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## X-Ray Microanalytical Study on *Cyclotella meneghiniana* (Bacillariophyceae) as a Bio-indicator for Metal Pollution in Marine and Fresh Water Environments

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**Abstract:** Many industries in Alexandria discharge loads of heavy metals without treatment to Lake Mariut and the Mediterranean Sea leading to massive metal pollution. The present study was carried out on *Cyclotella meneghiniana*; the most common phytoplankton dominating the water of the hypertrophic polluted Lake Mariut and the north coast of Alexandria at El-Mex over three successive seasons at each environment. The study aimed to investigate the possibility of using this diatom as a bio-indicator organism for metal pollution. X-ray emission spectra obtained from *C. meneghiniana* revealed a wide range of detectable elements. Clear seasonal and spatial variations in the elemental composition of *C. meneghiniana* were observed. Concerning heavy metals, clear peaks of Fe and Cu were routinely detected in *C. meneghiniana* collected from the two environments, with relatively higher levels in L. Mariut compared to the sea. Lower levels of Mn, Co and Ni were also detected in *C. meneghiniana*, but with relatively higher levels in the marine environment. Levels of the accumulated metals in *C. meneghiniana* were correlated with their levels in the surrounding environment and a comparison was made between the two environments. However, results indicated that *C. meneghiniana* could be efficiently used as a bio-indicator organism to detect metal pollution due to its wide distribution and frequent occurrence though different seasons, in addition to its ability to uptake, a wide range of metals.

**Key words:** X-ray microanalysis, Metals, *Cyclotella meneghiniana*, bio-indicator

### Introduction

The role of phytoplankton in aquatic ecosystems is a key role being the base for all food chains and primary production. Moreover, they possess high ability in the distribution and cycling of different pollutants entering aquatic environments. Diatoms and blue green algae among other phytoplankton are known to occur in highly polluted water systems (El-Bestawy, 1993; El-Naggar *et al.*, 1998). L. Mariut, a close brackish water lake located south of Alexandria is a highly polluted water body and is classified as a hypertrophic system according to the levels of dissolved nutrients (nitrogen, phosphorus). It is subjected to a continuous discharge of untreated agricultural wastewater and primary treated domestic and industrial wastewater in huge quantities. Thus, not only nutrients but also biodegradable organic matter, pathogenic organisms (sewage); fertilizers and pesticides (agricultural wastewater); hazardous metals with other toxic synthetic chemicals (industrial effluents) are transported to the lake and finally accumulated in the sediments and living organisms causing severe problems in the food chains. As a result, the entire ecological system of L. Mariut is unbalanced and the water quality is highly deteriorated.

In a recent study (El-Bestawy, 1993), species composition of bacterial and algal populations in L. Mariut were studied with emphasis on their role in the cycling of heavy metals, which are available in levels far exceeding those reported in the most polluted lakes worldwide. Among the different species of phytoplankton, *C. meneghiniana* was the most dominant species present in a very high distribution frequency at the most polluted sites throughout the annual cycle. Results also revealed more metal content in that diatom as detected by X-ray microanalysis compared with other algal species. This reflects a high resistance to pollutants and particularly to metal pollution. Not only in L. Mariut, but also *C. meneghiniana* found to dominate the population in highly polluted sites at El-Mex (marine environment), which is subjected to heavy industrial discharges. Its wide occurrence beside the high

ability to tolerate extreme environmental conditions has led to study its manipulation for the production of lipid-derived "biodiesel" fuel, because it was found that lipid accumulation in phytoplankton occurs during periods of environmental stresses (Roessler *et al.*, 1994).

Some studies were carried out on the effect of toxic pollutants on fatty acid composition in *C. meneghiniana*. The pollutants included penta-chlorobenzene in comparison to trichlorobenzene isomers (Sicko-Goad *et al.*, 1989a); 1,2,3-trichlorobenzene (Sicko-Goad *et al.*, 1989b); 1,3,5-trichlorobenzene (Sicko-Goad *et al.*, 1989c); 1,2,4-trichlorobenzene (Sicko-Goad *et al.*, 1989d). These studies on lipophilic toxicants and the response of *C. meneghiniana* to such stress suggested that the daily diurnal variation in the lipid content of *C. meneghiniana* may be an important consideration for using it in evaluating the presence and effects of lipophilic toxicants in the environment (i.e. indicator organism). Many other biochemical studies on *Cyclotella* are available; such as the effect of silicon deficiency (Roessler, 1988), uptake of amino acids (Liu and Hellebust, 1974); utilization of amino acids as nitrogen sources and their effects on nitrate reductase in *Cyclotella* (Liu and Hellebust, 1974) and the oxidation of glycolic acid (Paul and Volcani, 1974) and many more. However, very few studies were found concerning the uptake, accumulation and effects of heavy metals on *Cyclotella* (Giri and Devi, 1992; El-Bestawy, 1993; El-Bestawy *et al.*, 1996). The main objective of the present study was to investigate the ability of *C. meneghiniana* for metals uptake and accumulation from polluted systems (fresh and marine). Also to monitor the changes in the elemental composition of this diatom under different heavy metals loads in order to use this alga as indicator organism for metal pollution. Electron-probe X-ray microanalysis was used to determine the elemental composition of *C. meneghiniana* at different sites and seasons in each environment. This technique has many advantages and allows the simultaneous monitoring of a wide range of detectable elements but has the disadvantage of low sensitivity where only metals in concentration above 10<sup>-8</sup> g

## E. El-Bestawy: *Cyclotella meneghiniana* a metal bio-indicator

are detectable, (Pedersen *et al.*, 1981). It has been extensively used in environmental studies for the whole cell preparations of bacteria and algae (Booth *et al.*, 1987; Bistricki and Munawar, 1982; Lehman, 1985; El-Bestawy *et al.*, 1996) and for sectioned algal cells (Sicko-Goad *et al.*, 1975; Jensen *et al.*, 1982).

### Materials and Methods

**Study Area:** Figure 1 presents the sampling sites in L. Mariut and Mediterranean coast of Alexandria. which. hold more than 40% of the Egyptian. industries. Wastewater from. different industries is discharged untreated or only primary treated either to L. Mariut, south of Alexandria or to the coast at El-Mex. Loads of heavy metals are discharged: to these environments that led to. massive metal pollution.

**Sampling:** Samples were collected from 6 different sites along: the main basin of L. Mariut during summer, autumn 96 and winter 96-97. In the marine environment, 2 sites at El-Mex in addition to one site at Abou Talat, west of Alexandria and away from waste discharges were selected for sampling during winter, spring and autumn 97. Sampling technique followed the standard procedure described in the "Standard Methods for Examination of Water and Wastewater" (Greenberg *et al.*, 1995). Subsurface water samples were collected in pre-acid: washed polyethylene bottles (1L) for determination of heavy metals. For qualitative and quantitative analysis of *Cyclotella*, samples were taken using, a trawl, net with a mesh size of 64  $\mu\text{m}$ .

**Preparation of Samples for Metal Determination:** One liter water samples collected in a polyethylene bottles were filtered through cellulose filters of 5  $\mu\text{m}$  and 0.22  $\mu\text{m}$  pore size to retain all the phytoplankton and pico-biomass, respectively. The filtrate was collected in polyethylene bottles that had been washed in 10% Analar nitric acid for 24 hours and double rinsed with glass distilled water. Immediately after filtration, the filtrate was acidified by adding 2 ml of 2 M Analar nitric acid till analysis using Atomic Absorption spectrophotometer (AAS), Smith-Heftji II.

**X-ray Microanalysis of *Cyclotella*:** Net samples collected from the lake and seawater were filtered through 5  $\mu\text{m}$  nucleopore membrane filters: Filters: Filters were washed up with glass-distilled water to remove any salts or debris. Filters with the phytoplankton were air-dried, after which they were stuck onto aluminum stubs covered with carbonaceous membranes to increase the electrical Contact between them. Samples were then coated with carbon using a Ploron E6432 Carbon Coater with continuous evaporation at 8 volt and 100 amps. *C. meneghiniana* that dominated the phytoplankton. population in all samples was selected and examined for elemental composition using scanning electron microscope (SEM). Samples were analyzed using a scanning electron microscope (SEM360), associated with LINK AN 10.000 analyzer. Analysis of *C. meneghiniana* cells was carried out using the following operational parameters: live time, 100 sec; accelerating voltage, 15keV; detector specimen working. distance, 25 mm; detector take-off angle, 35°. Probe current was set at 500p amps but was normally adjusted to give a count rate of approximately 1000 cps. Quantitation of the produced spectra from different cells was carried out using the LINK ZAF/PB program for elements within a protein matrix, using cobalt as

reference element. For each phytoplankton sample, mean elemental mass fractions were calculated from analysis of 5 randomly chosen cells of *C. meneghiniana*.

### Results and Discussion

**Spatial Variation in the Elemental Composition of *C. menaghiirtiana* 1st. Fresh Water Environment:** During the summer of 1996, sites that are located near out falls, especially 2 and 3, had the highest levels of most of the detected elements compared: to site 6 near N.ubaria canal the less polluted zone (Fig. 1) At site 6, only Na, Mg and Cl were considerably detected at high concentration levels in *C. meneghiniana*, while heavy metals were fairly low (Table 1, Fig. 2). The same trend was also detected at site 6 during autumn in *C. meneghiniana* with highest levels of the essential elements including Na, Mg, Cl and K compared to other sites. On the other hand, heavy metals and other element were detected at their highest levels in *C. meneghiniana* isolated from the polluted sites 1, 2 and 3.

During winter 96 and as a result of rough weathering. conditions, higher plants like water hyacinth and *Fragmites* completely blocked certain parts of the lake. Thus, samples from site 6 cannot be collected: during that season. Site 2, in front of the El-Qalaa drain, was characterized by containing the highest levels of Na, S, K, Mn, Fe, Co, Ni and Cu. This is expected because site 2 that discharges huge amounts of wastewater ( $5 \times 10^5 \text{ m}^3/\text{day}$ ), with  $3.5 \times 10^5 \text{ m}^3/\text{day}$  and agricultural discharge containing a lot of nutrients, fertilizers, and pesticides, while the remaining is domestic and industrial discharges. At the front of the outfall of the west treatment plant (site 5), was mixture of primary treated domestic and industrial effluent is discharged into L. Mariut with a rate of  $7.5 \times 10^5 \text{ m}^3 \text{ day}$ . These discharges brought heavy loads of heavy metals, pathogenic microorganisms, biodegradable organic matter, and synthetic chemicals. Species composition of lake phytoplankton certainly affected by such pollutants and only species that are resistant to heavy pollution we predominating in this ecosystem (El-Bestawy, 1993; El-Bestawy *et al.*, 1996). This is true for *C. meneghiniana* that dominated the algal population of L. Mariut during this study. It is occurred with very high frequency at the most polluted sites (2.5) containing. high levels of most elements under investigation including heavy metals. These results are in agreement with results obtained by El-Naggar *et al.* (1998), where *C. meneghiniana* was among the most common genera occurred in treated sewage discharged into L. Manzala, Egypt.

**Marine Environment:** Table 2 and Fig. 3 represent the elemental mass fractions of *C. meneghiniana* at 3 different sites at Alexandria coast in the marine environment. A significant spatial variation was also noticed at the marine environment. Site 1 is located in the far west of Alexandria at Abou Talat area while sites 2 and 3 located at El-Mex, where heavy loads of industrial effluents are discharged without treatment. This variation was clearly shown by the highest content of most of the elements including heavy metals at sites 2 and 3, compared to site 1 (Table 2, Fig. 3). This was a general trend. in the three seasons, indicating a positive correlation between the internal elemental content of *C. meneghiniana* and the external environmental metal levels. These results also confirmed the high ability of this diatom to be a good biosensor or bio-indicator for detecting metal pollution even at low metal concentration.

E. El-Bestawy: *Cyclotella meneghiniana* a metal bio-indicator

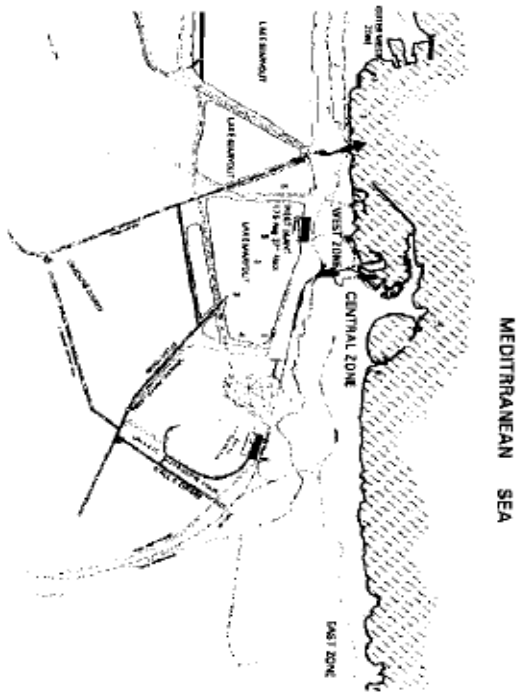


Fig. 1: Study area and sampling sites

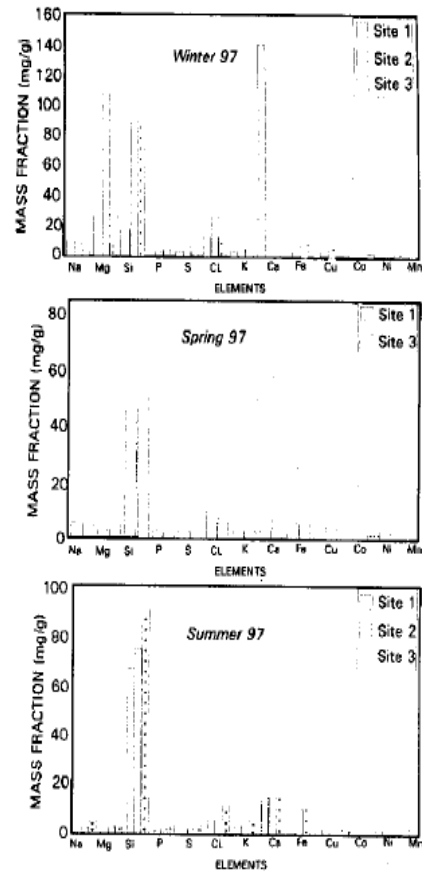


Fig. 3: Spatial variation in the elemental composition of *C. meneghiniana* in marine environment during different seasons

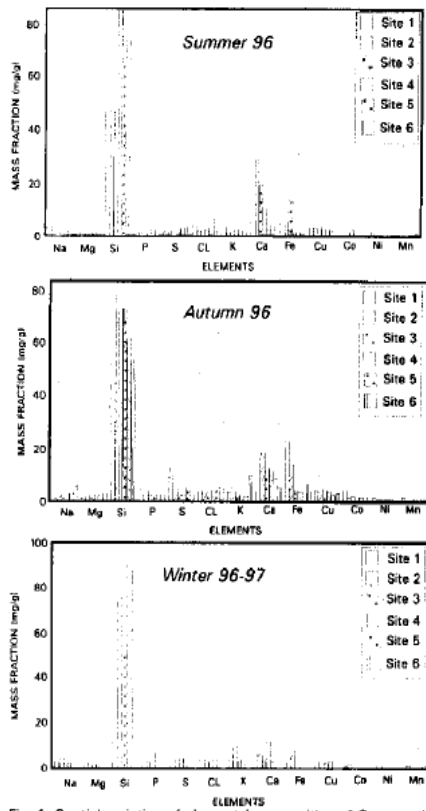


Fig. 2: Spatial variation of elemental composition of *C. meneghiniana* in fresh water environment during different seasons

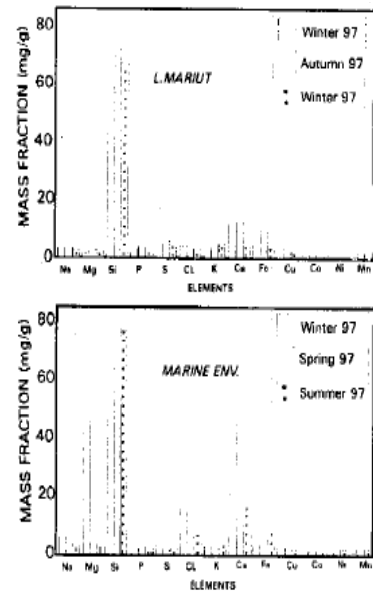


Fig. 4: Seasonal variations in the elemental composition of *C. meneghiniana* in freshwater and marine environment

E. El-Bestawy: *Cyclotella meneghiniana* a metal bio-indicator



Fig. 5: SEM micrograph of *Cyclotella meneghiniana* (air dried preparation) (a) Two cells, (b) Few cells

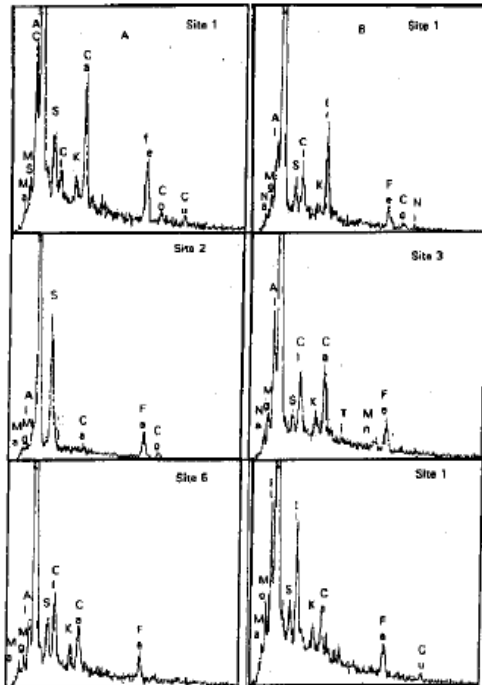


Fig. 6: X-ray emission spectra from whole cells of *C. meneghiniana* (a) from L. Mariut water, (b) from marine water

**Seasonal Variation in the Elemental Composition of *C. meneghiniana*:** Results of X-ray microanalysis showed significant variation in the elemental composition of *C. meneghiniana* at either environment (fresh water or sea). At L. Mariut, *C. Meneghiniana* isolated during autumn characterized by its high content of elements under investigation including the heavy metals, followed by summer, then winter (Fig. 4). At the marine ecosystem, there was a different trend where different elements showed their maximum level at the different seasons. Most of the heavy metals including Fe, Mn and Co were accumulated in *C. meneghiniana* during summer as well as Si and K. Nickel and copper were detected at their highest levels in the diatoms cells during spring, while Na, Mg, S, Cl and Ca showed their maximum absorption in *C. meneghiniana* during winter (Fig. 4). This trend at both environments is mainly attributed to the temperature, which regulates the growth and the metabolic activity of microorganisms. It is well known that there is a proportional relation between temperature and metals uptake. Algae including diatoms show high capacity for metal uptake at high temperatures than at low temperatures (Sharma and Azeez, 1988). In that study, binding of Cu, Cd, Pb, Zn, Cr, and Au to *Spirulina* was found to be temperature dependent. In another study, massive reduction of Au (III) to Au (0) occurred after one hour of agitation with the algae at 55 °C (Greene and Darnall, 1988).

**Comparison Between the Elemental Composition of *C. meneghiniana* in Fresh and Marine Environment:** Elemental mass fractions of *C. meneghiniana* were significantly different between the two environments (Table 3). In the marine environment, the average concentrations of Na, Mg, Cl and Ca in the cells of *C. meneghiniana* were much higher than those reported in the cells isolated from L. Mariut. In the later, much higher levels of P, S and K were detected in the diatom. This is mainly attributed to the composition of water at each environment and to the bio-availability of such elements in the surrounding environment. Iron and copper were detected at higher levels in the diatom cells that were isolated from L. Mariut, while Mn, Co and Ni were higher in the cells inhabiting seawater. This is attributed to some factors including the kind and the amount of heavy metals, metal species, bioavailability, prevailing and optimum conditions for metals uptake and finally the presence of other competitors (Abbas and Edwards, 1989; Prosi, 1989) (Fig. 5, 6).

**Bio-concentration of Heavy Metals:** Plankton and macroalgae appear to be unable to regulate their uptake and/or output of metals from the ambient water up to several thousands times (Beijer and Jernelov, 1986). The presence of *Cyclotella*, throughout the sampling periods at both environments, enabled elemental data to be analyzed. It showed a clear tendency to concentrate heavy metals and was detected at high frequency in both polluted and less polluted areas. It was of great help using X-ray microanalysis during the present study to investigate the possibility of using dominant member of the phytoplankton as a bio-monitor to detect metal pollution that prevailed in fresh-water and seawater environments. The relatively short lifetime of phytoplankton (few days to a few months), makes them very susceptible to the abiotic stresses such as heavy metals. Due to the high turnover in metal concentration as shown by Srivastava and Prakash (1991), metal concentration can vary considerably in the cells within a short time of growth. Therefore, *C. meneghiniana* showed high ability to respond and to

## E. El-Bestawy: *Cyclotella meneghiniana* a metal bio-indicator

Table 1: Average elemental mass fractions of *C. meneghiniana* in freshwater environment (mg/g ± SE)

Site	Metals												
	Na	Mg	Si	P	S	Cl	K	Ca	Mn	Fe	Co	Ni	Cu
Summer Aug. 96													
1	4.1 ± 0.7	-	46.9 ± 7.1	1.6 ± 0.9	1.6 ± 0.5	3.411.6	3.3 ± 1.7	5.0 ± 2.7	0.2 ± 0.1	4.3 ± 2.1	-	-	2.8 ± 0.4
2	1.9 ± 0.3	1.6 ± 0.5	29.6 ± 7.0	1.4 ± 0.3	1.4 ± 0.5	1.8 ± 0.6	1.0 ± 0.5	28.4 ± 10.5	0.5 ± 0.2	2.5 ± 1.2	0.2 ± 0.1	0.9 ± 0.2	2.4 ± 0.3
3	1.2 ± 0.3	0.2 ± 0.1	47.6 ± 5.9	2.5 ± 0.3	12.5 ± 6.1	1.7 ± 0.2	2.911.3	19. ± 1 4.1	0.3 ± 0.1	13.5 ± 8.8	0.3 ± 0.2	0.2 ± 0.08	3.110.3
4	3.9 ± 0.4	1.6 ± 0.3	84.3 ± 9.6	2.6 ± 0.3	3.910.6	5.2 ± 0.8	2.4 ± 0.7	9.911.8	0.2 ± 0.1	0.7 ± 0.1	0.5 ± 0.2	0.2 ± 0.1	2.5 ± 0.6
5	1.7 ± 0.4	1.1 ± 0.4	66.6 ± 7.5	2.2 ± 0.2	2.9 ± 0.5	1.810.6	0.5 ± 0.2	1.910.3	0.2 ± 0.1	1.0 ± 0.3	0.6 ± 0.3	0.5 ± 0.2	1.9 ± 0.4
6	4.1 ± 1.0	2.7 ± 1.2	72.9 ± 6.8	2.1 ± 0.5	3.2 ± 1.1	6.4 ± 2.0	1.5 ± 0.8	3.9 ± 1.3	0.2 ± 0.1	0.6 ± 0.2	0.1 ± 0.01	0.3 ± 0.1	1.6 ± 0.5
Autumn Oct. 96													
1	1.1 ± 0.4	0.9 ± 0.3	80.1 ± 7.8	4.5 ± 0.4	12.7 ± 3.5	3.4 ± 0.6	3.1 ± 0.8	15.9 ± 3.7	0.8 ± 0.4	22.2 ± 4.8	0.6 ± 0.2	0.5 ± 0.2	3.2 ± 1.2
2	1.7 ± 0.7	1.8 ± 0.6	72.7 ± 4.8	4.8 ± 0.6	4.1 ± 0.6	3.8 ± 0.8	5.3 ± 1.1	18.1 ± 3.7	-	13.7 ± 4.6	0.7 ± 0.3	0.2 ± 0.1	3.91 0.9
3	2.610.8	2.6 ± 6.7	82.2 ± 3.2	2.3 ± 0.3	2.7 ± 0.4	3.34 ± 0.7	1.7 ± 0.3	11.7 ± 3.1	0.1 ± 0.09	3.7 ± 1.1	1.110.6	0.2 ± 0.1	3.4 ± 1.0
4	3.4 ± 0.9	1.1 ± 0.3	74.7 ± 9.0	2.3 ± 0.2	2.6 ± 0.3	4.1 ± 0.5	2.2 ± 0.6	12.0 ± 2.3	0.70 ± 0.3	3.0 ± 1.0	1.0 ± 0.6	0.3 ± 0.1	3.1 ± 1.5
5	2.9 ± 0.6	2.5 ± 0.1	62.9 ± 2.3	3.42 ± 0.6	5.4 ± 0.4	5.4 ± 0.4	1.7 ± 0.5	8.2 ± 1.3	0.610.1	2.6 ± 0.9	0.8 ± 0.6	0.2 ± 0.1	2.0 ± 0.6
6	6.2 ± 1.3	3.0 ± 1.8	54.7 ± 11.6	2.1 ± 0.3	2.1 ± 0.6	5.5 ± 2.1	9.5 ± 6.6	4.8 ± 3.1	0.5 ± 0.1	6.2 ± 4.2	0.8 ± 0.4	0.1 ± 0.09	2.5 ± 0.4
Winter Dec. 96													
1	1.8 ± 0.5	2.2 ± 0.6	47.5 ± 9.6	2.0 ± 0.6	2.3 ± 0.8	3.2 ± 0.9	2.81 0.8	4.9 ± 1.4	-	3.41 1.7	0.3 ± 0.1	0.11- 0.06	1.310.2
2	3.8 ± 0.0	1.31 0.6	75.8 ± 19.1	0.36 ± 0.2	7.2 ± 4.1	2.9 ± 1.8	8.8 ± 7.4	2.7 ± 1.0	0.5 ± 0.2	6.2 ± 3.5	0.7 ± 0.3	0.2 ± 0.1	2.0 ± 0.3
4	1.4 ± 0.6	1.3 ± 0.8	54.2 ± 4.6	6.2 ± 1.7	3.3 ± 0.5	1.7 ± 0.3	2.0 ± 0.2	11.3 ± 0.8	0.3 ± 0.03	3.6 ± 0.8	0.0	0.0	1.7 ± 0.1
5	2.2 ± 0.3	1.4 ± 0.2	89.3 ± 6.5	4.1 ± 1.3	3.910.2	2.41 0.6	3.2 ± 0.33	2.0 ± 0.4	0.7 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.01	2.0 ± 1.2

Table 2 : Average elemental mass fractions of *C. meneghiniana* in marine environment (mg/g ± SE)

Site	Metals												
	Na	Mg	Si	P	S	Cl	K	Ca	Mn	Fe	Co	Ni	Cu
Winter Feb. 97													
1	8.5 ± 1.7	25.3 ± 1.5	15.6 ± 2.1	0.1 ± 0.05	1.9 ± 0.2	10.7 ± 1.9	1.1 ± 0.4	139.4 ± 7.0	1.0 ± 0.6	1.4 ± 0.6	0.2 ± 0.07	1.4 ± 0.5	1.5 ± 0.3
2	7.57 ± 1.0	105.9 ± 4.1	87.5 ± 4.0	2.0 ± 0.2	5.1 ± 0.8	28.9 ± 2.0	2.9 ± 1.1	5.0 ± 1.2	0.1 ± 0.05	0.1 ± 0.08	0.03 ± 0.01	-	1.1 ± 0.63
3	2.3 ± 0.9	7.1 ± 1.54	88.2 ± 6.2	2.3 ± 0.1	1.7 ± 0.5	9.5 ± 0.2	2.6 ± 0.4	4.9 ± 0.3	1.8 ± 0.6	5.8 ± 0.5	0.1 ± 0.08	0.2 ± 0.1	2.4 ± 0.3
Spring Mar 97													
1	2.9 ± 0.7	2.4 ± 0.5	47.2 ± 0.3	2.5 ± 0.2	1.8 ± 0.1	8.9 ± 1.0	1.8 ± 0.6	1.9 ± 0.8	1.1 ± 0.1	4.7 ± 1.3	0.7 ± 0.3	0.4 ± 0.2	1.9 ± 1.1
3	4.3 ± 0.6	3.8 ± 1.2	55.1 ± 11.4	1.3 ± 0.03	0.9 ± 0.2	5.0 ± 2.0	1.8 ± 0.3	6.0 ± 1.1	1.2 ± 0.5	4.1 ± 0.5	0.3 ± 0.1	1.3 ± 0.4	3.1 ± 1.1
Summer Aug. 97													
1	1.97 ± 0.5	1.9 ± 0.6	66.6 ± 19.5	0.4 ± 0.2	0.6 ± 0.1	1.3 ± 0.6	2.4 ± 0.1	14.1 ± 1.4	0.4 ± 0.2	0.4 ± 0.2	0.6 ± 0.3	-	1.3 ± 0.3
2	2.2 ± 0.5	2.8 ± 0.7	74.5 ± 10.2	1.3 ± 0.3	1.9 ± 0.8	6.9 ± 0.5	3.3 ± 0.4	18.5 ± 3.6	1.7 ± 0.7	8.9 ± 1.9	0.8 ± 0.6	0.6 ± 0.4	1.5 ± 0.7
3	4.7 ± 1.0	3.8 ± 1.2	90.8 ± 2.7	3.0 ± 0.5	2.8 ± 0.6	10.7 ± 1.7	4.6 ± 0.6	16.1 ± 2.7	1.5 ± 0.6	12.5 ± 3.3	3.5 ± 2.5	1.1 ± 0.7	0.6 ± 0.3

Table 3: Comparison between freshwater and marine water regarding the elemental composition of *C. meneghiniana*; mg/g biomass/SE

Environment	Elements												
	Na	Mg	Si	P	S	Cl	K	Ca	Mn	Fe	Co	Ni	Cu
Fresh water	2.7 ± 0.2	1.6 ± 0.2	65.3 ± 3.2	2.8 ± 0.3	4.5 ± 0.2	3.4 ± 0.4	3.4 ± 0.6	9.5 ± 1.7	0.3 ± 0.05	5.2 ± 1.4	0.5 ± 0.1	0.2 ± 0.05	2.4 ± 0.3
Marine	4.2 ± 0.8	17.3 ± 11.7	64.1 ± 6.2	1.6 ± 0.1	2.0 ± 0.4	10.2 ± 2.5	2.5 ± 0.4	23.3 ± 11.2	1.1 ± 0.1	4.7 ± 1.2	0.8 ± 0.4	0.7 ± 0.1	1.8 ± 0.3

Table 4: Concentration factors of metals in *C. meneghiniana* isolated from L. Mariut water and marine water at Alexandria coast

Ecosystem	Metals			
	Fe	Cu	Co	Ni
<b>Fresh water</b>				
Summer 96	1.0 × 10 <sup>4</sup>	4.69 × 10 <sup>4</sup>	2.3 × 10 <sup>3</sup>	7.5 × 10 <sup>3</sup>
Autumn 96	8.7 × 10 <sup>3</sup>	1.2 × 10 <sup>5</sup>	4.7 × 10 <sup>3</sup>	4.9 × 10 <sup>3</sup>
Winter 96	9.0 × 10 <sup>3</sup>	2.7 × 10 <sup>4</sup>	3.3 × 10 <sup>3</sup>	2.9 × 10 <sup>3</sup>
<b>Marine water</b>				
Winter 97	6.2 × 10 <sup>3</sup>	7.7 × 10 <sup>3</sup>	5.6 × 10 <sup>2</sup>	2.5 × 10 <sup>3</sup>
Spring 97	9.3 × 10 <sup>3</sup>	1.2 × 10 <sup>4</sup>	3.1 × 10 <sup>3</sup>	4.8 × 10 <sup>3</sup>
Summer 97	9.4 × 10 <sup>3</sup>	1.5 × 10 <sup>4</sup>	1.0 × 10 <sup>4</sup>	4.0 × 10 <sup>2</sup>

\*Concentration factor = (μg/g) dry biomass/(μg/ml) dissolved metals in water

concentrate metals from the surrounding water, although the low sensitivity of X-ray microanalysis hindered the detection of other metals that previously detected at relatively higher concentration such as Hg, Pb, Cr and Zn (El-Bestawy, 1993). Table 4 presents the concentration factors of elements that were detected at both environments in *C. meneghiniana* using X-ray microanalysis. At both environments, summer was the season that metals were highly concentrated in *C. meneghiniana*, followed by spring and finally winter. This is directly related to the effect of temperature and the consequent increase in the microbial metabolic activities including metal uptake. The magnitude order of metal accumulation was merely the same at both ecosystems at all seasons, with few exceptions. The order was Cu > Fe > Ni > Co, except for winter samples in L. Mariut, where Co recorded the highest

concentration level than Ni, while Co in summer in the marine environment showed its highest level in *C. meneghiniana* followed by Fe then Ni, However, the concentration factors in fresh water were higher than in seawater.

It is surprising that Cu which is a highly toxic metal to algae, is the most concentrated metal in *C. meneghiniana* with a concentration factor ranged between  $7.7 \times 10^3$  to  $1.2 \times 10^5$ -fold higher than Cu level in the surrounding water. This could be a result of an acclimatization process through which *C. meneghiniana* became tolerant or resistant to the toxic effect of Cu (Lawrence *et al.*, 1989; Shengjun and Holcombe, 1990). Iron was the second metal accumulated at high levels in *C. meneghiniana* with concentration factors ranging between  $6.2 \times 10^3$  and  $1 \times 10^4$ -fold increase than in water. This was followed by Ni and Co with concentration factors ranging between  $4 \times 10^2$  to  $7.5 \times 10^3$  and from  $5.6 \times 10^2$  to  $1 \times 10^4$ -fold increase, respectively. This showed that the bio-accumulation of such metals depends on the environmental conditions and to the metal selectivity by the diatom.

### Conclusion

Results obtained during this investigation indicated that metal accumulation by *C. meneghiniana* is proportional to the relative metal content in the surrounding water and the diatom *C. meneghiniana* could be efficiently used as a bio-indicator to detect metal pollutants in aquatic ecosystems.

## E. El-Bestawy: *Cyclotella meneghiniana* a metal bio-indicator

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