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Short-term changes in phytoplankton assemblages and their potential for heavy metal bioaccumulation – a laboratory study

by

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Abstract

This study focused on phytoplankton changes in polluted waters of Lake Manzala and the assessment of heavy metal bioaccumulation capacity during the 15-day laboratory experiment. Phytoplankton samples were analyzed every day and the concentration of zinc, iron and lead in water, in phytoplankton and in filtrate – every fifth day of the experiment. Significantly higher phytoplankton abundance was recorded in water from the El-Boom station (basin I) compared to the New Bahr El-Baqar drain (basin II), followed by distinct differences in its composition and chlorophyll content. However, the most abundant species were the same in both basins, i.e. *Chroococcus minor*, *Microcystis aeruginosa*, *Actinoptychus octonarius*, *Aulacoseira granulata*, *Pantocsekiella ocellata*, *Kirchneriella obesa* and *Nephrocytium limneticum*. Water in basin I was more polluted with heavy metals compared to basin II. Basin I was characterized by the dominance of cyanobacteria and high relative abundance of chlorophytes compared to basin II, where either cyanobacteria and/or diatoms dominated in the phytoplankton. In the former basin, the highest uptake factors (UFs) were recorded for iron and zinc and the lowest UF for lead. In basin II, the highest UF was determined for zinc, but relatively high UFs were recorded also for iron and lead. The presented results suggest that phytoplankton can contribute to natural biosorbents of heavy metals in Egyptian lakes.

Key words: heavy metals, bioaccumulation, chlorophyll, Cyanobacteria, diatoms, chlorophytes

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Introduction

At present, significant changes in lake ecosystems occur worldwide due to the impact of various climatic and anthropogenic factors. This applies also to the Egyptian lakes (El-Shabrawy et al. 2015; Shadrin et al. 2016). The pollution with heavy metals is one of the major environmental problems in recent years (Fu & Wang 2011), which affects i.e. Lake Manzala in Egypt (Zahran et al. 2015), but also other lakes in most countries around the world (Jaishankar et al. 2014). Consequently, water pollution affects the natural balance of aquatic ecosystems (Kosygin et al. 2007), which is largely associated with industrial, sewage or agricultural drainage (Sathware et al. 2007). The Bahr El-Baqar drain, considered as one of the most heavy-metal polluted drains in Egypt (Abdel-Shafy & Aly 2002), receives and carries most of the wastewater (3 billion m^3 per year or about 60 m^3 s^{-1} according to Stahl et al. 2009) into Lake Manzala. The drain runs through a very densely populated area of the Eastern Delta, i.e. through Qalyubia, Sharkia, Ismailia and Port Said Governorate. Zahran et al. (2015) demonstrated that Lake Manzala is highly contaminated with iron (Fe), cadmium (Cd), lead (Pb) and chromium (Cr), because of the continuous supply of various pollutants. The highest heavy metal concentrations were recorded in the northeastern and southern parts of the lake near the Bahr El-Baqar drain. Heavy metals, which are directly related to environmental pollution, have a major impact on all organisms (Mason 2002). This applies in particular to the biological toxicity of iron, copper (Cu), lead, cadmium, mercury (Hg), nickel (Ni), zinc (Zn) and manganese (Mn). The above metals tend to bio-accumulate and are ultimately transferred to higher levels of the food chain (Gustav 1974; Jaishankar et al. 2014). Some metals, such as Cu, Mn, Fe, and Zn, are considered essential micronutrients but become toxic when present in higher concentrations than those needed for normal growth (Nies 1999). Other heavy metals (e.g. Cd, Hg, and Pb), which play an unknown role in living organisms, are even toxic at very low concentrations (Wood 1974; Nies 1999).

The non-degradability and toxicity of heavy metals (Kathal et al. 2016) may cause serious damage to the health of organisms inhabiting a given ecosystem or to human health through the food chain (Angelone & Bini 1992; Chan et al. 2003; Tchounwou et al. 2012; Ali et al. 2016). Heavy metals can be directly accumulated by some organisms, e.g. phytoplankton. Such bioaccumulation capacities are often considered good indicators of exposure and risk, and they have been extensively used to assess contamination levels of heavy metals in polluted ecosystems (Phillips & Rainbow 1994). Several studies were undertaken to quantify the use of phytoplankton in either bioindication of environmental changes or heavy metal bioaccumulation (e.g. Hussian et al. 2015; Ali et al. 2016; Goher et al. 2016). Aquatic environments are usually rich in phytoplankton resources, which are relatively inexpensive to process and capable of accumulating high levels of metals, and can also

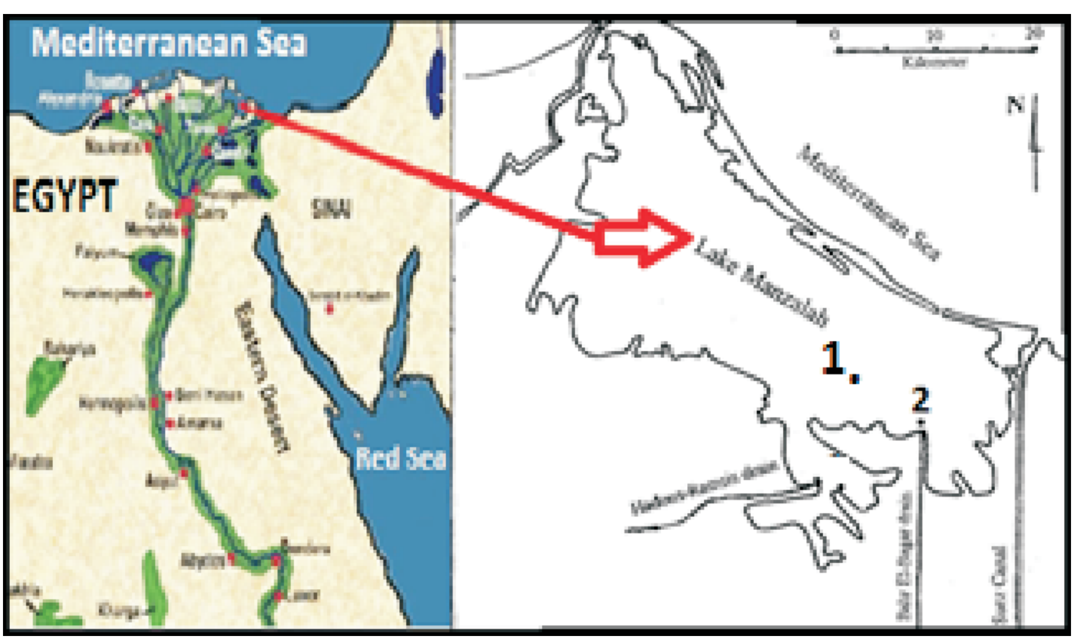

Figure 1

Water sampling sites: 1 – El-Boom station, 2 – New Bahr El-Baqar drain in Lake Manzala in Egypt be used as inexpensive biosorption materials in environmentally friendly technologies. The use of living phytoplankton to remove toxic metals from contaminated waters can be widespread, because phytoplankton is ubiquitous in almost all parts of the world. Metals can be removed from the surrounding environment through the accumulation in cells during both nonmetabolic-dependent (adsorption) and metabolic-dependent (absorption) processes (Perez-Rama et al. 2002; Malik 2004; Mehta & Gaur 2005; Perales-Vela et al. 2006; Topperwien et al. 2007; Lavoie et al. 2009). Furthermore, the direct or indirect presence of phytoplankton in the diet of many herbivores and predators contributes significantly to the bio-transfer of heavy metals to higher trophic levels. Thus, the phytoplankton and the assessment of its potential for heavy metal bioaccumulation are of particular concern. The amount of heavy metals in planktonic organisms may depend on their content in water and partly in sediment, as well as several environmental factors (Elmaci et al 2007). According to the findings of Bahnasawy et al. (2011), the concentrations of Cu, Zn, Cd, and Pb were much higher in phytoplankton than in water. This may be related to the active metabolism of planktic microorganisms characterized by rapid adsorption, the high surface-area-to-mass ratio, or even the fact that some microalgal species can also protect themselves by accumulating some pollutants, such as heavy metals, in their polysaccharide walls (Ravera 2001). Furthermore, some factors, including e.g. productivity and physicochemical properties of water bodies, quantitative and qualitative features of planktic assemblages, absorption capacity of heavy metals or even the seasons can affect the content of heavy metals in phytoplankton (Elmaci et al 2007).

The objective of this study was to describe the abundance and structure of phytoplankton in the polluted waters and to assess its ability to accumulate the selected heavy metals, primarily zinc, iron and lead regarded as the main pollutants in Lake Manzala.

Materials and methods

Water sampling and experiment setting

Water samples were collected from two sites: the El-Boom station (1) and the New Bahr El-Baqar drain (2) in Lake Manzala in Egypt (Fig. 1), in spring 2017. Lake Manzala is a coastal lake of the northern Nile Delta, surrounded with wetlands. It is one of the most important lakes with high fish production (about 30% of the total catch in Egypt). The Bahr El-Baqar drain is located in the eastern part of the Nile Delta (Abdel-Fattah & Helmy 2015). It runs for about 170 km from Cairo to Lake Manzala (Abdel-Shafy & Aly 2002), discharging approximately 1122 million m^3 of drainage waters with a salinity of 1237 g m^{-3} into the lake. Furthermore, the Bahr El-Baqar drain is considered as one of the most polluted drains with industrial and urban sewage, and with water unsuitable for reuse (Soleiman et al. 1994). Bahr El-Baqar with coordinates of 31°7′0″N and 32°6′0″E is divided into the new and old Bahr El-Baqar drains, which are connected with the lake in the south-eastern part. The El Boom station is located at the main west channel of the Bahr El-Baqar drain.

Both sampling sites were polluted with heavy metals (Stahl et al. 2009). Furthermore, they differed in terms of physicochemical parameters, primarily nutrients (Table 1). The concentrations of mineral nitrogen and phosphorus were generally higher at the New Bahr El-Baqar drain, while salinity lower than at the El-Boom station. The highest differences were related to ammonium (30 times), nitrates (7 times) and nitrites (3 times), and only about 1.5 times in the case of phosphorus. However, nutrient enrichment and low Secchi disk depth also indicated the hypertrophic conditions at both sampling sites.

Each 500 l water sample was transported to the aquarium. Basin I contained a water sample from the El-Boom station and basin II – a water sample from the New Bahr El-Baqar drain. Each basin was additionally divided into two 250 l glass subbasins to get more replications. The experiment was carried out without any additional treatments, supported only with air pumps and neon light, and then incubated at a laboratory temperature of 25°C for 15 days.

To follow up quantitative and qualitative changes in phytoplankton in the two basins during the experiment, daily water samples were collected to measure the total chlorophyll as a phytoplankton biomass proxy. Other samples were preserved to investigate the species composition and its density.

To evaluate the uptake for some heavy metals, the interval water sampling (on the 1st, 5th, 10th and 15th day) starting from the beginning to the end of the experiment was applied to each basin. The three kinds of samples for measurements of the total heavy metals: (1) in water – before the filtration process, (2) in phytoplankton (fresh weight) and (3) in filtrate – after filtration through GF/F filter paper, were collected. The samples were preserved with 15% nitric acid (HNO3) and placed into a freezer (at –20°C). They were stored in a refrigerator for atomic absorption measurements.

Table 1

Physicochemical parameters of waters at two sampling sites in Lake Manzala in spring 2017

Parameters	Units	Sampling sites	
		El-Boom	New Bahr El-Baqar Drain
Temperature	°C	26.4	26.2
Secchi disk depth	m	0.3	0.2
Total solids	$g\ l^{-1}$	3.71	3.03
Total dissolved solids	$g\ l^{-1}$	3.46	2.65
Total suspended solids	$g\ l^{-1}$	0.25	0.39
Electrical conductivity	$mS\ cm^{-1}$	5.41	4.12
pH		7.98	7.78
Dissolved oxygen	$mg\ l^{-1}$	14.2	1.2
Biological oxygen demand	$mg\ l^{-1}$	31.5	47.5
Chemical oxygen demand	$mg\ l^{-1}$	2.8	8.8
Nitrites	$\mu g\ l^{-1}$	21.12	68.57
Nitrates	$\mu g\ l^{-1}$	15.17	110.1
Ammonium	$\mu g\ l^{-1}$	325.37	9862.26
Orthophosphates	$\mu g\ l^{-1}$	324.53	445.08
Total phosphorus	$\mu g\ l^{-1}$	331.94	480.38
Silicon dioxide	$mg\ l^{-1}$	10.98	10.67
Salinity	PSU	2.66	2.12
TSI (Trophic State Index)[a]		82.7 (hypertrophy)	86.7 (hypertrophy)

[a]TSI calculations according to Carlson, Simpson (1996)

Analyses of chlorophyll, phytoplankton and heavy metals

A known volume of water samples was filtered in situ through a glass microfiber filter GF/F. The filter paper with filtrate was wrapped in aluminum foil and preserved in a dark ice box. In the laboratory, chlorophyll was extracted by soaking the filter in 5 ml acetone (90%) and preserved in dark at 20°C overnight. The samples were shaken thoroughly and centrifuged. The clear acetone extract was siphoned carefully and then measured spectrophotometrically to estimate chlorophyll *a*, *b* and *c*, using 90% acetone as blank. To measure pheophytins, the extract was acidified with 2 drops of 4N HCl and re-measured to calculate pheophytins. The concentrations of chlorophyll *a*, *b*, *c* and pheophytins were calculated according to APHA (1992).

For quantitative and qualitative analysis of phytoplankton, 250 ml of water samples was preserved with 4% neutral formalin and Lugol's iodine solution, which was then transferred into a glass cylinder with extra Lugol's iodine solution added to faint tea color, covered with aluminum foil and allowed for 5 days to settle (APHA 2012). Ninety percent of the supernatant fluid was siphoned off, the sample volume was adjusted to fixed volume (25 ml) and transferred to a small plastic vial for microscopic examination. The drop method was applied for counting and identification of phytoplankton species (APHA 2012) and triplicate samples (2 or 5 µl) were collected and examined under an inverted microscope ZEISS IM 4738, with a magnification of 400× and 1000× (with oil immersion). Results of phytoplankton density were presented as a number of cell per liter (cell l^{-1}). The main references used in the identification included: Krammer & Lange-Bertalot (1991), Popovsky & Pfiester (1990), Ettl & Gärtner (1988), Tikkanen (1986), Prescott (1978), Starmach (1974), Deskachary (1959), Hannford & Britton (1952) and Huber & Pestalozzi (1942). Taxonomic names of species were compared with Guiry & Guiry (2017) and currently accepted names are given in this study.

Analysis of heavy metals was carried out according to APHA (2012), using atomic spectrometry: inductively coupled plasma optical emission (ICP-OES), model Agilent 5100 Synchronous Vertical Dual View (SVDV) in water and quality control samples from National Institute Standards and Technology (NIST).

Numerical and Statistical analyses

Trophic conditions in Lake Manzala were assessed according to the Trophic State Index (TSI), which was calculated from the Secchi disk depth and concentrations of chlorophyll *a* and total phosphorus (Carlson & Simpson 1996). The uptake factor (expressed in %) was used to assess the bioaccumulation of heavy metals by phytoplankton as follows:

$$UF = \frac{C_{Ph}}{C_W} \times 100\%$$

where: C_{Ph} – concentration of heavy metals in phytoplankton (fresh weight) after filtration, C_W – concentration of heavy metals in water before filtration.

Significant changes in chlorophyll and phytoplankton abundance in the two experimental basins were tested using the U Mann-Whitney test, which is used to compare two independent groups. Furthermore, multiple comparisons of mean ranks for all samples (ANOVA, Kruskal-Wallis test) were applied to chlorophyll concentration. The statistical analyses were provided at the 0.05 significance level (StatSoft, Inc. v. 12). A cluster analysis based on the percentage similarity of taxonomic groups and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was applied to determine the similarity of phytoplankton assemblages' structure on each day of the experiment in both basins (Multi-Variate Statistical Package, MVSP, Kov. Comp. Serv. 1985-2009). The existence of any relationships between the features of phytoplankton and its capacity for heavy metal bioaccumulation were additionally tested using principal component analysis (PCA) and canonical correspondence analysis CCA.

Results and discussion

Chlorophyll, phytoplankton abundance and structure during the experimental period

Changes in the chlorophyll content (as a phytoplankton biomass proxy), phytoplankton density/abundance and structure in each experimental basin were analyzed on a daily basis. The concentration of chlorophyll ranged from 126.5 μg l^{-1} to 557.6 μg l^{-1} and from 130.3 μg l^{-1} to 785.3 μg l^{-1} in basin I and basin II, respectively (Fig. 2), during the whole experimental period, with no treatments and supported only with aeration, neon light and temperature of 25°C. The larger range of concentration in water of basin II from the New Bahr El-Baqar drain corresponded also to higher levels of mineral nitrogen and phosphorus. Nonetheless, general changes in the chlorophyll concentration in both experimental basins were not statistically significant, because of similar average chlorophyll concentrations of approximately 272.4 μg l^{-1} in both basins. Whereas different medians and coefficients of variation suggested a variable phytoplankton growth pattern for both experimental

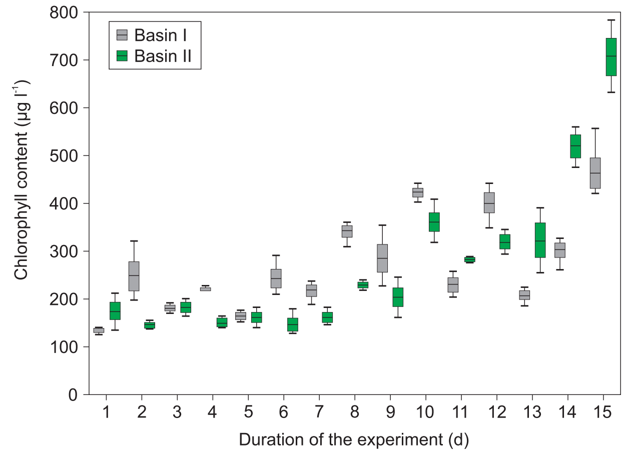

Figure 2

Chlorophyll content (mean ± SE, min.–max) in basin I (El-Boom station) and basin II (New Bahr El-Baqar drain) during the experimental period

basins. The multiple comparisons for each day of the experiment confirmed the significant differences ($p < 0.05$) between the 1st, 3rd, 5th and 10th, 12th, 15th, and between the 2nd, 4th, 6th, and 14th, 15th day of the experiment in basin I and basin II, respectively.

During the experiment, phytoplankton density changed from 190 × 10^4 cell l^{-1} to 780 × 10^4 cell l^{-1} and from 215 × 10^4 cell l^{-1} to 705 × 10^4 cell l^{-1} in basin I with the water sample from the El-Boom station and basin II with the water sample from the New Bahr El-Baqar drain, respectively (Fig. 3). The maximum phytoplankton abundance was recorded on the 10th (basin I) and 11th (basin II) day of the experiment, whereas the minimum abundance almost at the end

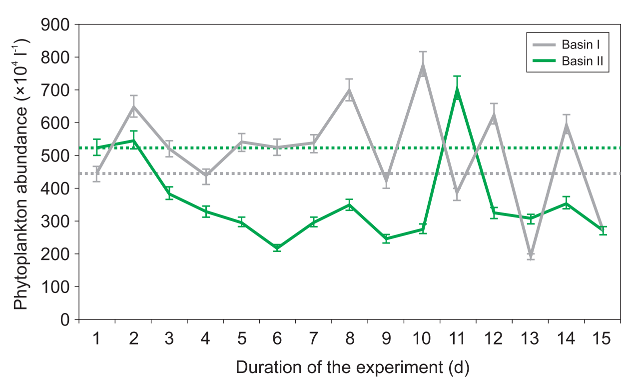

Figure 3

Phytoplankton abundance in basin I (El-Boom station) and basin II (New Bahr El-Baqar drain) during the experimental period; dotted lines indicate the abundance output levels of the experiment in each basin

Table 2

General features of phytoplankton (total abundance and abundance of dominant groups, No. × 10^4 cell l^{-1}) in both experimental basins

Abundance	Basin I			
	Total	Cyanophyceae	Chlorophyceae	Baccillariophyceae
Minimum	190	90	35	40
Maximum	780	345	270	205
Median	525	250	175	90
Mean	507.3	242.3	163.7	94.3
Standard deviation	157.1	69.0	75.3	47.8
Coefficient of variation (%)	31	28	46	51
Abundance	Basin II			
	Total	Cyanophyceae	Chlorophyceae	Baccillariophyceae
Minimum	215	75	35	25
Maximum	705	380	165	260
Median	325	145	70	105
Mean	361.3	181.3	71.3	105.0
Standard deviation	132.1	91.2	30.3	64.3
Coefficient of variation (%)	37	50	43	61

of the experiment. The mean and median values of the total abundance were higher and the coefficient of variation was lower in basin I compared to basin II (Table 2). The differences in the phytoplankton abundance changes in both experimental basins were statistically significant (the Mann-Whitney U test, U = 54.00, p = 0.016), indicating different growth patterns of phytoplankton.

The phytoplankton structure was primarily due to the high contribution of three main groups, i.e. Cyanophyceae, Chlorophyceae and Bacillariophyceae (Fig. 4). Cyanobacteria were always the most dominant group in basin I, accounting for 42–57% of the total abundance. The next group, chlorophytes, accounted for 13–47% and the last group, diatoms, accounted for 8–34% of the total abundance. In basin II, on the other hand, the structure of phytoplankton varied. In the first seven days of the experiment, phytoplankton was dominated by cyanobacteria (41–73%), while later (on the 8th, 10th, 12th, 14th and 15th day) – mainly by diatoms (36–46%) or cyanobacteria (on the 9th, 11th and 13th day – 43–57%). Chlorophytes played then a less important role in phytoplankton, with an average contribution of 21% in basin II and 31% in basin I. The representatives of the remaining phytoplankton groups were noted sporadically in both basins and their contribution was up to 4%. All this may indicate that the dominance of cyanobacteria in Egyptian lakes is a persistent problem of global eutrophication and health. Furthermore, the high abundance of cyanobacteria in Lake Manzala is significantly above the WHO's moderate risk threshold (Chorus & Bartram 1999) or even higher thresholds (Napiórkowska-Krzebietke et al. 2015) with cyanotoxin hazard.

In general, phytoplankton assemblages in basin I were represented by a total of 68 species, belonging

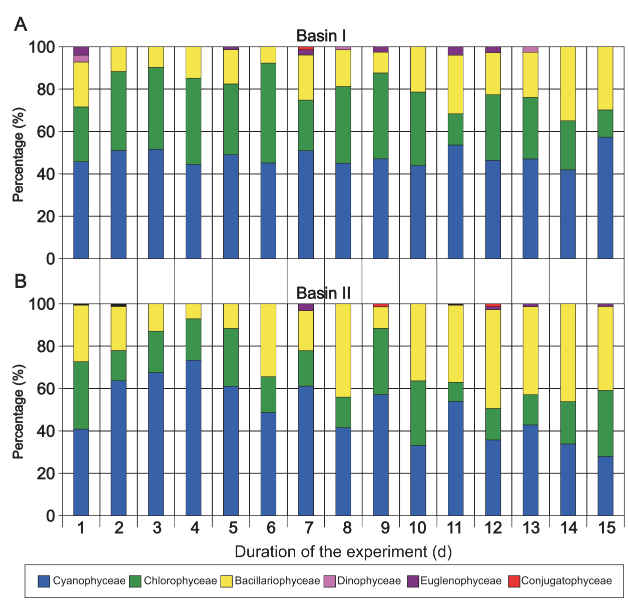

Figure 4

Phytoplankton structure in basin I – El-Boom station (A) and basin II – New Bahr El-Baqar drain (B) during the experimental period to 6 classes (Table 3). The highest species richness was determined for the Chlorophyceae class (25 species) with the highest abundance of *Kirchneriella obesa* (West) West & G.S. West and *Nephrocytium limneticum* (G.M. Smith) G.M. Smith. Next, the Bacillariophyceae class was represented by 18 species with the most abundant: *Actinoptychus octonarius* (Ehrenberg) Kützing, *Aulacoseira granulata* (Ehrenberg) Simonsen and *Pantocsekiella ocellata* (Pantocsek) K.T.Kiss & E.Ács. The Cyanophyceae class was represented by 16 species, and *Chroococcus minor* (Kützing) Nägeli and *Microcystis aeruginosa* (Kützing) Kützing were the most abundant. Furthermore, 7 species represented Euglenophyceae, whereas the lowest species richness was recorded for Dinophyceae and Conjugatophyceae, i.e. only one species for each group.

The species richness of phytoplankton in the second basin was similar to that in the first basin: 67 phytoplankton species belonging to 6 classes. However, the Chlorophyceae class was represented by a larger number of species (28), while Bacillariophyceae and Euglenophyceae classes by a smaller number of species (17 and 4, respectively) compared to basin I. The two other classes were represented by only one species. Furthermore, the same species of chlorophytes, cyanobacteria and diatoms were the most abundant as in basin I.

The cluster analysis by the UPGMA method based on the percentage similarity showed considerable differences in the daily phytoplankton structure and between both experimental basins (Fig. 5A, B). Three different groups were distinguished in basin I, starting from the 4th and 9th, 2nd and 8th, 3rd and 5th experimental day with the highest phytoplankton similarity of over 95% (Fig. 5A). Samples collected on other experimental days: the 1st and 11th, 12th and 10th, 6th, 7th and 14th day with 87–94% similarity were then included among these three groups, respectively. The least similar (77%) were samples collected on the 13th and 15th day, forming the fourth group. In basin II, three groups could be distinguished (Fig. 5B). In contrast to basin I, the highest similarity of 97% was found between samples collected on the 10th and 15th day of the experiment. The most similar phytoplankton assemblages developed also on the 8th, 14th, 12th and 13th day, co-forming together the first group with the overall similarity of 85%. The "center of the aggregation" of the second group were samples collected on the 5th and 7th day of the experiment with similarity of 95%, followed by a high similarity with samples from the 9th and from the 3rd and 4th day of the experiment. The remaining samples, i.e. from the 1st, 2nd and 11th day, comprised the last group.

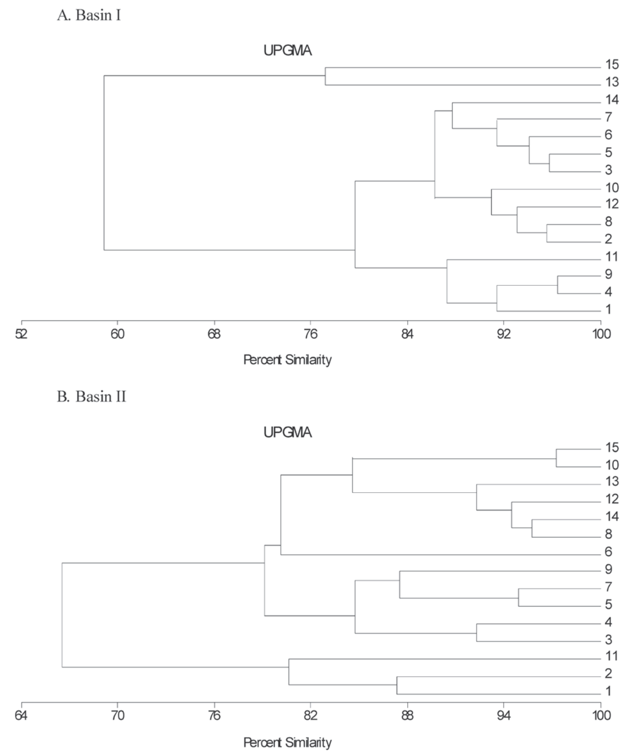

Figure 5

Cluster analysis based on similarity of phytoplankton taxonomic structure in basin I (A – water from El-Boom station) and basin II (B – water from New Bahr El-Baqar drain) during the 15-day experimental period; 1–15 consecutive days of the experiment

Bioaccumulation of heavy metals by phytoplankton

During the experiment, the three kinds of samples, i.e. one before filtration (water samples) and two after filtration (phytoplankton fresh weight samples and filtrate samples) were collected at 5-day intervals (the 1st, 5th, 10th and 15th day) and analyzed separately for heavy metals. The highest concentration of zinc (39.3–49.0 μg l^{-1}) and approximately 4 times lower concentration of iron (7.5–13.1 μg l^{-1}) were recorded in water of both basins during the whole experiment period (Fig. 6). Lead was then characterized by the lowest concentrations, i.e. 0.18–0.47 μg l^{-1} and 0.09–0.18 μg l^{-1} in basin I and II, respectively. The average concentrations of Zn, Fe and Pb in water usually reached 43.5 μg l^{-1}, 10.7 μg l^{-1} and 0.2 μg l^{-1}, respectively. These concentrations did not exceed their permissible limits according to the World Health Organization (WHO 2003a,b,c). Furthermore, their mean concentrations in a sequence of Zn > Fe >

Table 3

List of phytoplankton species investigated both in basin I (El-Boom) and basin II (New Bahr El-Baqar drain) during the experimental period (dominant species are in bold)

No.	Species	El-Boom	New Bahr El-Baqar drain
	CHLOROPHYCEAE		
1	*Acutodesmus acuminatus* (Lagerheim) P.M. Tsarenko	+	+
2	*Ankistrodesmus falcatus* (Corda) Ralfs	+	+
3	*Ankistrodesmus fractus* (West & G.S.West) Collins	+	+
4	*Chlorella vulgaris* Beyerinck	+	+
5	*Coelastrum microporum* Nägeli	+	+
6	*Crucigenia tetrapedia* (Kirchner) Kuntze	+	+
7	*Desmodesmus bicaudatus* (Dedusenko) P.M. Tsarenko	–	+
8	*Desmodesmus maximus* (West & G.S.West) Hegewald	–	+
9	*Elakatothrix gelatinosa* Wille	+	+
10	*Franceia ovalis* (Francé) Lemmermann	+	+
11	*Fusola viridis* J.W. Snow	+	+
12	*Golenkinia radiata* Chodat	+	-
13	*Kirchneriella aperta* Teiling	+	+
14	*Kirchneriella major* C. Bernard	+	+
15	***Kirchneriella obesa* (West) West & G.S. West**	**+**	**+**
16	*Monoraphidium convolutum* (Corda) Komárková-Legnerová	+	+
17	***Nephrocytium limneticum* (G.M. Smith) G.M. Smith**	**+**	**+**
18	*Oedogonium americanum* Transeau	+	+
19	*Oocystis pyriformis* Prescott	+	+
20	*Pseudopediastrum boryanum* var. *longicorne* (Reinsch) Tsarenko	–	+
21	*Pseudokirchneriella elongata* (G.M.Smith) F.Hindák	–	+
22	*Quadrigula chodatii* (Tanner-Füllemann) G.M. Smith	–	+
23	*Scenedesmus armatus* (Chodat) Chodat	+	–
24	*Scenedesmus bijugus* (Turpin) Lagerheim	–	+
25	*Scenedesmus obtusus* Meyen	+	+
26	*Schroederia jadayi* G.M. Smith	+	–
27	*Selenastrum bibraianum* Reinsch	+	+
28	*Tetradesmus dimorphus* (Turpin) M.J. Wynne	+	+
29	*Tetraëdron minimum* (A. Braun) Hansgirg	+	+
30	*Tetraëdron trigonum* (Nägeli) Hansgirg	+	+
31	*Tetraselmis suecica* (Kylin) Butcher	+	+
	BACILLARIOPHYCEAE		
1	***Actinoptychus octonarius* (Ehrenberg) Kützing**	**+**	**+**
2	***Aulacoseira granulata* (Ehrenberg) Simonsen**	**+**	**+**
3	*Biddulphia biddulphiana* (J.E. Smith) Boyer	+	+
4	*Ceratoneis closterium* Ehrenberg	+	+
5	*Chaetoceros lorenzianus* Grunow	–	+
6	*Cocconeis neodiminuta* Krammer	+	+
7	*Cyclotella meneghiniana* Kützing	+	+
8	*Cymbella tumida* (Brébisson) van Heurck	+	–
9	*Entomoneis alata* (Ehrenberg) Ehrenberg	+	+
10	*Fragilaria vaucheriae* (Kützing) J.B. Petersen	+	+
11	*Lyrella lyra* (Ehrenberg) Karajeva	+	+
12	*Nitzschia acicularis* (Kützing) W. Smith	+	+
13	*Nitzschia frustulum* var. *perpusillum* (Rabenhorst) van Heurck	–	+
14	*Nitzschia linearis* W. Smith	+	+
15	*Nitzschia palea* (Kützing) W. Smith	+	+
16	***Pantocsekiella ocellata* (Pantocsek) K.T. Kiss & E.Ács**	**+**	**+**
17	*Pinnularia major* (Kützing) Rabenhorst	–	+

Tabele 3 continued

18	*Pleurosigma elongatum* W. Smith	+	–
19	*Triceratium favus* Ehrenberg	+	–
20	*Triceratium grande* var. *septangulata* (Kitton) Schmidt	+	–
21	*Ulnaria ulna* (Nitzsch) Compère	+	+
	CYANOPHYCEAE		
1	*Anabaena wisconsinensis* Prescott	+	+
2	*Anabaenopsis circularis* (G.S. West) Woloszynska & V. Miller	+	+
3	*Aphanocapsa grevillei* (Berkeley) Rabenhorst	+	+
4	***Chroococcus minor* (Kützing) Nägeli**	**+**	**+**
5	*Chroococcus minutus* (Kützing) Nägeli	+	+
6	*Chroococcus pallidus* Nägeli	+	+
7	*Dactylococcopsis raphidioides* Hansgirg	+	+
8	*Glaucospira laxissima* (G.S. West) Simic, Komárek & Dordevic	+	–
9	*Gloeobacter violaceus* Rippka, J.B. Waterbury & Cohen-Bazire	+	+
10	*Gloeocapsa punctata* Nägeli	+	+
11	*Gomphosphaeria aponina* Kützing	+	+
12	*Leptolyngbya tenuis* (Gomont) Anagnostidis & Komárek	+	+
13	*Merismopedia tenuissima* Lemmermann	+	+
14	***Microcystis aeruginosa* (Kützing) Kützing**	**+**	**+**
15	*Oscillatoria tenuis* C. Agardh ex Gomont	+	+
16	*Phormidium inundatum* Kützing ex Gomont	+	+
17	*Rhabdoderma lineare* Schmidle & Lauterborn	+	+
	EUGLENOPHYCEAE		
1	*Euglena minuta* Prescott	+	+
2	*Lepocinclis buetschlii* Lemmermann	+	–
3	*Lepocinclis ovum* (Ehrenberg) Lemmermann	+	+
4	*Phacus longicauda* (Ehrenberg) Dujardin	+	–
5	*Trachelomonas armata* (Ehrenberg) F. Stein	+	+
6	*Trachelomonas hispida* (Perty) F. Stein	+	+
7	*Trachelomonas cylindracea* (Playfair) T.G. Popova	+	–
	CONJUGATOPHYCEAE		
1	*Euastrum binale* var. *gutwinskii* (Schmidle) Homfeld	+	–
2	*Cosmarium laeve* Rabenhorst	–	+
	DINOPHYCEAE		
1	*Prorocentrum tsawwassenense* Hoppenrath & B.S. Leander	+	+

+ present, – absent

Pb were ranked in a different way compared to the results from 31 sampling sites in Lake Manzala [Fe (29.6 μg l^{-1}) > Zn (5.2 μg l^{-1}) > Pb (4.7 μg l^{-1})] acquired during the previous studies (Zahran et al. 2015). In general, the water from the El-Boom station in basin I with its higher phytoplankton abundance and significantly varying chlorophyll content (on the 1st, 3rd, 5th and 10th, 12th, 15th day) was more polluted with heavy metals compared to water from the New Bahr El-Baqar drain in basin II, characterized by higher content of nutrients. Furthermore, only the concentrations of Zn and Fe statistically significantly varied (zinc p = 0.043; iron p = 0.030) in both basins.

General trends included the gradual decrease in the content of heavy metals in water and their rapid (especially at the beginning) increase in phytoplankton throughout the experimental period. Clear differences in bioaccumulation are reflected in the uptake factor (UF) of heavy metals in phytoplankton, including ach day of the experiment and each basin. In basin I, zinc and iron had the highest UF starting from the 5th day up to the end of the experiment (90–92% and 81–99%, respectively) (Fig. 7) along with the increase in the total phytoplankton abundance up to the 10th day. The bioaccumulation of lead in phytoplankton was the lowest up to the 10th day (16–32%), and it reached 83% only on the last day of the experiment, i.e. on the 15th day when the total abundance dropped below its initial value. Cyanobacteria dominated in phytoplankton and their abundance was approximately two times higher compared to diatoms and chlorophytes every fifth day of the experiment. The process of bioaccumulation was clearly different during the 15-day experiment in basin II. This may be due to the differences in phytoplankton abundance and structure, i.e. the lower total abundance and

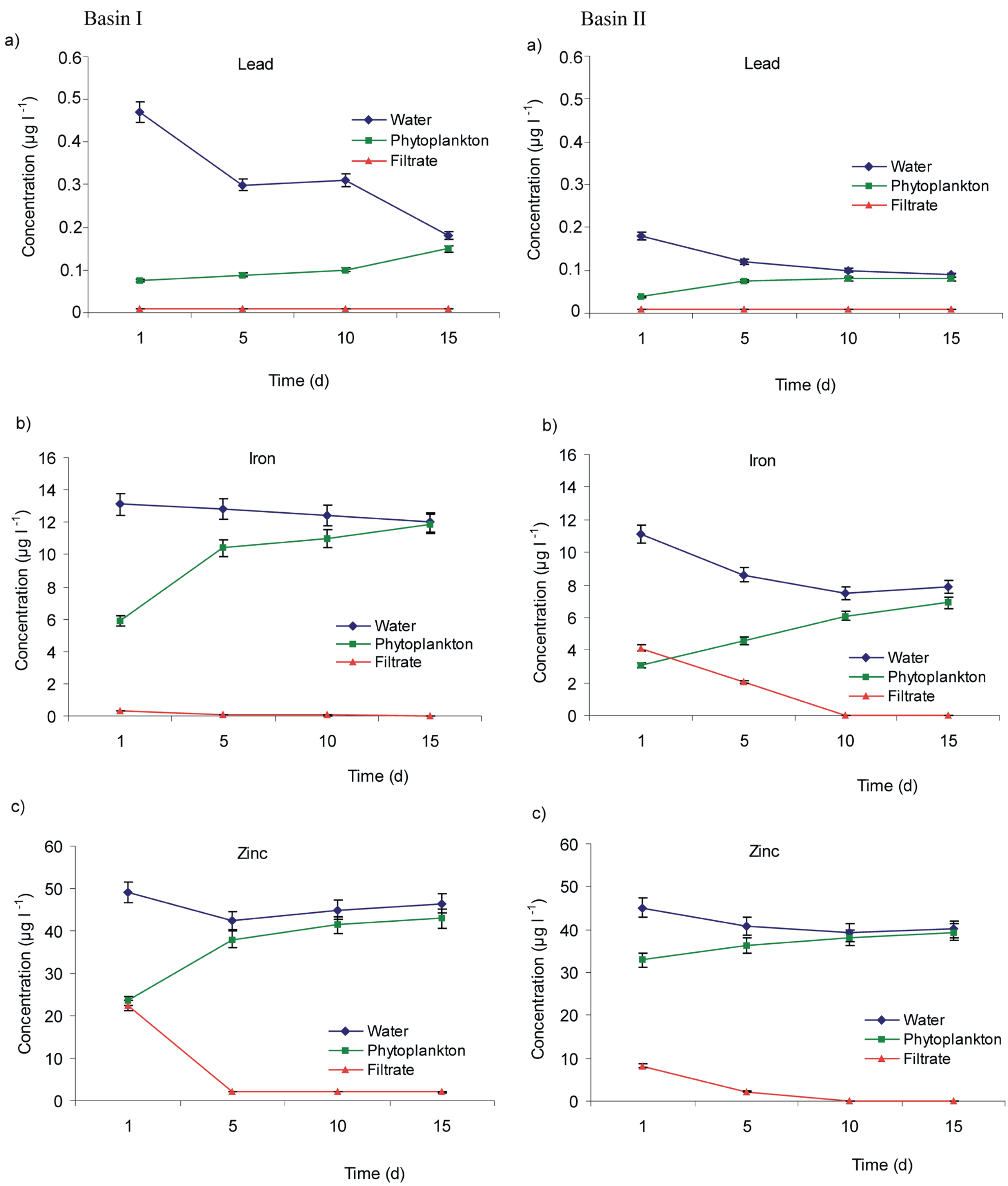

Figure 6

Uptake of heavy metals: lead (a), iron (b) and zinc (c) by phytoplankton in basins I and II during the experimental period

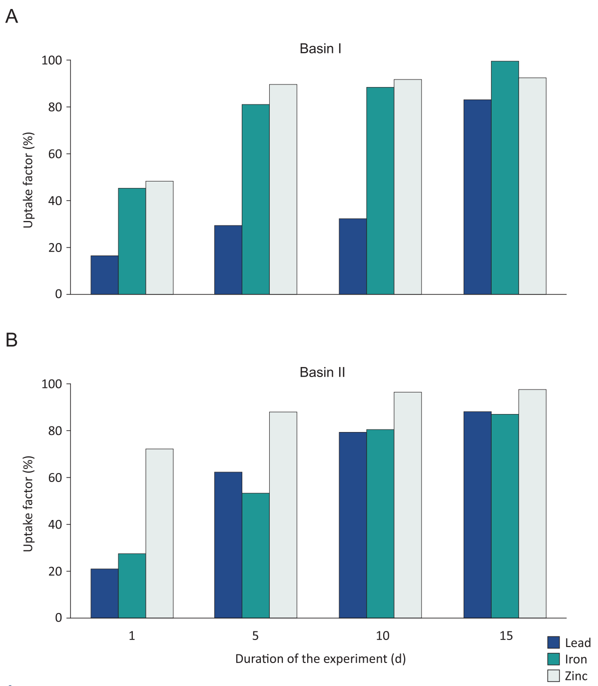

Figure 7

Uptake factor (%) used to assess the rate of heavy metal bioaccumulation by phytoplankton in basin I (A) and basin II (B)

abundance of cyanobacteria and chlorophytes and the higher abundance of diatoms in basin II compared to basin I. Starting from the 5th day to the end of the experiment, the uptake factors were higher in the case of zinc (89–98%) and lead (63–89%), and lower in the case of iron (53–87%). Cyanobacteria dominated in phytoplankton only on the 5th day, followed by the dominance of diatoms (on the 10th and 15th day) and chlorophytes (on the 15th day). Furthermore, the total phytoplankton abundance gradually decreased up to the 10th day of the experiment along with the rapid increase in the uptake of all heavy metals. Such a different course of the bioaccumulation process due to differences in phytoplankton is consistent with findings of Elmaci et al. (2007). On the other hand, the different bioaccumulation degree can also results from the existence of two stages: the first stage of initial passive and rapid uptake of heavy metals and the second stage of active and slow uptake (Shanab et al. 2012). The present results did not show such sensitivity of cyanobacteria to heavy metals as that found by Shanab et al. (2012), maybe because the phytoplankton in Lake Manzala was dominated by chroococcalean and not by nostocalean cyanobacteria.

Furthermore, the PCA revealed the existence of correlation between the total abundance of phytoplankton and its taxonomic groups, and the content of heavy metals and bioaccumulation potential (Fig. 8A). The first two factors (PC1 and PC2) explained 73% of the total variability. The total phytoplankton abundance and the abundance of Cyanophyceae, Dinophyceae and Chlorophyceae were strongly and positively correlated with PC1, which explained 53% of the total variability. Similar relationships were recorded for the content of heavy metals in water which, in turn, was closely related to phytoplankton. The uptake factors of heavy metals were strongly and negatively-correlated with PC1, whereas such relations with PC2, which explained only 20% of the total variability, were weaker. Furthermore, the relations between phytoplankton and heavy metals were additionally checked for the samples collected on the 1st, 5th, 10th and 15th day of the experiment (Fig. 8B). It was possible to distinguish two groups, one group with samples from basin I and

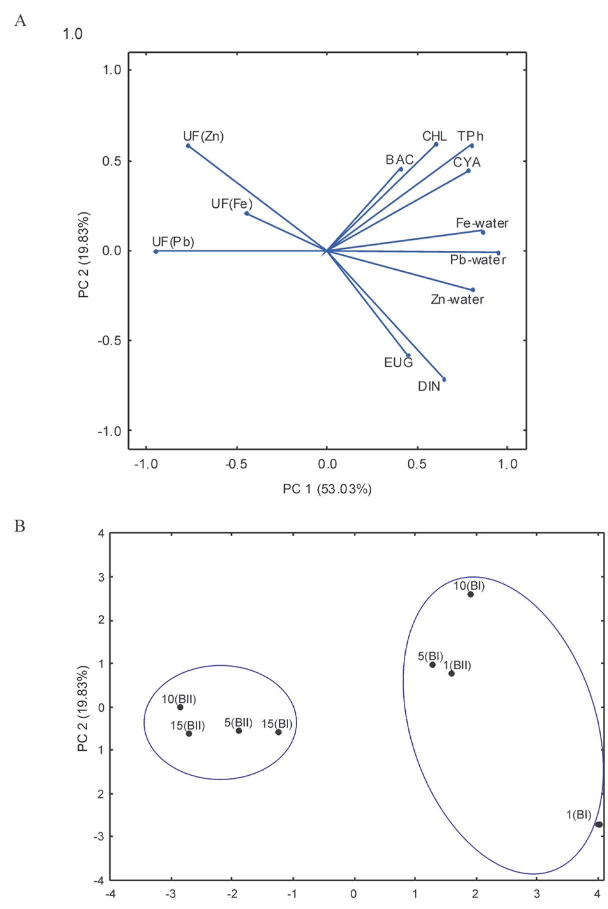

Figure 8

PCA-based relationships between phytoplankton and heavy metal bioaccumulation (A) and ordination of samples (B) during the 15-day experiment in basin I (BI) and basin II (BII)

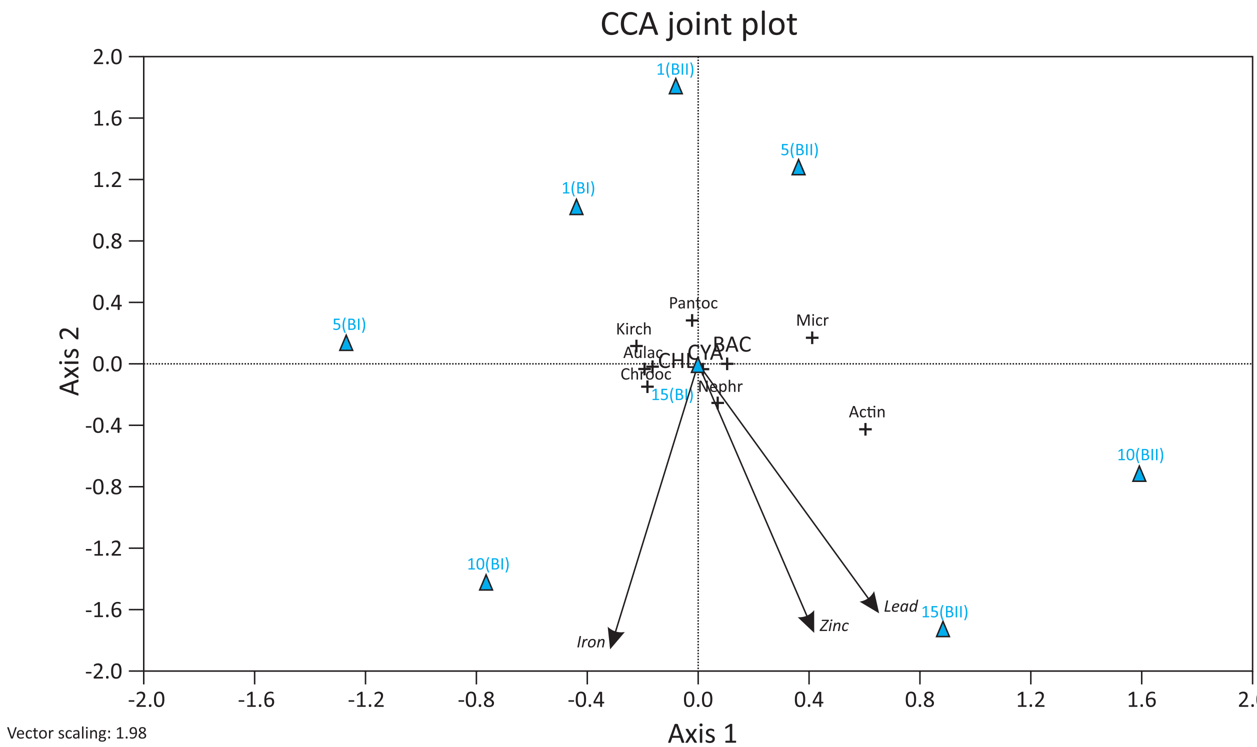

Figure 9

CCA joint plot of relationships between dominant phytoplankton species and classes and iron, zinc and lead bioaccumulation capacity on the 1st, 5th, 10th and 15th day of the experiment in basin I (BI) and basin II (BII) based on Canonical Correspondence Analysis. Kirch – *Kirchneriella obesa*, Nephr – *Nephrocytium limneticum*, Actin – *Actinoptychus octonarius*, Aulac – *Aulacoseira granulata*, Pantoc – *Pantocsekiella ocellata*, Chrooc – *Chroococcus minor*, Micr – *Microcystis aeruginosa*, CHL – Chlorophyceae, BAC – Bacillariophyceae, CYA – Cyanophyceae

the other group with samples from basin II, except for samples 1(BII) and 15(BI). CCA analysis revealed also some relationship between the dominant species and the taxonomic class of phytoplankton and bioaccumulation of heavy metals (Fig. 9). The eigenvalues were 0.037 and 0.015 for the first and the second axis, respectively. The cumulative percentage reached 40.2% of the total variation for both axes. The species-environment correlations were 0.925 and 0.795 for the 1st and 2nd axis, respectively. The grouping of the samples was very similar to that obtained with PCA.

Cyanobacteria with *Chroococcus minor* and *Microcystis aeruginosa*, chlorophytes with *Kirchneriella obesa* and *Nephrocytium limneticum* and diatoms with *Actinoptychus octonarius*, *Aulacoseira granulata* and *Pantocsekiella ocellata* played the main role in the bioaccumulation of heavy metals in Lake Manzala as the most abundant species in phytoplankton. In basin I, the dominant cyanobacteria contributed primarily to the very high bioaccumulation level of iron and zinc and the reduced bioaccumulation level of lead. Chlorophytes also played an important role. In basin II, initially cyanobacteria and then diatoms primarily contributed to a similarly high UF of iron and lead and the accelerated UF of zinc.

Lake Manzala is one of the most important resources for fishing in Egypt, but it receives treated and untreated wastewaters of municipal, industrial and agricultural origin (Zahran et al. 2015). The results of the present experimental studies suggest that high cyanobacteria abundance can contribute to either nuisance problems or natural biosorbents of heavy metals in Egyptian lakes. The applicable and effective technologies including bioremediation or biosorption for removal of heavy metals (e.g. Champagne 2009; Baskaran et al. 2010; Mann & Mandal 2014; Goher et al. 2016) are still needed. Therefore, studies that have been conducted to investigate the levels of heavy metals in aquatic environments, their bioaccumulation level in organisms and factors that affect the bioaccumulation process by various organisms (Machiwa 1992; 2000; Ferletta et al. 1996; Engdahl et al. 1998) should be continued.

Conclusions

Nutrient-rich water contaminated with heavy metals delivered with wastewaters of municipal, industrial and agricultural origin was collected for further studies from two selected sites in Lake Manzala. The 15-day experiment with no additional treatments, supported only with aeration, neon light and temperature of 25°C was aimed at describing the abundance and structure of phytoplankton, and assessing the bioaccumulation potential. The water sample from the El-Boom station (basin I) was characterized by significantly higher phytoplankton abundance compared to water sample from the New Bahr El-Baqar drain (basin II) throughout the experiment period. The main phytoplankton groups were dominant Cyanophyceae and co-dominant Bacillariophyceae and Chlorophyceae. Considering the day-to-day changes in the chlorophyll content, the significant differences were recorded between selected days (the 1st, 3rd, 5th and 10th, 12th, 15th day of the experiment in basin I and the 2nd, 4th, 6th, and 14th, 15th day of the experiment in basin II). The concentrations of Zn, Fe and Pb were usually higher in basin I than in basin II. The differences were also related to the degree of bioaccumulation (expressed as an uptake factor) in phytoplankton which, in turn, was connected with clearly different structures in both basins. The dominance of Cyanobacteria with a high contribution of chlorophytes contributed to the highest bioaccumulation of iron and zinc in basin I, whereas cyanobacteria with/or primarily diatoms had an important contribution to the highest UF of zinc and similarly high UF of iron and lead in basin II.

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