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Seasonal variation in microcystin levels of river Nile water at Sohag City, Egypt

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Keywords : Cyanobacteria, cyanotoxins, microcystin, river Nile, Egypt.

The river Nile is the main source of drinking water in Egypt. Nutrient loading coupled with year-round warm weather favor the growth of cyanobacteria, several of which can produce cyanotoxins, especially the potent liver toxins called microcystins. Most microcystin resides inside or closely attached to cyanobacteria cells, and are released into the water column as cells lyse from senescence or chemical treatment. The present study evaluated microcystin levels, as measured by immunoassay (ELISA), in both raw and finished drinking water of the river Nile, during the warm season (May-October), near the drinking water intake for Sohag City, Egypt.

The results showed that microcystin content within the cells correlated better with type of microcystin-producing cyanobacteria (*Gomphosphaeria, Microcystis, Oscillatoria*) rather than chlorophyll a. Microcystin concentration in cell-free water correlated significantly with that measured within the cells, with maximum values being recorded in September (0.4-0.78 $\mu g\ l^{-1}$). Microcystin levels in the finished drinking water were low (56.1- 87.1 $ng\ l^{-1}$) and were detected only in May and June. The study indicates that microcystin is present in the raw and finished drinking water at Sohag City but that levels did not exceed the World Health Organization (WHO) drinking water guideline level of $1\mu g\ l^{-1}$ during May-October 1999.

Variations saisonnières des concentrations en microcystines dans les eaux du Nil à Sohag, Egypte

Mots clés : cyanobactéries, cyanotoxines, microcystines, Nil, Egypte.

Le Nil est la principale source d'approvisionnement en eau potable en Egypte. Les apports en nutriments, couplés à un climat chaud permanent favorisent la croissance des cyanobactéries dont plusieurs espèces peuvent produire des cyanotoxines, et spécialement des microcystines potentiellement toxiques pour le foie. La plupart des microcystines sont contenues ou fortement attachées dans les cellules et sont relarguées dans la colonne d'eau aussi bien par lyse au moment de la sénescence qu'après traitement chimique. Cette étude évalue les concentrations en microcystines, mesurées d'après des immunoessais (ELISA), dans les eaux brutes du Nil et après traitement destiné à la potabilité, durant la saison chaude (mai-octobre), près de la prise d'eau de la ville de Sohag.

Les résultats montrent que le niveau en microcystines dans les cellules est mieux corrélé avec le type cyanobactéries productrice de microcystine (*Gomphosphaeria, Microcystis, Oscillatoria*) qu'avec la chlorophylle a. La concentration en microcystine dans l'eau (cellules exclues) est significativement corrélée avec celle mesurée dans les cellules, avec un maximum observé en septembre (0,4-0,7 μgl^{-1}). Les concentrations de microcystines dans l'eau, produites en vue de la distribution d'eau potable, étaient basses (56,1-87,1 ngl^{-1}) et seulement détectées en mai et juin. L'étude indique que la microcystine est présente dans les eaux brutes et dans les eaux après traitement à Sohag. Cependant, durant la période mai-octobre 1999, les valeurs observées n'excèdent pas le niveau guide de 1 μgl^{-1} recommandé par l'Organisation Mondiale de la Santé (OMS).

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1. Introduction

In the seasonal cycle of freshwater phytoplanton occurring in temperate lakes, the appearance of cyanobacteria is probably due to increased light and temperature at the end of spring (Reynolds et al. 1981). In warmer climates where annual temperatures and light intensities are higher, cyanobacteria can dominate in high nutrient waters (Hallegraeff 1993). In marine water, harmful algae are almost exclusively the red tide organisms called dinoflagellaes, while in fresh and brackish waters, they are the cyanobacteria (Carmichael 1996).

Cyanobacteria are well-known producers of potent toxins that may cause death of domestic animals and wildlife (Skulberg et al. 1984, Gorham & Carmichael 1988, Carmichael 1997), and jeopardize human health (Billings 1981, Bourke et al. 1983, Falconer et al. 1983, Turner et al. 1990, Hayman 1992, Jochimsen et al. 1998). They produce two major groups of toxins, alkaloid neurotoxins and peptide hepatotoxins (Carmichael 1988, 1992). Of particular importance are the hepatotoxic cyclic peptides called microcystins (MCYSTs) (Skulberg et al. 1993, Carmichael 1997).

Toxic cyanobactria have been commonly observed in reservoirs and rivers used as drinking water sources ; and in recreational waters worldwide (Watanabe et al. 1989, Sivonen et al. 1990, Carmichael 1992, Carmichael & Falconer 1993, Brittain et al. 2000). Although Mohamed (1998) isolated and charcterized MCYSTs from toxic species of cyanobacteria in river Nile irrigation canals and fish ponds in Egypt, no data has been published on MCYST levels in the drinking water sources. Therefore, we undertook this study to determine levels of MCYSTs within the phytoplankton and surrounding water in the river Nile as well as in finished drinking water during the main part of the water-bloom forming season for cyanobacteria in Egypt.

2. Materials and methods

2.1. Collection of samples

Samples were collected monthly using a 20 μm meshnet during the warm season (May — October 1999) from the river Nile close to the intake of two water treatment stations built on the river Nile at Sohag city (Fig. 1). Each sample was a composite of three subsamples collected at different distances around the intake of each water treatment station. Finished drinking water samples were collected from the tap water of three houses which received water from these water treatment stations.

2.2. Measurement of chlorophyll

Chlorophyll a as a measure of biomass, was determined spectrophotometrically in a methanol extract of a known volume of the river Nile water sample according to the method of Talling & Driver (1963). Phytoplankton cells were counted using a haemocytometer, and algal species were identified according to Presccot (1978).

2.3. Determination of MCYST levels

MCYST concentrations were determined in phytoplankton by the enzyme-linked immunosorbent assay (ELISA) according to An & Carmichael (1994). The amount of MCYST was calculated in relation to the amount of chlorophyll a instead of dry weight because of the presence of other particles in the river Nile water that may affect the biomass weight. MCYST levels in the river Nile (cell free) and drinking water were also determined by ELISA, after passing the filtered river Nile water as well as tap water through C18 cartridges (solid—phase extraction) (Brittain et al. 2000). The relationships among MCYSTs content in phytoplankton, chlorophyll a, potable river Nile water and the number of cyanobacterial species were examined by correlation analysis.

3. Results

The results of this study did not differ significantly between the samples collected at station I and station II. The different cyanobacterial species found were *Gomphospaeria lacustris, Merismopedia incerta, Microcystis aeruginosa,* and *Oscillatoria agardhii* (Table 1, 2). Chlorophyll a contents of the water samples differed dramatically during all time periods sampled (May-October) (Fig. 2). MCYST concentration in the phytoplankton collected at the two stations, did not correlate with chlorophyll a content ($r = -0.249$). The highest values of MCYST were recorded in July and September (Fig. 3). On the other hand, MCYST levels in phytoplankton had a positive correlation with cell number of *Microcystis* ($r = 0.6$), *Gomphosphaeria* ($r = 0.7$) and *Oscillatoria* ($r = 0.6$) at both sample collection sites.

Microcystin contents in the cell-free river water had a positive correlation with those in the phytoplankton ($r = 0.44$). The maximum levels of MCYST, in the cell-free water, were detected in September for both stations, and were 0.78 and 0.4 μg L^{-1}, respectively (Fig. 4). MCYSTs in finished drinking water were very low (56.1-87.1 ng L^{-1}) and were detected only in May and June (Fig. 5).

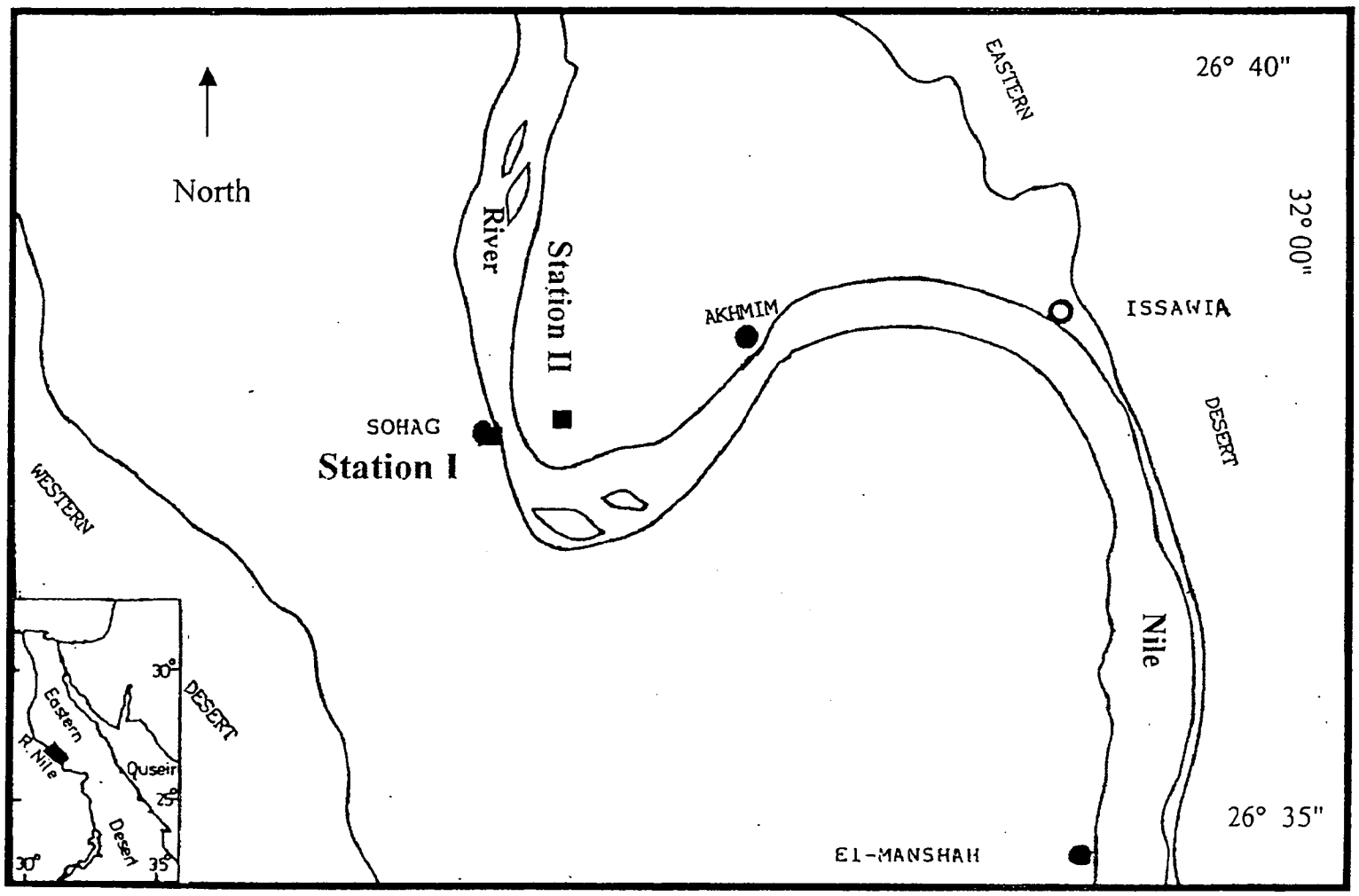

Fig. 1. Map showing locationof sampling points from the river Nile..
Fig. 1. Carte donnant la localisation des points étudiés sur le Nil.

4. Discussion

When MCYSTs occur in water bodies that are used for drinking water, they present a hazard and potential risk to human and animal health (Chorus & Bartram 1999, Hitzfeld et al. 2000). The high variability in the toxicity of cyanobacteria within and between years, and even within a water body on a particular day, makes assessment of the potential risk to users a major problem (Kotak et al. 1995).

Phytoplankton are monitored by using biomass measurements and direct counting. The biomass most frequently used is chlorophyll a, with peak values for an oligotrophic water being about 1.5-10.5 µg/L, while in eutrophic waters it can be about 300 µg/L (Carmichael 1996). Our study showed that the river Nile lies within the range of oligotrophic waters.

Most studies on seasonal variability of MCYST content in phytoplankton, show that MCYST concentrations correlate with the abundance of certain cyanobacteria species in the phytoplankton rather than chlorophyll a. Tsuji et al. (1996) found that there is no relationship between the production of MCYST and chlorophyll a in Lake Sagami, Japan. Kotak et al. (1996) showed that MCYST concentrations were associated with the abundance of *M. aeruginosa* in the phytoplankton while Henriksen & Moestrup (1997) found that the highest concentrations of MCYSTs were detected in samples dominated by *Planktothrix agardhii* when collected from lake Skt.jorgen Sd, Denmark. The results of our present study agree with these previous findings, in that we found the highest concentrations of MCYSTs in the phytoplankton did not correlate with chlorophyll a content, but did correlate strongly with the abundance of *Microcystis aeruginosa, Gomphosphaeria lacustris* and *Oscillatoria agardhii* in the phytoplankton.

Microcystins are normally confined within the cells and released into the surrounding water as a result of

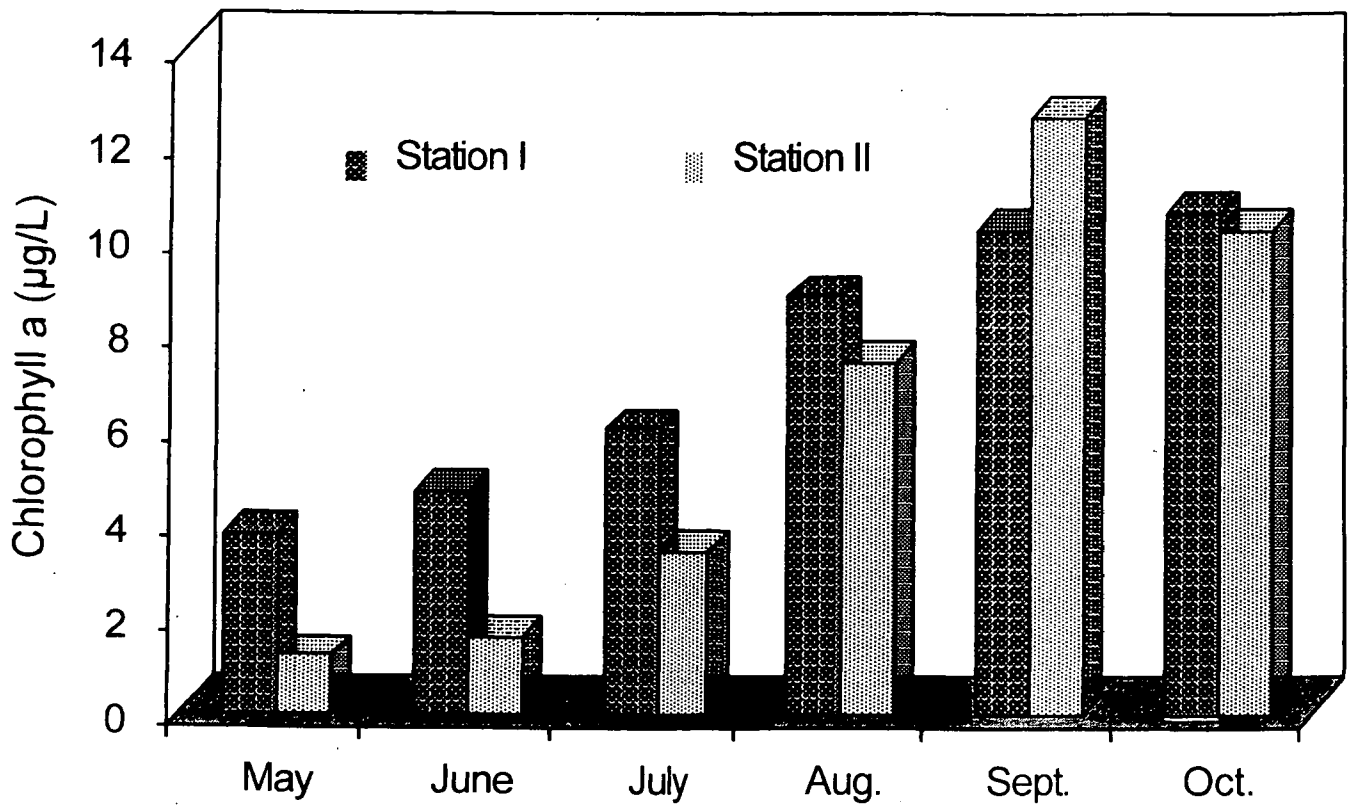

Fig. 2. Chlorophyll a contents (µg L^{-1}) of water samples collected from the river Nile during May-October, 1999.
Fig. 2. Concentrations en chlorophylle a (µg. l^{-1}) des échantillons collectés sur le Nil entre mai et octobre 1999.

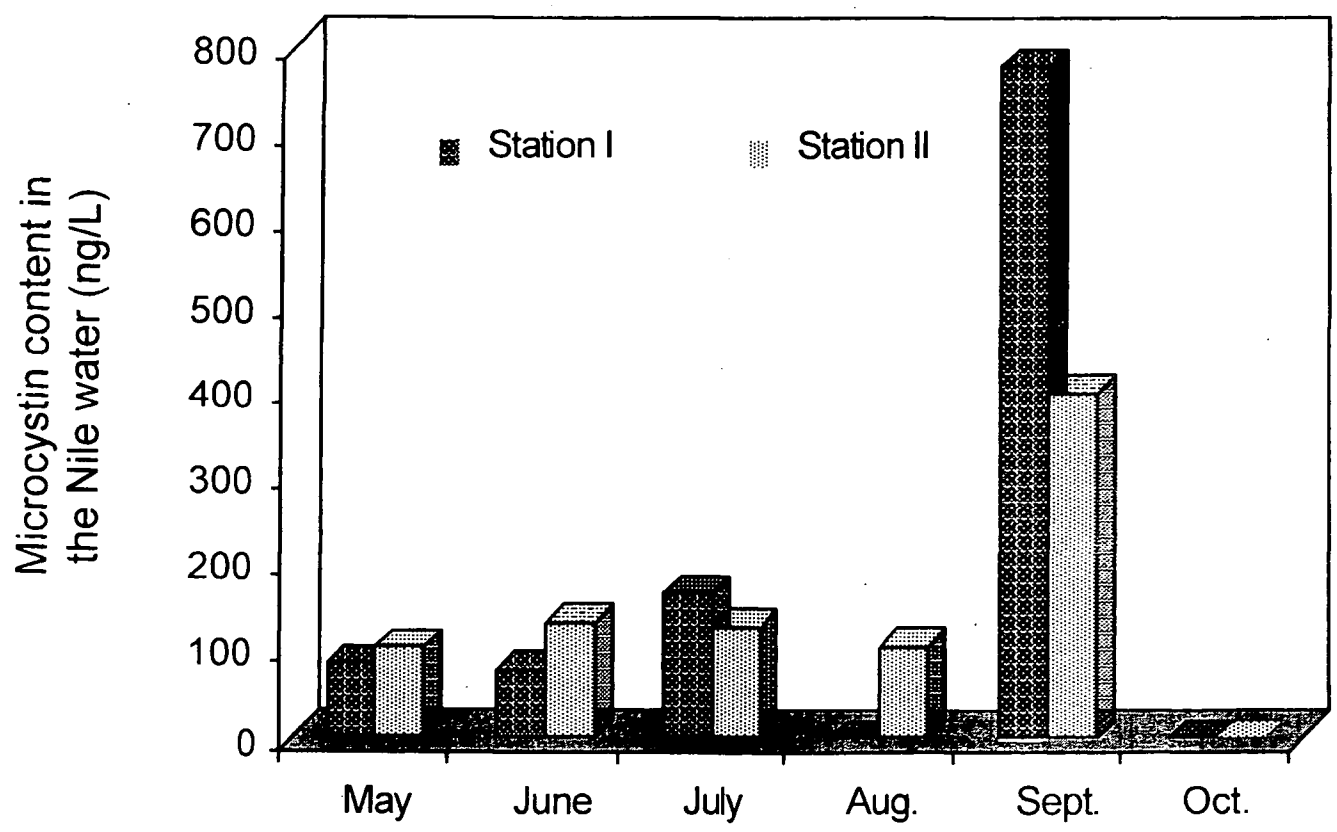

Fig. 3. Microcystin concentrations (ng/µg^{-1} chl a) within the cells present in the water samples collected from the river Nile in May-October 1999.

Fig. 3. Concentrations en microcystine (ng/µg^{-1} chl a) dans les cellules présentes dans les échantillons collectés dans le Nil entre mai et octobre 1999.

Table 1. Composition and Number (organisms/l) of phytoplankton species in the river Nile at station I during the study period.
Tableau 1. Composition du phytoplancton en nombre de cellules par litre, dans le Nil à la station I pendant la période étudiée.

Algal Species	May	June	July	August	September	October
Cyanobacteria						
Aphanizomenon flos-aqua (L.) Ralfs	150	350	450	300	100	—
Chroococcus limneticus Lemm.	—	100	300	—	—	—
Gomphosphaeria lacustris Chodat	208	353	606	504	458	404
Merismopedia incerta Lemm.	1430	1680	2502	1040	8065	7030
Microcystis aeruginosa Kuetz.	2200	3566	5300	2060	8520	6508
Oscillatoria agardhii Gom.	5080	8067	10000	8086	12040	9000
Chlorophyta						
Ankistrodesmus falcatus Ralf.	5088	2450	1000	300	58	—
Dictyosphaerium pulchellum Wood.	1000	436	—	—	—	—
Pediastrum simplex Lemm.	1810	476	—	—	—	—
Diatoms						
Fragillaria sp.	3000	4100	2588	3200	1500	2020
Melosira sp.	5030	8008	7060	5500	8100	6563
Dinoflagellates						
Ceratium sp.	56	100	—	—	—	—

Table 2. Composition and number (organisms/l) of phytoplankton species in the river Nile at station II during the study period.
Tableau 2. Composition du phytoplancton en nombre de cellules par litre, dans le Nil à la station II pendant la période étudiée.

Algal Species	May	June	July	August	September	October
Cyanobacteria						
Aphanizomenon flos-aqua (L.) Ralfs	58	123	—	—	—	—
Gomphosphaeria lacustris Chodat	220	338	600	486	428	412
Merismopedia incerta Lemm.	1560	1308	3540	900	10100	8880
Microcystis aeruginosa Kuetz.	2506	2048	5039	1034	4586	3548
Oscillatoria agardhii Gom.	4078	9077	12032	8065	13044	9200
Chlorophyta						
Dictyosphaerium pulchellum Wood.	879	384	152	134	186	230
Pediastrum simplex Lemm.	1066	973	465	384	420	388
Diatoms						
Melosira sp.	6089	7030	6088	6540	7057	7534
Synedra sp.	1490	1400	312	334	889	1282

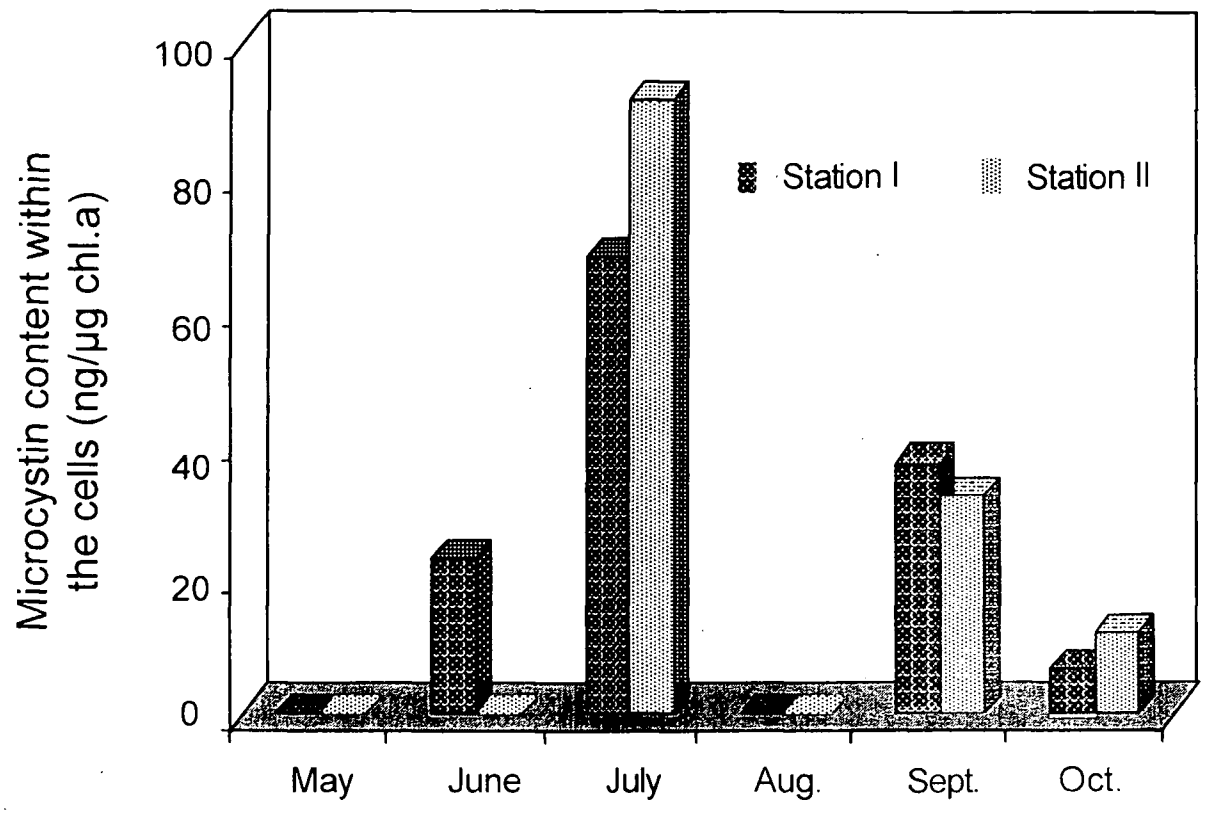

Fig. 4. Microcystin concentrations (ng L^{-1}) in the cell-free water of samples collected from the river Nile in May-October 1999.

Fig. 4. Concentrations en microcystine (ng. l^{-1}) dans l'eau (cellules exclues) des échantillons collectés dans le Nil entre mai et octobre 1999.

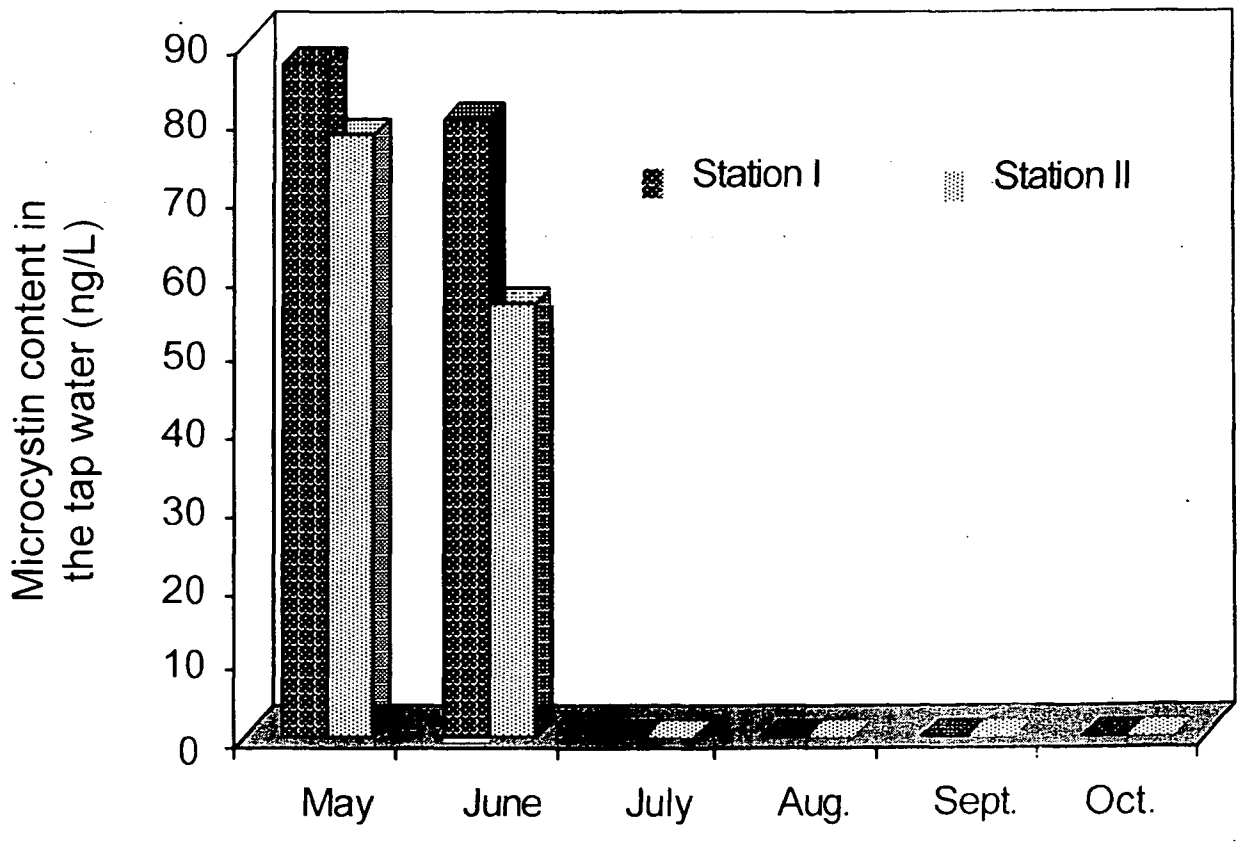

Fig. 5. Microcystin levels (ng L^{-1}) in finished drinking water samples collected from houses receiving water from water treatment stations in May-October 1999.

Fig. 5. Concentrations en microcystine (ng. l^{-1}) dans les échantillons d'eau potable collectés dans les maisons recevant l'eau en provenance des stations de traitement des eaux entre mai et octobre 1999.

chemical treatment (Kenefick et al. 1993, Lam & Prepas 1997, Lam et al. 1995) or natural senescence (Berg et al. 1987). During our study released MCYSTs into the river Nile water increased as MCYST levels in the phytoplankton increased and reached their maximum in September (0.4 - 0.78 µg/L). Thus, our results agree with those obtained by Kotak et al. (1996), when they found that MCYSTs increased over the summer, peaking at 0.344 µg/L in mid-August, and were correlated with MCYST in the phytoplankton. Earlier, Tsuji et al. (1996) detected MCYSTs in cell-free water of Lake Sagami and Lake Tsukui, Japan at a level of 0.02-2.64 µg/L.

ELISA is a very sensitive technique for detecting trace amounts of MCYSTs in drinking water at the ng level (Chu et al. 1990, Carmichael & An, in press). Using this technique enabled us to detect low amounts of MCYSTs in the finished drinking water of the river Nile. A previous study on seasonal variation of MCYST content in drinking water supplies in Haimen (China) in 1994, showed that MCYSTs were present only during June-September plus one occurrence in May (Ueno et al. 1996).

Our study showed that MCYST levels in finished drinking water supplies at Sohag city did not exceed the new World Health Organization guideline level (1 µg/L). However, the study did confirm that MCYSTs are present in river Nile water, both before and after treatment. This leads us to recommend that drinking water supplies at other sites in Egypt, using river Nile water, should be tested for MCYSTs, either by monitoring cell counts so that they do not exceed the threshold level of 5000 cells of cyanobacteria /ml or by direct measurement of MCYSTs so that they do not exceed 1 µg L^{-1} (Chorus & Bartram 1999).

In conclusion, this study found that :

* Microcystins contents in the phytoplankton correlated positively and significantly with cell numbers of *Microcystis, Gomphosphaeria* and *Oscillatoria*, rather than with chlorophyll a content (biomass) of phytoplankton samples.

* Microcystin levels in finished drinking water supplies at Sohag City, Egypt did not exceed the new World Health Organization (WHO 1998) guideline level (1 µg/L).

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