion concentrations were determined using flame photometer (Flame Photometer M 71 D type Nr/ LPG075) [7].

3. Determination of calcium and magnesium

The complexometric titration method [8] was employed for both calcium and magnesium determinations. For calcium determination, freshwater sample (1 mL) was diluted by 5 mL of distilled water and KOH solution (2 mL, 10% w/v). The mixture was titrated against Na_2-EDTA (0.005 N) in the presence of muroxide as an indicator until a purple end point. The 0.005 N EDTA is equivalent to 0.1 mg of calcium. Similarly, for the determination of magnesium, freshwater sample (1 mL) was diluted by distilled water (5 mL) and titrated with Na_2-EDTA (0.005 N) in the presence of Erichrome Black T as an indicator until a blue end point. The amount of EDTA consumed is equivalent to Ca^{2+} plus Mg^{2+}. The 0.005 N of EDTA is equivalent to 0.06 mg of magnesium.

4. Determination of water chlorinity

Chlorinity was analyzed by the method of [9]. Water sample (1 mL) was mixed with 10 mL distilled water followed by adding 1 mL of $K_2Cr_2O_7$ (5% w/v) as indicator. The solution was titrated against $AgNO_3$ (0.05 N) until orange color appeared.

5. Determination of nitrate

Nitrate was spectrophotometrically determined by chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulphonic acid disodium salt) in concentric sulfuric acid [10]. The reagent solution was obtained by dissolving 0.05 g of chromotropic acid disodium salt dihydrate in 50 mL of concentric sulfuric acid (95%). Chromotropic acid solution (1 mL, 0.05 %) and 3 mL of concentric sulfuric acid (95%) were added carefully, drop by drop to 1 mL of water sample in clean and dry test tubes. The tubes were left for 30 min, and the absorbance was measured at 412 nm. The nitrate concentrations of the unknown samples were determined using a calibration graph with different concentrations of $NaNO_3$.

6. Determination of inorganic phosphate

A spectrophotometric method was employed for the determination of inorganic phosphates [11]. Ammonium molybdate tetrahydrate (5g) was dissolved in 42.6 mL of concentric sulfuric acid in a 250 mL measuring flask then completed to the mark with distilled water. Stannous chloride (0.2% w/v) was prepared in hydrochloric acid solution (2% v/v). One milliliter of the freshwater sample was mixed with ammonium molybdate reagent (1 mL) and $SnCl_2$ solution (1 mL). After 15 min, the developed blue color was measured at 660 nm by a spectrophotometer against a blank. Different concentrations of KH_2PO_4 were used as standard.

7. Determination of sulfate

Sulfates were determined according to the method described by Sheen [12]. One milliliter of water sample mixed with 2 mL of distilled water, and 1 mL of acidic-NaCl solution (24 g of NaCl + 2.5 mL of HCl (6N) + 100 mL of distilled water) and 1 mL of $BaCl_2$ solution (10% w/v). After 5 min, 1 mL of acacia gum solution (0.5%) was added, then the tubes were allowed to stand for 20 min. The absorbance of the turbid $BaSO_4$ solution was measured at 420 nm by a spectrophotometer.

8. Determination of the total organic matter content

According to Walkley and Black [13], the total organic matter (OM) of the collected water samples was estimated by oxidation using potassium dichromate

($K_2Cr_2O_7$) and sulfuric acid (H_2SO_4). Water sample (50 mL) was evaporated until complete drying at 40 °C, then $K_2Cr_2O_7$ (10 mL, 0.167 % w/v) and concentric H_2SO_4 (20 mL) were added. The solution was then mixed with boric acid (10 ml, 85% w/v) and diphenylamine (1.0 mL, 0.16% w/v) aqueous solutions. The unreacted dichromate was then estimated by volumetric titration against ammonium ferrous sulfate solution (0.5 M).

9. Determination of phenol

The determination of phenol in the water samples was based on the method of [14] with slight modification. Briefly, to a 5 mL water sample, sodium nitroprusside (0.01 M, 100 µL) was added followed by hydroxylamine hydrochloride (0.04 M, 100 µL) and NaH_2PO_4/NaOH buffer (pH 12, 300 µL). After shaking, the mixture was left for 15 min. The absorbance of the developed color was measured spectrophotometrically at 700 nm against a suitable blank without phenol.

10. Determination of algal pollution index

Algal pollution index (API) was determined using modified Palmer index [15] according to the following equation:

$$API = \frac{\sum_{j=1}^{n} p_j \times c_j}{\sum_{j=1}^{n} c_j}$$

where $\boldsymbol{p_j}$ and $\boldsymbol{c_j}$ are the pollution index of algal genera [16] and the relative number of algal assemblages of j^{th} species, respectively. The API is ranged from 0 to 4, where values closer to 4 indicate high organic pollution and near to 0 show low organic pollution.

Identification of microalgae

Different freshwater microalgal taxa were identified morphologically using light microscope (40X) based on different descriptions reported by [17–20]. Estimation of the microalgal counts was performed using a hemocytometer under light microscope and was expressed as the number of individuals per mL of water sample. Five replicates were counted for each site. The identified microalgal taxa were grouped into specific functional groups according to Reynolds et al. and Padisák et al. [21,22]. These groups were not specific to a definite taxonomic group but were associated with the preference of taxa to a certain habitat as described by [21,22,24,25].

Community structure analysis

Several biodiversity indices were utilized in the analysis including species richness (Margalef's index, d), which represent the number of taxa identified in the site, species diversity (Shannon–Wiener's H′, log base e), which indicate the strength of diversity, evenness (Pielou's J′), which is related to the even distribution of the individuals between the different studied sites. Furthermore, the variation in the taxomonic structure in the phtoplankton community was indicated by different indices including, taxonomic diversity (D), taxonomic distinctness (D*), average taxonomic distinctness (D^+), variation in taxonomic distinctness (L^+) and total taxonomic distinctness (sD^+) [24, 26].

In vitro effect of phenol on phytoplankton diversity

Ten liters of surface water sample from El-Ibrahimiya canal, Assiut region, Egypt were collected and settled for 24 hours. This site was chosen based on negligible phenol contamination. The precipitated phytoplankton was collected by centrifugation (4800 g, 15 min) and washed thrice with distilled water. The collected phytoplankton were inoculated into 250 mL Erlenmeyer conical flasks containing 100 mL of Bold's Basal

medium (BBM) [23] and different phenol concentrations (0, 0.05, 0.2 and 0.35 mg L^{-1}). The phenol treatments and control were cultivated in triplicates for 6 days under continuous illumination (48.4 µmol $m^{-2}s^{-1}$) at 25 °C. Ten milliliters of the treatments were withdrawn after each two days for the examination and identification of algal community using light microscope. The effect of phenol treatment on any index of biodiversity compared to the control was calculated using the following equation:

$$\text{Difference (\%)} = \frac{I_t - I_c}{I_c} \times 100$$

where, I_t and I_c are the results of each calculated index in the phenol treated and control cultures, respectively.

Statistical analysis

The biodiversity indices were calculated using the DIVERSE routine of the PRIMER package (Primer V. 6.0, Primer-E). A distance-based redundancy analysis (dbRDA) was utilized to reflect the correlation between different microalgal groups and the estimated physico-chemical parameters of water samples and different biodiversity indices using PERMANOVA+ in PRIMER v6 software.

RESULTS

Physico-chemical analysis of freshwater samples

Different physical and chemical water parameters were analyzed and listed in Table 1. The investigated sites were generally alkaline (pH ranged from 7.95 to 8.85). The temperature of the collected samples was ranged between 25 and 28 °C. EC was fluctuated between 292.00 ± 3.00 and 1109.67 ± 18.15 µS cm^{-1}. The concentrations of sodium and potassium ions were also measured and varied from 30.5 to 154 mg L^{-1} and from 3.06 to 12.3 mg L^{-1}, respectively. Maximum water chlorinity was observed at site 2 which was 236.4 ± 27.08 mg L^{-1}. Calcium and magnesium concentrations were ranged from 50.00 ± 10.00 to 76.67 ± 15.28 mg L^{-1} and from 8.00 ± 12.49 to 82.00 ± 6.93 mg L^{-1}, respectively. Nitrate contents were fluctuated between 15.88 and 41.68 mg L^{-1}, while the range of phosphate was 0.08 − 1.50 mg L^{-1} and sulphate concentrations were 1.32 − 6.71 mg L^{-1}. Organic matter content ranged from 33.60 to 127.20 mg L^{-1}. Phenol as an organic pollutant in the investigated sites was fluctuated between 0.05 and 0.2 mg L^{-1} (Table 1).

Community structure of different phytoplankton

A total of 137 species of phytoplankton were identified from ten different sites. The algal community was grouped into five taxonomic classes: Cyanophyceae (10 genera and 20 species), Chlorophyceae (31 genera and 56 species), Charophyceae (5 genera and 10 species), Bacillariophyceae (17 genera and 41 species) and Euglenophyceae (5 genera and 10 species). Chlorophyta and Bacillariophyta members were the most predominant algal groups in the studied sites compared to other groups. The frequency of occurrence and abundance of microalgae varied greatly in different sites, reflecting a spatial heterogeneity (Table 2).

The predominant taxa with high occurrence remark (% OR) were *Merismopedia tenuissima*, *Microcystis* sp.(1), and *Pseudanabaena* sp. (1) from Cyanobacteria and

Chlorococcum hypnosporum, Coelastrum astroideum, Coelastrum cambricum, Dictyosphaerium reniforme, Nephrocytium schilleri, Scenedesmus quadricauda, Tetraëdron minimum from Chlorophyceae as well as *Aulacoseira granulata, Aulacoseira italica, Cyclotella meneghiniana, Nitzschia acicularis, Nitzschia fruticosa, Nitzschia palea,* and *Ulnaria ulna* from Bacillariophyceae (diatoms) and *Staurastrum chaetoceras* from Charophyta (Table 2).

Table 1: Physico-chemical analysis of water samples from different investigated sites and their GPSs.

Site	GPS	T	pH	EC	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	NO_3^-	PO_4^-	SO_4^{2-}	OM	Phenol	API
		°C		μScm^{-1}	mg L^{-1}										
S1	N 27°23'2.3", E 30°53'41"	26	8.85 ± 0.02	323.67 ±15.37	33.6	4.4	112.29 ±27.08	76.67 ±15.28	42.00 ±18.00	20.24 ±3.83	0.60 ±0.01	2.85 ±1.11	110.40 ±27.15	0.14 ±0.01	1.75
S2	N 27°17'54", E 30°59'1"	26	8.59 ± 0.03	321.67 ±6.11	30.5	3.06	236.4 ±27.08	63.33 ±15.28	82.00 ±6.93	15.88 ±4.41	0.79 ±0.15	1.64 ±0.16	33.60 ±20.36	0.09 ±0.01	2.11
S3	N 27°15'25", E 31°1'36"	27	8.79 ± 0.02	315.33 ±5.86	33.6	4.48	177.3 ±35.46	53.33 ±15.28	78.00 ±18.00	21.69 ±7.63	0.21 ±0.02	1.47 ±0.09	88.80 ±3.39	0.08 ±0.01	2.22
S4	N 27°14'34", E 31°4'38"	25	8.36 ±0.02	409.33 ±11.59	35.8	6.83	124.11 ±35.46	70.00 ±20.00	58.00 ±19.29	41.68 ±3.83	0.57 ±0.09	1.85 ±0.10	96.00 ±27.15	0.20 ±0.02	3.16
S5	N 27°14'44", E 31°4'5"	28	8.13 ±0.02	349.33 ±1.53	35.7	6.64	135.93 ±27.08	63.33 ±15.28	44.00 ±13.86	18.78 ±4.92	0.08 ±0.02	1.68 ±0.06	79.20 ±10.18	0.09 ±0.01	2.14
S6	N 27°11'51", E 31°6'7"	25	7.95 ±0.01	696.33 ±32.88	76.1	12.3	100.47 ±27.08	66.67 ±15.28	42.00 ±15.87	19.51 ±4.36	0.91 ±0.16	1.99 ±0.06	79.20 ±10.18	0.20 ±0.01	2.51
S7	N 27°10'11", E 31°9'27"	26	8.84 ±0.03	292.00 ±3.00	33.6	4.32	88.65 ±35.46	56.67 ±5.77	32.00 ±9.17	33.32 ±7.66	0.45 ±0.10	1.73 ±0.11	55.20 ±23.76	0.05 ±0.01	2.00
S8	N 27°8'26", E 31°14'19"	26	8.64 ±0.03	329.67 ±5.69	38.9	4.86	165.48 ±27.08	73.33 ±15.28	20.00 ±12.49	37.32 ±5.59	0.18 ±0.04	1.81 ±0.08	36.00 ±37.34	0.06 ±0.03	2.16
S9	N 27°6'37", E 31°13'32"	25	8.04 ±0.02	1109.67 ±18.15	154	11.4	171.39 ±36.91	50.00 ±10.00	38.00 ±6.93	19.15 ±6.39	1.50 ±0.15	6.71 ±0.21	127.20 ±10.18	0.20 ±0.03	2.74
S10	N 27°6'5", E 31°12'42"	27	8.66 ±0.04	304.00 ±4.58	35.7	4.37	118.20 ±27.08	60.00 ±10.00	8.00 ±12.49	36.95 ±6.07	0.36 ±0.04	1.32 ±0.07	67.20 ±6.79	0.07 ±0.01	2.05

T: temperature; EC: electrical conductivity; OM: organic matter, API: algal pollution index.

On the other hand, some species such as *Microcystis* sp.(2), *Oscillatoria* sp., *Pseudanabaena* sp. (2) and *Spirulina* sp.(2) from Cyanobacteria as well as *Chlorella* sp., *Closterium* sp., *Closterium acerosum* var. *tumidum, Closterium moniliferum, Actinotaenium globosum, Ankistrodesmus arcuatus, Oonephris obesa, Pseudopediastrum boryanum, Scenedesmus acutus, Scenedesmus insignis, Staurastrum dorsidentiferum, Staurastrum furcigerum, Tetraëdron caudatum, Tetraspora* sp. and *Ulothrix* sp. from Chlorophyta and *Phacus* sp. and *Phacus warszewiczii* from Euglenophyta and *Cocconeis* sp., *Cymatopleura elliptica, Cymbella* sp., *Fragilaria capucina, Fragilaria crotonensis* and *Ulnaria delicatissima* from Bacillariophyta were characterized by rare occurrence remarks.

Table 2: List of identified microalgal species in different study sites along with their occurrence remarks (% OR) and functional groups (FG)

Algal taxa	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	OR	FG
Cyanobacteria												
Anabaena sp.			+					+			R	H1
Chroococcus turgidus (Kützing) Nägeli	+		+		+		+	+			M	Lo
Coelosphaerium sp.	+						+				R	Lo
Coelosphaerium minutissimum Lemmermann	+	+	+				+	+			M	Lo
Gomphosphaeria sp.				+		+				+	L	Lo
Kamptonema formosum (Bory ex Gomont) Strunecký, Komárek & J.Smarda.			+	+	+	+		+	+		M	MP
Merismopedia minima G.Beck					+		+				R	Lo
Merismopedia tenuissima Lemmermann	+	+	+		+	+	+	+		+	H	Lo
Microcystis sp.(1)	+	+	+	+	+	+	+	+		+	H	M
Microcystis sp.(2)			+								R	M
Microcystis aeruginosa Kützing	+	+									R	M
Oscillatoria limosa C.Agardh ex Gomont					+	+				+	L	MP
Oscillatoria sp.								+			R	MP
Oscillatoria tenuis C. Agardh ex Gomont				+	+	+					L	MP
Phormidium sp.			+	+	+	+				+	M	S1
Pseudanabaena sp. (1)	+	+	+	+	+	+	+	+		+	H	S1
Pseudanabaena sp. (2)						+					R	S1
Spirulina sp.(1)						+		+			R	S2
Spirulina sp.(2)						+					R	S2
Spirulina major Kützing ex Gomont				+			+	+	+		L	S2
No. of Cyanophyta	**7**	**5**	**9**	**7**	**9**	**11**	**8**	**10**	**2**	**6**		
% of Cyanophyta	**15.91**	**9.62**	**15.52**	**16.28**	**15.52**	**19.64**	**16.00**	**17.24**	**12.50**	**10.34**		
Chlorophyta												
Actinastrum hantzschii Lagerheim		+	+		+	+	+	+		+	M	J
Ankistrodesmus arcuatus Korshikov						+					R	J
Ankistrodesmus densus Korshikov	+	+	+				+	+			M	X1
Ankistrodesmus falcatus (Corda) Ralfs							+				R	X1
Ankistrodesmus fusiformis Corda	+	+	+		+		+	+		+	M	X1
Ankistrodesmus spiralis (W.B.Turner) Lemmermann	+	+					+				L	X1
Chlamydomonas sp.		+	+	+	+					+	M	X2
Chlorella sp.						+					R	X1
Chlorella vulgaris Beijerinck				+	+	+					L	X1
Chlorococcum hypnosporum Starr		+	+	+	+	+	+	+	+	+	H	MP
Closteriopsis longissima (Lemmermann) Lemmermann	+	+	+		+		+			+	M	P

Coelastrum astroideum De Notaris	+	+	+	+	+		+	+		+	H	J
Coelastrum cambricum W.Archer	+	+	+		+	+	+	+		+	H	J
Coelastrum microporum Nägeli		+	+		+	+		+		+	M	J
Desmodesmus insignis (West & G.S.West) E.Hegewald	+										R	J
Dictyosphaerium granulatum Hindák	+	+	+		+		+	+		+	M	F
Dictyosphaerium reniforme Bulnheim		+	+	+	+	+	+	+		+	H	F
Eudorina sp.		+									R	G
Eudorina elegans Ehrenberg				+		+					R	G
Golenkinia paucispina West & G.S.West		+	+				+	+		+	M	J
Lagerheimia citriformis (J.W.Snow) Collins				+	+		+			+	L	J
Lagerheimia longiseta (Lemmermann) Printz	+	+									R	J
Lemmermannia tetrapedia (Kirchner) Lemmermann			+		+	+		+		+	M	J
Micractinium pusillum Fresenius		+			+	+				+	L	F
Monactinus simplex var. echinulatum (Wittrock) Pérez, Maidana & Comas		+	+							+	L	J
Monoraphidium circinale (Nygaard) Nygaard				+	+	+					L	X1
Monoraphidium contortum (Thuret) Komárková-Legnerová				+		+		+		+	L	X1
Monoraphidium griffithii (Berkeley)			+	+		+				+	L	X1
Monoraphidium komarkovae Nygaard	+	+	+	+	+	+		+			M	X1
Mucidosphaerium pulchellum (H.C.Wood) C.Bock, Proschold & Krienitz	+	+					+				L	F
Nephrocytium limneticum (G.M.Smith) G.M.Smith					+	+				+	L	F
Nephrocytium lunatum West	+	+	+		+	+	+	+		+	H	F
Oocystis borgei J.W.Snow		+	+		+	+	+	+		+	M	F
Oonephris obesa (West & G.S.West) Fott			+								R	F
Pandorina morum (O.F.Müller) Bory				+	+	+		+			L	G
Pediastrum duplex Meyen		+				+	+	+		+	M	J
Pediastrum simplex Meyen			+		+		+	+		+	M	J
Pediastrum simplex va	+	+									R	J

r. *clathratum* Schröter												
Pseudopediastrum boryanum (Turpin) E.Hegewald	+										R	J
Scenedesmus ellipticus Corda	+		+		+		+	+			M	J
Scenedesmus gonzalez-guerreroi Molinari & Guiry				+	+			+			L	J
Scenedesmus quadricauda (Turpin) Brébisson	+	+	+	+	+	+	+	+		+	H	J
Stauridium tetras (Ehrenberg) E.Hegewald								+		+	R	J
Tetradesmus dimorphus (Turpin) M.J.Wynne		+		+	+	+				+	M	J
Tetradesmus incrassatulus (Bohlin) M.J.Wynne					+					+	R	J
Tetradesmus lagerheimii M.J.Wynne & Guiry	+	+					+				L	J
Tetradesmus obliquus (Turpin) M.J.Wynne				+							R	J
Tetraëdron caudatum (Corda) Hansgirg			+								R	J
Tetraëdron minimum (A.Braun) Hansgirg	+	+	+	+	+		+	+		+	H	J
Tetraëdron trigonum (Nägeli) Hansgirg				+		+					R	J
Tetraspora sp.						+					R	J
Tetrastrum elegans Playfair				+							R	J
Tetrastrum peterfii Hortobágyi				+	+						R	J
Tetrastrum staurogeniiforme (Schröder) Lemmermann								+			R	J
Ulothrix sp.									+		R	MP
Willea apiculata (Lemmermann) D.M.John, M.J.Wynne & P.M.Tsarenko		+	+	+	+	+				+	M	F
No. of Chlorophyta	**18**	**29**	**25**	**21**	**29**	**25**	**22**	**24**	**2**	**28**		
% of Chlorophyta	**40.91**	**55.77**	**43.10**	**48.84**	**50.00**	**44.64**	**44.00**	**40.68**	**12.50**	**48.28**		
Charophyta												
Actinotaenium globosum (Bulnheim) Kurt Förster							+				R	J
Closterium acerosum var. *tumidum* O.Borge				+							R	P
Closterium sp.									+		R	P
Closterium moniliferum Ehrenberg ex Ralfs										+	R	P
Cosmarium bioculatum Brébisson ex Ralfs	+										R	N
Mougeotia sp.			+								R	T
Staurastrum anatinum Cooke & Wills	+							+			R	N
Staurastrum chaetoceras (Schröder)	+	+	+		+		+	+	+	+	H	N

G.M.Smith												
Staurastrum dorsident iferum West & G.S.West						+					R	N
Staurastrum furcigeru m (Brébisson) W.Archer								+			R	N
No. of Charophyta	**3**	**1**	**2**	**1**	**1**	**1**	**2**	**3**	**2**	**2**		
% of Charophyta	**6.82**	**1.92**	**3.45**	**2.33**	**1.72**	**1.79**	**4.00**	**5.08**	**12.50**	**3.45**		
Euglenophyta												
Discoplastis spathirhy ncha (Skuja) Triemer						+					R	W1
Euglena gracilis G.A. Klebs				+							R	W1
Euglena oblonga F.Sc hmitz					+	+					R	W1
Lepocinclis acus (O.F. Müller) B.Marin & Melkonian				+	+	+					L	W1
Lepocinclis oxyuris (S chmarda) B.Marin & Melkonian					+	+					R	W1
Monomorphina trypan on (Pochmann) Marin & Melkonian					+	+				+	L	W1
Phacus sp.		+									R	W1
Phacus arnoldii var. ovatus Popova						+					R	W1
Phacus glaber Pochm ann				+	+	+					L	W1
Phacus longicauda (E hrenberg) Dujardin				+	+	+					L	W1
No. of Euglenophyta	**0**	**1**	**0**	**4**	**6**	**8**	**0**	**0**	**0**	**1**		
% of Euglenophyta	**0.00**	**1.92**	**0.00**	**9.30**	**10.34**	**14.29**	**0.00**	**0.00**	**0.00**	**1.72**		
Bacillariophyta												
Ardissonea fulgens (Greville) Kanjer, Kusber & Van de Vijver			+								R	D
Aulacoseira granulata (Ehrenberg) Simonsen	+	+	+	+	+	+	+	+	+	+	H	P
Aulacoseira italica (E hrenberg) Simonsen	+	+	+	+	+	+	+	+	+	+	H	B
Cocconeis sp.										+	R	MP
Craticula cuspidata (Kutzing) D.G.Mann	+		+	+	+		+	+		+	M	MP
Cyclotella sp.								+	+		R	A
Cyclotella striata (Kützing) Grunow	+						+				R	A
Cyclotella atomus Hus tedt		+	+	+	+					+	M	A
Cyclotella meneghinia na Kützing	+	+	+	+	+	+	+	+		+	H	C
Cymatopleura elliptic a (Brébisson) W.Smith								+			R	MP
Cymbella sp.			+								R	MP
Fragilaria capucina D esmazières	+										R	D
Fragilaria crotonensis Kitton	+										R	D

Gomphonema sp.				+	+	+					L	MP
Gyrosigma sp.		+	+				+	+		+	M	MP
Gyrosigma acuminatum (Kützing) Rabenhorst	+	+	+				+	+		+	M	MP
Gyrosigma attenuatum (Kützing) Rabenhorst	+		+				+			+	L	MP
Gyrosigma scalproides (Rabenhorst) Cleve			+								R	MP
Navicula sp.		+						+			R	MP
Navicula gregaria Donkin		+	+		+	+	+			+	M	MP
Navicula lanceolata Ehrenberg			+				+	+			L	MP
Nitzschia sp.		+									R	D
Nitzschia acicularis (Kützing) W.Smith	+	+	+		+	+	+	+		+	H	D
Nitzschia eglei Lange-Bertalot		+	+				+	+		+	M	D
Nitzschia exilis Archibald, nom. illeg.							+		+		R	D
Nitzschia fruticosa Hustedt	+	+	+		+	+	+	+	+	+	H	D
Nitzschia kurzeana Rabenhorst		+						+			R	D
Nitzschia linearis W.Smith			+							+	R	D
Nitzschia palea (Kützing) W.Smith	+	+	+	+	+	+	+	+	+	+	H	D
Nitzschia reversa W.Smith		+	+		+		+	+	+	+	M	D
Nitzschia sigma (Kützing) W.Smith										+	R	D
Nitzschia thermalis (Ehrenberg) Auerswald								+	+		R	D
Nitzschia vermicularis (Kützing) Hantzsch	+										R	D
Pseudostaurosira brevistriata var. inflata (Pantocsek) M.B.Edlund							+	+			R	D
Stephanodiscus sp.			+	+	+	+		+		+	M	D
Surirella sp.	+							+			R	MP
Surirella patella Kützing										+	R	MP
Tryblionella apiculata W.Gregory		+	+	+		+				+	M	D
Ulnaria acus (Kützing) Aboal								+	+		R	D
Ulnaria delicatissima (W.Smith) Aboal & P.C.Silva	+										R	D
Ulnaria ulna (Nitzsch) Compère	+	+	+	+	+	+	+	+	+	+	H	D
No. of Bacillariophyta	**16**	**17**	**22**	**10**	**13**	**11**	**18**	**22**	**10**	**21**		
% of Bacillariophyta	**36.36**	**32.69**	**37.93**	**23.26**	**22.41**	**19.64**	**36.00**	**37.28**	**62.50**	**36.21**		

% OR = (number of sites in which an alga was recorded / total number of sites) × 100. (1 – 24% (rare, R); 25 – 49% (low, L); 50 – 74% (medium, M); 75 – 100% (high, H))

Phytoplankton diversity

The spatial complexity of the microalgal community was investigated using different alpha (α) diversity measures for the largest microalgal groups (Cyanobacteria, Chlorophyta, Bacillariophyta). Margalef's species richness (d) measures the total number of taxa in each site. Chlorophyta exhibited the highest d values followed by Bacillariophyta and Cyanobacteria (Table 3). Pielou's evenness index (J') reflects the variation in the abundance of each microalga in a specific site. The J' values ranges 0 and 1, and the higher values indicate high evenness. The J' values for green algae were ≥ 0.84 in different sites, while the J' values for diatoms and blue green algae were fluctuated between 0.68 to 0.79 and 0.66 to 0.93, respectively. Similarly, J' values for all the phytoplankton community in different sites were ≥ 0.68. This result implies that the studied sites have high phytoplankton evenness. On the other hand, Shannon–Wiener's index (H′) is a direct estimate of diversity and it considers both the number of taxa and their abundance in the studied site. In general, the H′ index values for Chlorophyta were higher than Cyanophyta and diatoms in most of the investigated sites which reflects a high species diversity between them (Table 3). The H′ values for all the community were ranged between 1.90 and 3.59, which indicated a high biodiversity.

Taxonomic diversity (D) and taxonomic distinctness (D*) indicate a biodiversity of a community in relation to the taxonomic distance between any two species in a given site. For the total phytoplankton, the D and D* values were ranged from 37.44 to 70.51 and from 47.72 to 73.94, respectively. In general, the D and D* values were higher in case of green algae and diatoms compared to Cyanobacteria. On the other hand, the average taxonomic distinctness (D^+) is another biodiversity index which can be defined as the average path length in the taxonomic tree between species and confirm the close association in the upper levels of taxonomy such as order, class etc. The variation in taxonomic distinctness (L^+) reflects the unevenness in the taxonomic structure. The total taxonomic distinctness (sD^+) values for cyanoprokaryotes were lower than the values of chlorophycean algae and diatoms. Generally, the sD^+ values for all phytoplankton were ranged between 1047.41 and 4076.63 (Table 3). The high variability in the L+ values compared to the D+ values between different sites indicated a low variability in the taxonomic evenness.

The dbRDA analysis plots is used to visualize any possible correlation between the composition of the phytoplankton species and the investigated environmental factors. The length of the vectors and their relative pointing in the same direction reflectes positive correlations, while the negative correlation detected by the arrows at the opposite direction. The dbRDA analysis showed that increasing phenol and total organic matter had direct negative effects on the structure and biodiversity of phytoplanktons. Species richness (d) and diversity (H′) of the major phytoplankton groups (Cyanobacteria, Chlorophyta and Bacillariophyta) are locted at an opposite direction to phenol and organic matter, which implied a negative relationship. Similarly, species eveness (J′) was negatively correlated with phenol and organic matter in case of Cyanobacteria and diatoms, while this trend was not obvious in case of green algae (Fig. 1). On the other hand, increasing phenol and OM concentrations exhibited direct adverse effects on the taxonomic diversity (D) of the major phytoplankton groups. Similarily, taxonomic distinctness (D*) exhibited negative correlations with phenol and OM in case of Cyanobacteria and diatoms, however, green algae showed an opposite trend.

Table 3: Phytoplankton diversity indices for different study sites.

	Site	S	N	d	J'	H'	D	D*	D+	sD+	L+
Cyanobacteria	**S1**	7	31000	0.58	0.82	1.59	27.34	38.61	38.10	266.67	77.27
	S2	5	30000	0.39	0.72	1.16	22.16	39.25	38.89	194.44	55.56
	S3	9	34500	0.77	0.93	2.03	33.01	39.23	41.36	372.22	52.20
	S4	7	113000	0.52	0.66	1.28	14.68	22.92	40.21	281.48	87.90
	S5	9	55500	0.73	0.91	2.00	32.69	39.09	37.96	341.67	132.89
	S6	11	169000	0.83	0.91	2.19	32.59	37.33	39.60	435.56	111.17
	S7	8	38500	0.66	0.90	1.87	32.49	40.31	38.49	307.94	83.62
	S8	10	51000	0.83	0.91	2.09	35.41	42.16	41.98	419.75	54.26
	S9	2	20500	0.10	0.71	0.49	13.96	44.44	44.44	88.89	0.00
	S10	6	46000	0.47	0.70	1.26	24.83	42.51	41.48	248.89	40.60
Chlorophyta	**S1**	18	55000	1.56	0.97	2.82	37.53	40.20	40.01	720.26	211.96
	S2	28	113500	2.32	0.88	2.95	37.64	41.80	42.92	1201.65	176.64
	S3	25	109500	2.07	0.84	2.71	35.56	41.19	43.11	1077.78	169.42
	S4	21	139000	1.69	0.84	2.57	36.91	41.96	39.31	825.56	170.60
	S5	29	213500	2.28	0.87	2.93	40.53	44.11	43.32	1256.35	193.66
	S6	25	241000	1.94	0.88	2.84	40.14	43.83	44.41	1110.19	164.20
	S7	23	101500	1.91	0.94	2.95	41.47	44.35	44.71	1028.28	225.37
	S8	24	132000	1.95	0.86	2.73	35.41	39.86	41.63	999.03	170.98
	S9	2	5500	0.12	0.99	0.69	27.55	55.56	55.56	111.11	0.00
	S10	28	115500	2.32	0.93	3.08	38.76	41.53	43.30	1212.35	177.34
Diatoms	**S1**	16	473500	1.15	0.70	1.94	39.27	49.44	46.11	737.78	149.49
	S2	17	500000	1.22	0.75	2.13	39.56	47.69	40.85	694.44	302.98
	S3	22	507500	1.60	0.76	2.36	40.04	46.62	43.63	959.79	204.02
	S4	10	120500	0.77	0.70	1.60	31.18	45.40	48.89	488.89	161.32
	S5	13	273500	0.96	0.79	2.01	39.79	47.82	46.87	609.26	195.15
	S6	11	187500	0.82	0.72	1.73	38.19	51.01	46.26	508.89	218.92
	S7	18	397000	1.32	0.77	2.23	41.23	48.37	43.43	781.70	208.76
	S8	22	467500	1.61	0.76	2.36	39.76	45.61	44.40	976.72	184.38
	S9	10	248000	0.72	0.68	1.57	25.31	34.55	41.23	412.35	349.09
	S10	21	422000	1.54	0.79	2.39	42.32	48.35	43.17	906.67	232.37
All phytoplankton	**S1**	45	570500	3.32	0.69	2.61	51.88	60.57	69.51	3127.78	426.53
	S2	52	649500	3.81	0.73	2.90	54.74	61.16	65.49	3405.66	453.16
	S3	58	656500	4.26	0.76	3.08	55.00	60.35	68.53	3974.66	422.17
	S4	43	446000	3.23	0.83	3.13	69.06	73.94	69.02	2967.72	447.15
	S5	58	599500	4.28	0.86	3.48	66.12	69.48	69.29	4019.10	418.28
	S6	56	672000	4.10	0.89	3.59	70.51	73.48	71.23	3988.69	398.00
	S7	50	541500	3.71	0.79	3.09	58.05	63.29	68.56	3428.12	436.72
	S8	59	660500	4.33	0.79	3.22	58.28	62.66	69.10	4076.63	421.83
	S9	16	279000	1.20	0.69	1.90	37.44	47.72	65.46	1047.41	542.79
	S10	58	594000	4.29	0.80	3.24	59.60	63.96	66.86	3877.97	415.89

S: total number of observed taxa; N: total number of individuals; d: Margalef's species richness; J': evenness; H': Shannon's diversity index; D: taxonomic diversity; D*: taxonomic distinctness; D+: average taxonomic distinctness; sD+: total taxonomic distinctness: L+: variation in taxonomic distinctness.

It can also be noted that the average taxonomic distinctness (D^+) and the total taxonomic distinctness (sD^+) of the major phytoplankton groups showed contradicting results, i.e., the former was increased by increasing phenol and OM contents (positive

correlation), while the later was decresed (negative correlation). In addition, the variation in taxonomic distinctness (L^+) was negatively correlated with phenol and OM in case of Cyanobacteria and green algae, while positive correlation was observed for diatoms. On the other side, the analysis of the total phytoplankton community indicated that increasing phenol and OM contents had adverse effects on D, D*, D+, and sD+.

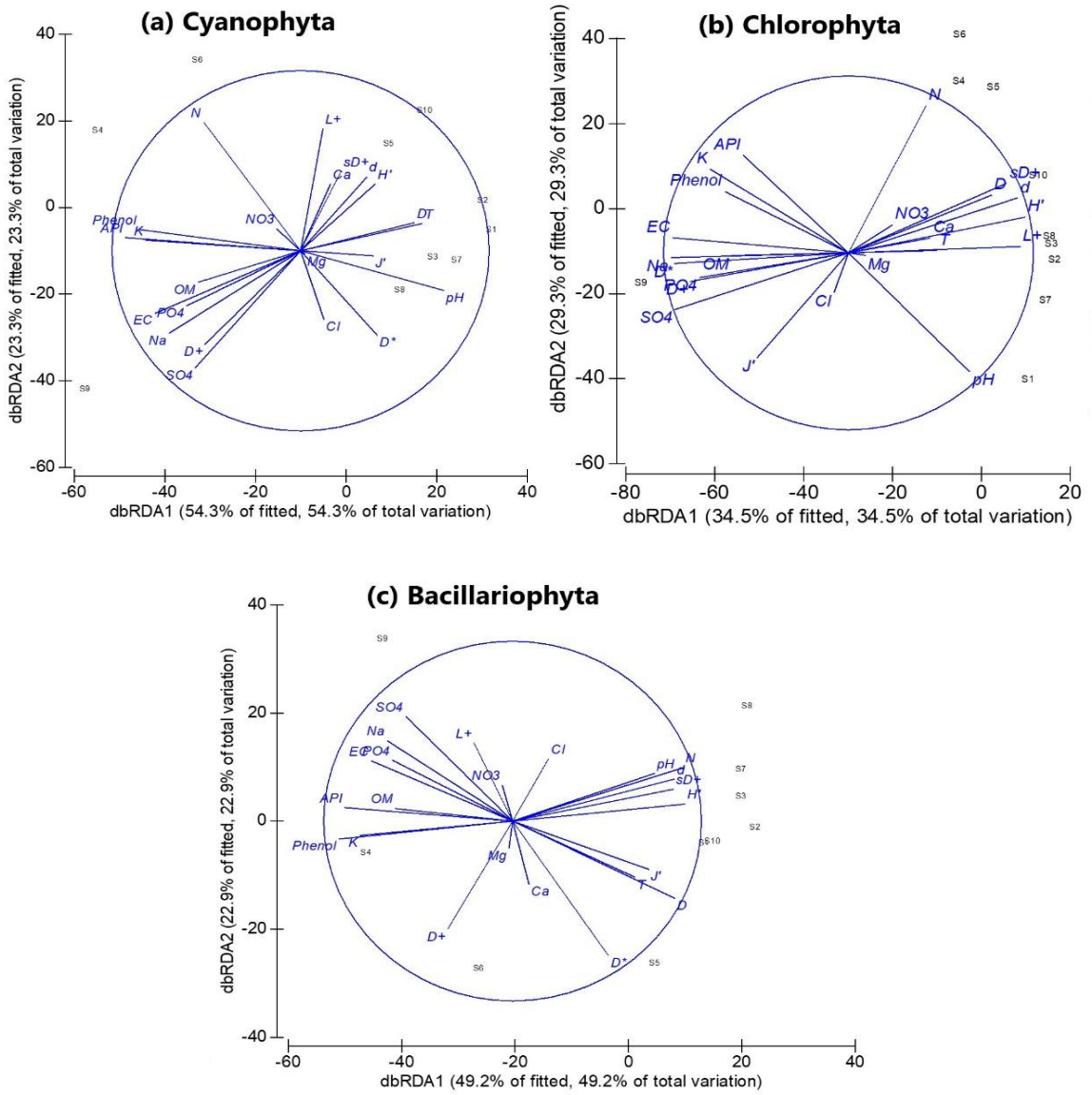

Fig. 1: Distance-based redundancy analysis for the community of (a) Cyanophyta, (b) Chlorophyta and (c) Bacillariophyta in different sites using Bray-Curtis similarity between samples and Spearman correlation with different abiotic factors and different biodiversity indices (T: temperature; OM: organic matter; Na: sodium; K: potassium; Ca: calcium; Mg: magnesium; NO_3: nitrate; SO_4: sulphate; PO_4: phosphate; API: algal pollution index; S: total number of observed species; N: total number of individuals; d: Margalef's species richness; J': evenness; H': Shannon's diversity index; D: taxonomic diversity; D*: taxonomic distinctness; D+: average taxonomic distinctness; sD+: total taxonomic distinctness: L+: variation in taxonomic distinctness).

On the other hand, different physico-chemical properties of water can directly affect phytoplankton diversity and structure as indicated by dbRDA. The analyis indicated that increasing PO_4^-, SO_4^{2-}, Na^+, K^+, and EC have direct negative effects on different diversity measures of the main phytoplankton groups. Furthermore, these parameters showed positive correlation with either D^+ values for Cyanobacteria and green algae or L^+ values of diatoms (Fig. 1).

The algal pollution index (API) was fluctuated betwwen 1.75 and 3.16, which indicated moderate to high organic pollution (Table 1). The API showed strong positive correlations with phenol pollution. This result indicated that this index can be effectively applied in the evironmental assement of water pollution by phenol.

Microalgal functional groups

The total phytoplankton community in the investigated sites was grouped into 19 functional groups (FGs) (H1, Lo, M, MP, S1, S2, J, X1, X2, N, P, F, G, T, W1, B, A, C and D) (Table 2, 4). The functional group with the highest number of recorded microalgal species was J, which included 10 – 16 species in different sites (except site 9, J was not represented by any species). In contrast, H1, X2, T, B, and C were characterized by low representation of 0 – 1 species in the different sites (Table 5).

Table 4: Definition of different identified microalgal functional groups in the present study as described by [21,22,25]

Codon	Environmental conditions
H1	Eutrophic, both stratified and shallow lakes with low nitrogen content.
Lo	Deep and shallow, oligo to eutrophic, medium to large lakes.
M	Eutrophic to hypertrophic, small- to medium-sized water bodies.
MP	Frequently stirred up, inorganically turbid shallow lakes.
S1	Turbid mixed environments. This codon includes only shade adapted cyanoprokaryotes.
S2	Warm, shallow, and often highly alkaline waters.
J	Shallow, mixed, highly enriched systems (including many low-gradient rivers).
X1	Shallow, eu-hypertrophic environments.
X2	Shallow, meso-eutrophic environments.
N	Continuous or semi-continuous mixed layer of 2–3 m in thickness. This association can be represented in shallow lakes where the mean depth is of this order or greater, as well as in the epilimnia of stratified lakes when the mixing criterion is satisfied.
P	Similar to that of codon **N** but at higher trophic states.
F	Clear, deeply mixed meso-eutrophic lakes.
G	Nutrient-rich conditions in stagnating water columns; small eutrophic lakes and very stable phases in larger river-fed basins and storage reservoirs.
T	Persistently mixed layers, in which light is increasingly the limiting constraint and thus optically deep, mixed environments including clear epilimnia of deep lakes in summer.
W1	ponds, even temporary, rich in organic matter from husbandry or sewages.
B	Mesotrophic small- and medium-sized lakes with species sensitive to the onset of stratification.
A	Clear, deep, base poor lakes, with species sensitive to pH rise.
C	Eutrophic small- and medium-sized lakes with species sensitive to the onset of stratification.
D	Shallow turbid waters including rivers.

Table 5: Total number of recorded microalgal species for each functional group in the investigated sites

FG	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
H1	0	0	1	0	0	0	0	1	0	0
Lo	4	2	3	1	3	2	5	3	0	2
M	2	2	2	1	1	1	1	1	0	1
MP	4	5	10	5	7	6	7	10	3	9
S1	1	1	2	2	2	3	1	1	0	2
S2	0	0	0	1	0	2	1	2	1	0
J	10	13	13	10	14	10	12	14	0	16
X1	4	4	4	5	4	6	4	4	0	3
X2	0	1	1	1	1	0	0	0	0	1
P	3	3	3	3	3	2	3	2	3	4
N	3	1	1	0	1	1	1	3	1	1
F	3	7	6	2	7	6	5	4	0	7
G	0	1	0	2	1	2	0	1	0	0
T	0	0	1	0	0	0	0	0	0	0
W1	0	1	0	4	6	8	0	0	0	1
B	1	1	1	1	1	0	1	1	1	1
C	1	1	1	1	1	1	1	1	0	1
D	8	9	10	4	6	6	8	11	7	9

To identify possible relationships between phytoplankton FGs and phenol pollution, dbRDA was performed considering the abundance of each functional group. The analysis indicated that phenol exerts adverse effects on the abundance for most of the identified FGs. In addition, the abundance of only three FGs (C, S1 and W1) was positively affected by phenol and organic matter (Fig. 2). On the other hand, the dbRDA revealed that increasing PO_4^{-}, SO_4^{2-}, Na^{+}, K^{+}, and EC have direct negative effects on the abundance and number of species of most of the identified FGs, which agreed with species diversity (Fig. 2, 3).

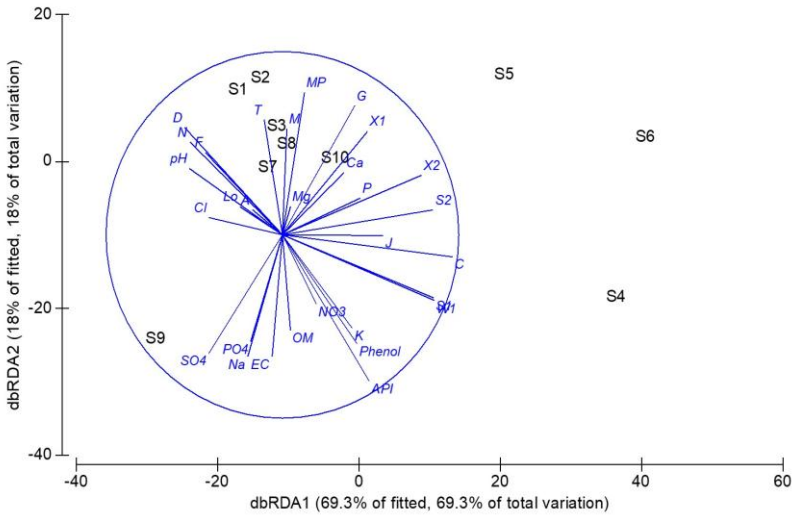

Fig. 2: Distance-based redundancy analysis indicating the variation in the abundance of different phytoplankton functional groups in different sites using Bray-Curtis similarity between samples and Spearman correlation with different abiotic factors and different biodiversity indices (T: temperature; OM: organic matter; Na: sodium; K: potassium; Ca: calcium; Mg: magnesium; NO_3: nitrate; SO_4: sulphate; PO_4: phosphate; API: algal pollution index). Different functinal groups (H1, Lo, M, MP, S1, S2, J, X1, X2, N, P, F, G, T, W1, B, A, C and D) as identified in Table 4.

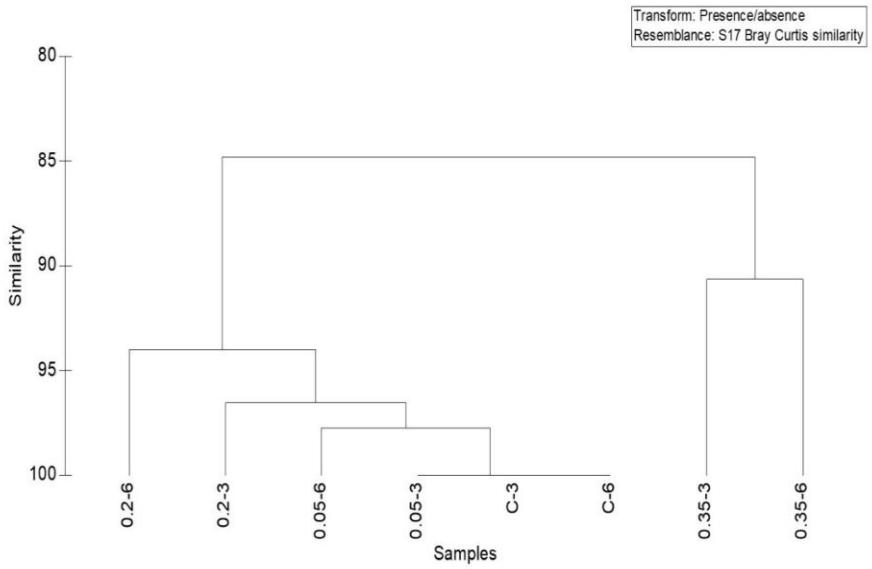

Fig. 3: Cluster analysis showing the effects of different phenol treatments. Analysis was performed using Bray-Curtis similarity index between species in different treatments. C: control; 0.05, 0.2, 0.35: phenol concentrations; 3: 3-day, 6: 6-day.

In vitro effect of phenol on microalgal diversity

The adverse effects of short-time toxicity of phenol towards the biodiversity of phytoplankton was investigated under *in vitro*-small scale experiment. The intolerable taxa to the investigated phenol concentrations were *Chroococcus turgidus* and *Gomphonema* sp. While the tolerable taxa were *Merismopedia tenuissima, Microcystis* sp.(1), *Microcystis* sp.(2), *Microcystis aeruginosa, Ankistrodesmus arcuatus, Ankistrodesmus fusiformis, Ankistrodesmus spiralis, Chlorella vulgaris, Chlorococcum hypnosporum, Coelastrum astroideum, Dictyosphaerium granulatum, Micractinium pusillum, Monoraphidium griffithii, Pseudopediastrum boryanum, Scenedesmus quadricauda, Scenedesmus ellipticus, Tetrastrum peterfii, Aulacoseira granulate, Aulacoseira italica, Craticula cuspidate, Cyclotella atomus, Cyclotella meneghiniana, Ulnaria ulna, Navicula gregaria, Nitzschia acicularis, N. fruticosa, N. linearis,* and *N. palea* (Table 6). These results indicated that the phenol toxicity is species specific. On the other hand, different phenol treatments were clustered into two main groups as indicated by the cluster analysis (Fig. 3). At one side, the controls, low and moderate concentrations of phenol (0.05 and 0.2 mg L^{-1}) represent the first group, while the second group contained the highest phenol concentration (0.35 mg L^{-1}). This result implied that increasing phenol concentration up to 0.35 mg L^{-1} had strong adverse effects on the algal community structure compared to low concentrations. The most sensitive microalgal taxa to 0.2 and/or 0.35 mg L^{-1} phenol were *Chroococcus turgidus*, *Gomphosphaeria* sp., *Actinastrum hantzschii*, *Coelastrum cambricum*, *Nephrocytium lunatum*, *Pediastrum simplex*, *Tetradesmus dimorphus*, *Staurastrum dorsidentiferum*, and *Gyrosigma attenuatum*. Moreover, exposure time to phenol stress plays a crucial role in structuring the phytoplankton community even at low concentrations.

Table 6: List of identified phytoplankton community and their occurrence in different phenol treatments.

Algal taxa	Control		0.05 mg L^{-1}		0.2 mg L^{-1}		0.35 mg L^{-1}		FG
	3-day	6-day	3-day	6-day	3-day	6-day	3-day	6-day	
Cyanobacteria									
Chroococcus turgidus (Kützing) Nägeli	+	+	+						Lo
Coelosphaerium sp.	+	+	+	+	+	+	+	+	Lo
Gomphosphaeria sp.	+	+	+	+					Lo
Merismopedia tenuissima Lemmermann	+	+	+	+	+	+	+	+	Lo
Microcystis sp.(1)	+	+	+	+	+	+	+	+	M
Microcystis sp.(2)	+	+	+	+	+	+	+	+	M
Microcystis aeruginosa Kützing	+	+	+	+	+	+	+	+	M
Pseudanabaena sp.	+	+	+	+	+	+	+		S1
No. of Cyanobacteria	**8**	**8**	**8**	**7**	**6**	**6**	**6**	**5**	
% Cyanobacteria	**17.78**	**17.78**	**17.78**	**16.28**	**14.29**	**15.38**	**17.14**	**17.24**	
Charophyta									
Staurastrum chaetoceras (Schröder) G.M.Smith	+	+	+	+	+	+	+		N
Staurastrum dorsidentiferum West & G.S.West	+	+	+	+	+	+			N
No. of Charophyta	**2**	**2**	**2**	**2**	**2**	**2**	**1**	**0**	
% Charophyta	**4.44**	**4.44**	**4.44**	**4.65**	**4.76**	**5.13**	**2.86**	**0.00**	
Chlorophyta									
Actinastrum hantzschii Lagerheim	+	+	+	+	+				J
Ankistrodesmus arcuatus Korshikov	+	+	+	+	+	+	+	+	J
Ankistrodesmus fusiformis Corda	+	+	+	+	+	+	+	+	X1
Ankistrodesmus spiralis (W.B.Turner) Lemmermann	+	+	+	+	+	+	+	+	X1
Chlorella vulgaris Beijerinck	+	+	+	+	+	+	+	+	X1
Chlorococcum hypnosporum Starr	+	+	+	+	+	+	+	+	MP
Coelastrum astroideum De Notaris	+	+	+	+	+	+	+	+	J
Coelastrum cambricum W.Archer	+	+	+	+	+	+			J
Dictyosphaerium granulatum Hindák	+	+	+	+	+	+	+	+	F
Lagerheimia longiseta (Lemmermann) Printz	+	+	+	+	+		+		J
Micractinium pusillum Fresenius	+	+	+	+	+	+	+	+	F
Monoraphidium griffithii (Berkeley)	+	+	+	+	+	+	+	+	X1
Nephrocytium limneticum (G.M.Smith) G.M.Smith	+	+	+	+	+	+	+		F
Nephrocytium lunatum West	+	+	+	+	+	+			F
Oocystis borgei J.W.Snow	+	+	+	+	+	+	+		F
Pediastrum simplex Meyen	+	+	+	+					J
Pseudopediastrum boryanum (Turpin) E.Hegewald	+	+	+	+	+	+	+	+	J
Scenedesmus quadricauda (Turpin) Brébisson	+	+	+	+	+	+	+	+	J
Scenedesmus ellipticus Corda	+	+	+	+	+	+	+	+	J
Tetradesmus dimorphus (Turpin) M.J.Wynne	+	+	+	+	+	+			J
Tetraëdron minimum (A.Braun) Hansgirg	+	+	+	+	+	+	+		J
Tetrastrum peterfii Hortobágyi	+	+	+	+	+	+	+	+	J
No. of Chlorophyta	**22**	**22**	**22**	**22**	**21**	**19**	**17**	**13**	

% Chlorophyta	**48.89**	**48.89**	**48.89**	**51.16**	**50.00**	**48.72**	**48.57**	**44.83**	
Bacillariophyta									
Aulacoseira granulata (Ehrenberg) Simonsen	+	+	+	+	+	+	+	+	P
Aulacoseira italica (Ehrenberg) Simonsen	+	+	+	+	+	+	+	+	MP
Craticula cuspidata (Kutzing) D.G.Mann	+	+	+	+	+	+	+	+	P
Cyclotella atomus Hustedt	+	+	+	+	+	+	+	+	MP
Cyclotella meneghiniana Kützing	+	+	+	+	+	+	+	+	B
Gomphonema sp.	+	+	+		+				C
Gyrosigma attenuatum (Kützing) Rabenhorst	+	+	+	+	+	+			MP
Navicula gregaria Donkin	+	+	+	+	+	+	+	+	MP
Nitzschia acicularis (Kützing) W.Smith	+	+	+	+	+	+	+	+	D
Nitzschia fruticosa Hustedt	+	+	+	+	+	+	+	+	D
Nitzschia linearis W.Smith	+	+	+	+	+	+	+	+	D
Nitzschia palea (Kützing) W.Smith	+	+	+	+	+	+	+	+	D
Ulnaria ulna (Nitzsch) Compère	+	+	+	+	+	+	+	+	D
No.of Bacillariophyta	**13**	**13**	**13**	**12**	**13**	**12**	**11**	**11**	
% Bacillariophyta	**28.89**	**28.89**	**28.89**	**27.91**	**30.95**	**30.77**	**31.43**	**37.93**	

The biodiversity indices (S, d, H′, D^+, sD^+, and L^+) showed remarkable variations between different phenol concentrations and exposure time in comparison to the controls (Table 7). The S, d, H′, D^+, and sD^+ values of the three phytoplankton groups were markdely reduced compared to the control especially at high phenol concentration (0.35 mg L^{-1}).

Furthermore, at this concentration, the variation in Cyanobacteria and Chlorophyta was markedly higher than diatoms (Fig. 4). On the other side, the variation in taxonomic distinctness (L+) for Cyanobacteria was increased to 23.24% at 0.05 mg L^{-1} (day 6) and reached 109% at 0.35 mg L^{-1} (day 6). In contrast the L+ values for Chlorophyta exhibited a slight variation, and that for diatoms was markedly increased to 15.5% at 0.35 mg L^{-1} in relation to the control (Fig. 4).

The variation in the taxonomic structure of the phytoplankton community was also analyzed using taxonomic dissimilarity (gamma+, Γ+) and grouped using cluster analysis (Fig. 5). The Γ+ can be defined as the mean of all taxonomic distances between all species in one treatment and their nearest relation in another treatment and is generally less affected by species richness.The cluster analysis of the Γ+ results grouped the treatments into two main groups which is quite similar to the cluster analysis of species based on Bray-Curtis similarity. It can be noted that the Γ+ values were markedly influenced by phenol concentration and exposure period. The highest dissimilarity was observed at 0.35 mg L^{-1} of phenol after 6-days (Fig.5).

Table 7: variation in different diversity indices of phytoplankton in response of *in vitro* effect of different phenol treatments

Samples	S	d	H'	D+	sD+	L+
Cyanobacteria						
C-3	8	3.366	2.079	36.9	295.2	106.3
C-6	8	3.366	2.079	36.9	295.2	106.3
0.05-3	8	3.366	2.079	36.9	295.2	106.3
0.05-6	7	3.083	1.946	36.51	255.6	131
0.2-3	6	2.791	1.792	35.56	213.3	167.9
0.2-6	6	2.791	1.792	35.56	213.3	167.9
0.35-3	6	2.791	1.792	35.56	213.3	167.9
0.35-6	5	2.485	1.609	33.33	166.7	222.2
Chlorophyta						
C-3	22	6.794	3.091	42.62	937.6	192.3
C-6	22	6.794	3.091	42.62	937.6	192.3
0.05-3	22	6.794	3.091	42.62	937.6	192.3
0.05-6	22	6.794	3.091	42.62	937.6	192.3
0.2-3	21	6.569	3.045	42.75	897.8	197
0.2-6	19	6.113	2.944	41.91	796.3	197.9
0.35-3	17	5.647	2.833	43.06	731.9	182.3
0.35-6	13	4.678	2.565	40.46	525.9	196.2
Diatoms						
C-3	13	4.678	2.565	46.3	601.9	178.6
C-6	13	4.678	2.565	46.3	601.9	178.6
0.05-3	13	4.678	2.565	46.3	601.9	178.6
0.05-6	12	4.427	2.485	45.29	543.4	177
0.2-3	13	4.678	2.565	46.3	601.9	178.6
0.2-6	12	4.427	2.485	45.29	543.4	177
0.35-3	11	4.17	2.398	44.85	493.3	206.3
0.35-6	11	4.17	2.398	44.85	493.3	206.3

S: total number of observed species; d: Margalef's species richness; H': Shannon's diversity index; D+: average taxonomic distinctness; sD+: total taxonomic distinctness: L+: variation in taxonomic distinctness. C: control; 0.05, 0.2, 0.35: phenol concentrations; 3: 3-day, 6: 6-day.

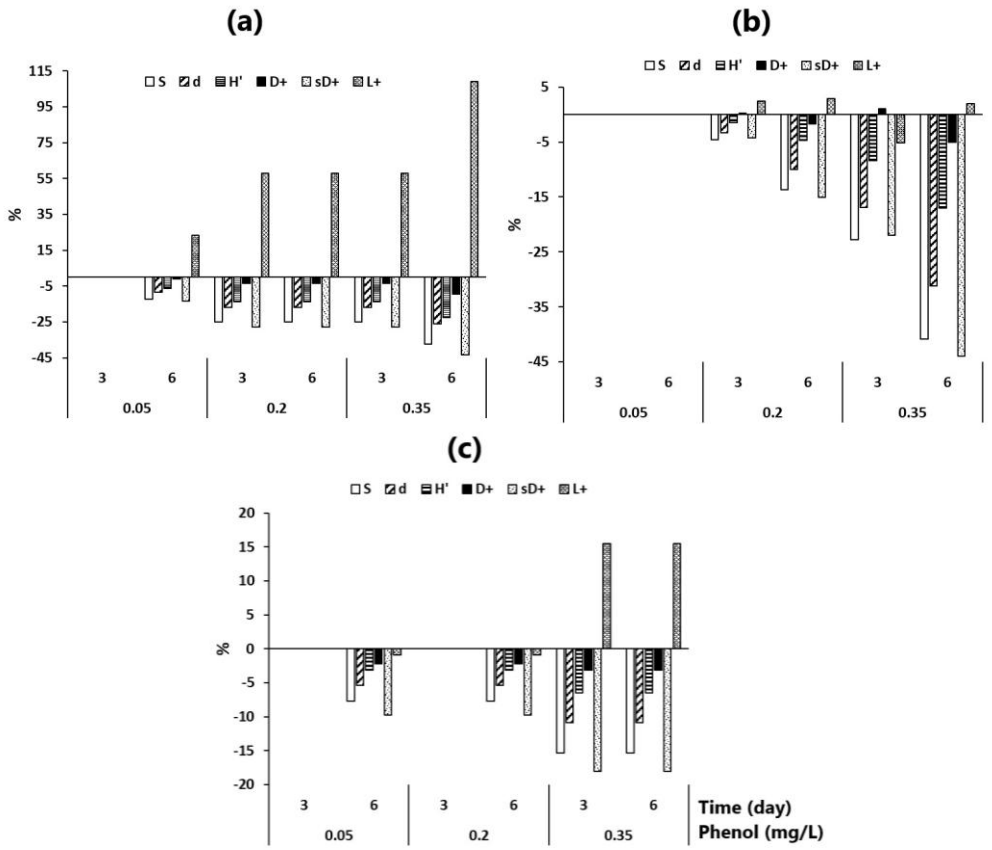

Fig. 4: Percentage variations in different diversity indices of (a) Cyanobacteria, (b) Chlorophyta, (c) Bacillariophyta compared to the control in response to different phenol treatments. S: total number of observed species; d: Margalef's species richness; H': Shannon's diversity index; D+: average taxonomic distinctness; sD+: total taxonomic distinctness: L+: variation in taxonomic distinctness.

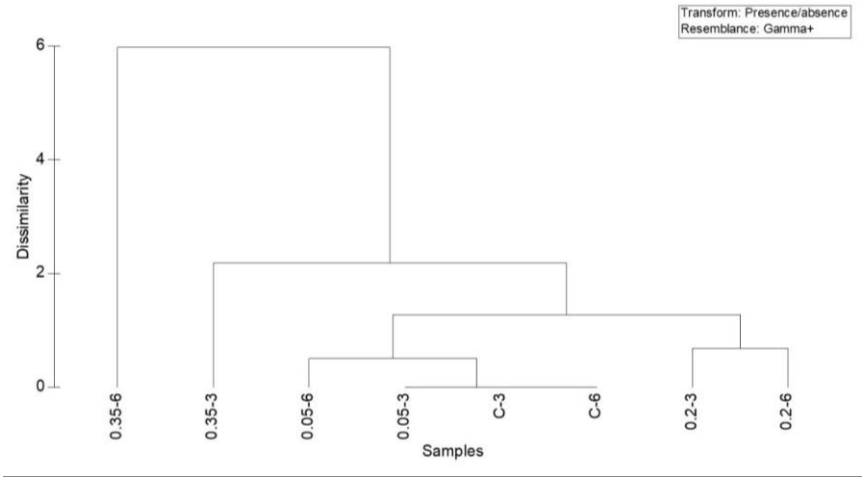

Fig. 5: Cluster analysis showing the effects of different phenol treatments on the taxonomic structure of the algal community. Analysis was performed using Gamma+ dissimilarity index between species in different treatments. C: control; 0.05, 0.2, 0.35: phenol concentrations; 3: 3-day, 6: 6-day.

DISCUSSION

Phenol is one of the well-known organic pollutants in the aquatic environments. However, little information is available in the literature regarding its negative effects on phytoplankton biodiversity. The concentration of phenol in the investigated sites were generally higher than 0.05 mg L^{-1}. Generally. the Environmental Protection Agency (EPA) indicated that the phenol concentration in potable and mineral waters should be below 0.5 µg L^{-1}, while the regulations for the discharged treated wastewater are 0.5 mg L^{-1} for surface waters and 1 mg L^{-1} for the sewage and industrial effluents [27].

In general, the existence of specific algal taxa in a polluted environment can be regarded as a bioindication of the type and extent of pollutants. For instance, *Nitzschia, Navicula, Scenedesmus, Oscillatoria, Microcystis,* and *Euglena* were continuously observed in habitats receiving high organic pollutants [28]. Similarly, the investigated sites in the present study contained pollution tolerant algal genera such as *Oscillatoria, Phormidium, Microcystis, Chlorella, Ankistrodesmus, Chlamydomonas, Pandorina, Micractinium, Scenedesmus, Closterium, Alaucoseira, Synedra, Ulnaria, Navicula, Nitzschia, Cyclotella, Euglena, Phacus* and *Lepocinclis*, which agreed with the list provided by Palmer [16]. Additionally, the prevalence of small centric diatoms, *Nitzschia* species, and small Chlorococcales is a bioindicator for organically polluted rivers [29]. Furthermore, the most dominant species with the highest number of individuals were *Scenedesmus quadricauda, Ulnaria ulna, Nitzschia palea, Alaucoseira granulata, Alaucoseira italica* and *Cyclotella meneghiniana*. These species were also reported previously as tolerant species to organic pollution [16]. In general, increasing organic pollution showed to have distinct negative impacts on richness, species and taxonomic biodiversity, and evenness of the phytoplankton community as indicated by the dbRDA analysis. Accordingly, the algal pollution index (API) was negatively correlated with richness and diversity of the main phytoplankton groups. This result was mainly related to the predominance of organic pollution-tolerant genera at the expense of sensitive ones. Furthermore, the present results indicated that phenol is one of the most hazardous organic pollutants that can affect biodiversity and composition of microalgae in the polluted habitat. However, the main phytoplankton groups showed different responses to the phenol pollution as indicated by the dbRDA. This behavior is consistent with the observations of Gomaa et al. [30] in relation to the organic pollution with the pharmaceutical compounds.

Generally, Chlorophyta were characterized by high species richness and diversity in the investigated sites, except site 9 followed by Bacillariophyta and Cyanobacteria. However, Bacillariophyta were characterized by the highest number of individuals. These results agreed with Elshobary and coauthors who reported that diatoms were the most dominant algal group in Ismailia canal, Egypt [31]. Additionally, the dbRDA analysis indicated a reduction in species richness and diversity of the major phytoplankton groups with increasing phenol and organic pollution. These results may be related to the disappearance of the most sensitive taxa to phenol pollution. However, this reduction in species richness and diversity was concomitant with increasing the number of individuals of green algae and Cyanobacteria, while that of diatoms was reduced. This effect may indicate that the most tolerant species to phenol stress belong to Chlorophyta and

Cyanobacteria. In other words, several green algae and Cyanobacterial species have been reported to utilize phenol as a carbon source for growth and metabolism [32–36] compared to diatoms [37]. In general, Cyanobacteria have been reported to flourish and show competitive interactions with different microalgal groups in organically contaminated environment, since they can effectively utilize several organic carbons for growth and metabolism [38].

Generally, phytoplankton species can adapt to different polluted environments by several structural and physiological changes [21]. The current results indicated that using phytoplanktons' FGs is an effective approach to estimate the adverse effects of organic pollution. *Ulnaria ulna* and *Nitzschia* spp. (group D) were recorded in all the investigated sites, however, their abundance was adversely affected by organic and phenol pollution. Similarly, Gomaa and coworkers observed a reduction in the abundance of this group as a consequence of the pharmaceutical pollution [30]. *Nitzschia* species are generally considered as cosmopolitan phytoplankton with high adaptability to pollution [15]. In contrast, the abundance of group S1 (*Phormidium* and *Pseudanabaena* spp.), group C (*Cyclotella meneghiniana*) and group W1 (Euglenozoa) showed a degree of positive correlation with increasing the organic and phenol pollution. Accordingly, these species may have adaptive strategies and can survive in organically polluted water, thus can be used as a bioindicators of phenol pollution.

On the other hand, several abiotic factors can possess direct effects on the phytoplankton diversity and structure. The dbRDA analysis indicated that increasing Na, K, EC, phosphate, and sulphate had adverse effects on the diversity and species richness of the major phytoplankton groups. In general, the environmental disturbance in water nutrients may contribute additional impacts with numerous drivers that can adversely affect the phytoplankton over time [39]. The change in the EC level gives direct information on the total dissolved solids, electrolytes, and degree of pollution [40,41]. The increase of EC of the polluted water was positively correlated with the API, which agreed with the observations of Çelekli [15]. Accordingly, the determination of EC and API in a given aquatic environment could provide direct estimations of organic pollution. The effects of phosphate on phytoplankton assemblage may be related to the indirect effect of alkaline pH and increasing calcium concentrations. As the pH rises above 7.0, most of the dissolved phosphates reacts with calcium to form insoluble compounds, which decreases the availability of phosphorus to phytoplankton, leading to a limited diversity [42].

The phytoplankton assemblage readily influenced in a short period when exposed to different phenol concentrations. A marked reduction in taxa richness and biodiversity was mainly attributed to the disappearance of the most sensitive taxa. As the concentration of phenol and exposure period increased, the structure of the phytoplankton community became more taxonomically dissimilar as indicated by the Γ+ analysis. This result indicated that most of the sensitive taxa are rather congeneric or emphasize their close association in the taxonomy, i.e., present in the same family or order.

CONCLUSION

The current investigation revealed the spatial variability in phytoplankton assemblage in relation to phenol pollution in different study sites at Assiut region, Egypt. In general, increasing phenol and organic pollution showed to have distinct adverse effects on richness, diversity, evenness, taxonomic diversity, and functional groups of the phytoplankton community as indicated by the dbRDA analysis. The algal pollution index (API) was fluctuated between 1.75 and 3.16, which indicated moderate to high organic pollution as well as it exhibited strong positive correlations with phenol pollution. *In vitro* phenol toxicity experiment showed that microalgal assemblage was altered as a consequence of short-term exposure to phenol stress. A distinct reduction in richness and biodiversity was mainly correlated to the disappearance of the most sensitive algal taxa. The results of the present study indicated that phenol is a toxic organic pollutant, which can adversely affect ecosystem functioning. Therefore, it is fundamental to monitor phenol pollution using phytoplankton as a bioindicator. Furthermore, proper water treatments are required to remediate phenol and reduce its toxic effects on the environment.

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