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SOME BIOLOGICAL RESPONSES OF THE DIATOM *NITZSCHIA CLOSTERIUM* (W. SMITH) TO COPPER AND ZINC

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ABSTRACT: Some biological responses of the marine diatom *Nitzschia closterium* to copper and zinc were studied. The species was isolated from the Gulf of Suez and grown in liquid culture with enriched filtered seawater over a maximum period of 10 days. The results indicated that low concentrations of copper (1 and 5 $\mu g.l^{-1}$) and zinc (10, 20 and 50 $\mu g.l^{-1}$) stimulated the growth, photosynthetic pigments (chlorophyll-a,c and carotenoids), total soluble proteins as well as 0_2-evolution in photosynthesis and dark respiration (0_2uptake) of the diatom as compared with the control culture. Whereas, higher concentrations of 50, 100, 200 and 300 $\mu g.l^{-1}$ copper and 100, 200, 300, 400 and 600 $\mu g.l^{-1}$ zinc inhibited the above metabolic processes of the organism (taking into consideration that the culture treated with 10 $\mu g.l^{-1}$ zinc produced the same O_2-uptake as in the control culture). Statistically, there exists reverse correlation between the different concentrations of copper and zinc and the metabolic processes of *Nitzschia closterium* as proved with linear regression equations.

KEYWORDS: *Anacystis, Chlorella,* Gulf of Suez, *Nitzschia*

INTRODUCTION

Heavy metals are among the most frequently identified pollutants in aquatic environments. From a biological point of view, heavy metals can be divided into two categories; essential and non-essential. However, many of the essential heavy metals are reported to be toxic at high concentrations and have a direct influence on various physiological and biochemical processes including reduction in growth, photosynthesis and chlorophyll content or inhibition of enzyme activities. Heavy metals are known to interfere with several photosynthetic functions. The extent to which phytoplankton transfer metals through the food chain depends, in part, on their abilities to accumulate and, to a large extent, to tolerate high concentrations of the metal before they themselves are obviously affected.

Copper is an essential element for higher plants and algae. At higher concentrations copper is toxic to most organisms, therefore, it is used in several fungicides, algicides, molluscicides and marine antifouling compounds. Copper inhibited the synthesis of chlorophylls (a and b) in the green alga, *Chlorella vulgaris* (Lam *et al.*, 1999) and also caused bleaching of phycocyanin in the blue-green alga, *Anacystis nidulans* (Gupta and Singhal, 1996). Generally, the most toxic physico-chemical form of copper is the free ion forms, whereas, copper bounded in organic compounds, which is dominant in the aquatic environment is less toxic than the free form (Newell and Sauders, 1986).

Zinc is well-known as essential mineral nutrient for growth of plants and algae as a component of several enzymes, e.g., superoxide dismutase, carbonpeptidase, carbonic anhydrase and a range of dehydrogenases (Coleman, 1998). Zinc deficiency in plants causes a remarkable reduction in electron transport and photophosphorylation (Schrotri *et al.*, 1981).

In general, it is possible to distinguish five different sources from which metal pollution of the environment originates: (1) geologic weathering, (2) industrial processing of ores and metals, (3) the use of metals and metal compounds, (4) leaching of metals from garbage and solid waste dumps and (5) animal and human excretions that contain heavy metals (Wittmann, 1981 and Abalde *et al*., 1995b).

The aim of the present work is to study the response of some biological processes of *Nitzschia closterium* to the different concentrations of copper and zinc, which are the most common heavy metals in the Gulf of Suez.

MATERIALS AND METHODS

Isolation and purification of the organism: One liter of surface seawater was collected from Ain Sukhna, Suez Gulf, in carefully cleaned polyethylene bottle and passed immediately through plankton net of I00- μm mesh size to eliminate the macrozooplankton. This water was supplemented in the laboratory with Erdschreiber medium (Starr, 1964), which containing 0.2 g $NaNO_3$, 0.03 g $Na_2HPO_4.12H_2O$ and 50 ml soil extract. The soil extract was prepared according to Pringschcim's soil water medium cited in Venkataraman (1969) by boiling 50 g of garden soil in one liter of seawater for one hour then cooled. The clear upper filtrate layer was taken and filtered again through a glass filter paper and kept in the refrigerator for subsequent use. This seawater supplemented with Erdschreiber solution was poured in several conical flasks of 250 ml capacity (120 ml were added in each one) and incubated for 17 days at 21 ± 1°C under day light fluorescent lamps of about 6400 Lux and with 12 light: 12 dark cycles.

The grown algae were examined under the binocular research microscope and the dominant cells of *Nitzschia closterium* (W. Smith) were picked up by a fine micropepitte and transferred into a new fresh Erdschreiber solution and incubated again for 17 days under the same previously mentioned conditions. The isolation process was repeated several times and in each one the cells of *Nitzschia closterium* were picked up and transferred into sterilized Erdschreiber solution till purified cells were obtained. These purified cells of *Nitzschia closterium* were then inoculated on a sterilized and modified marine medium-f (Guillard and Ryther, 1962), which supported faster growth for *N. closterium* as recommended by Stauber and Florence, 1989.

The effect of ionic copper and zinc on some metabolic processes of the organism: Since CU^{2+} and Zn^{2+} showed the highest concentrations among the detected heavy metals at the different stations of Suez Gulf (EI-Naggar *et al.,* 2002), their effects on some growth parameters of *Nitzschia closterium* were studied. Range concentrations of copper (1 to 500 $\mu g.1^{-1}$) and zinc (5 to 800 $\mu g.1^{-1}$) were investigated on the growth but only the effective values on the organism were chosen.

i. **Growth:** The growth was followed up by measuring the optical density of the algal suspension at 750 nm as described by Sriharan *et al.* (1989). Subsamples of one ml were taken every two days for growth assessment.

ii. **Photosynthetic pigments:** The chlorophylls (a and c) were determined according to the method recommended by Parsons and Strickland (1965) and carotenoid by the method of Jensen (1978).

iii. **Total soluble proteins:** After the extraction of pigments, the pellet containing the algal protein was extracted with 3 ml of 1 N NaOH in a boiling water bath for two hours, then cooled and centrifuged again for 10 minutes at 3000 rpm (Payne and Stewart, 1988). The total soluble proteins were determined quantitatively according to the method described by Lowry *et al.* (1951). The protein concentration was estimated as microgram per milliliter of algal suspension.

iv. **Photosynthetic activity and respiration:** The photosynthetic activity was measured polaro-graphically as oxygen evolution using a Clark type electrode (YSI. model 53), The actinic white light was obtained from a 150 W tungsten lamp. The algae were harvested during their exponential growth phase (5 days after incubation) and subsamples of 4 ml of

the algal suspension at room temperature (20 ± 1° C) were taken and the O_2-evolving capacity during light was measured. Respiration was measured in dark as O_2 uptake by the same sample. The oxygen evolved and that consumed was calculated as μmol $O_2.ml^{-1}.h^{-1}$

Statistical Analysis: Regression analysis was performed using the statistical package NCSS and PASS (Hintze, 2002).

Effect of copper and zinc on some metabolic processes of the organism

i. Growth: The response of *Nitzschia closterium* growth to different concentrations of copper and zinc is present in Table 1. The concentrations of 1 and 5 $\mu g.l^{-1}$ copper stimulated the algal growth by 12 and 17 %, respectively over the control culture after 10 days of incubation. While higher concentrations of 50, 100, 200 and 300 $\mu g.l^{-1}$ copper caused about 7, 22, 36 and 53 % growth reduction, respectively below the control culture. A significant inverse relation was observed between both growth and copper concentrations.

ii. Growth = - 0.0002 (Cu conc.) + 0.145, where, $R^2 = 0.1713$, n = 35 and $p \leq 0.05$ Zinc concentrations of, 10, 20 and 50 $\mu g.l^{-1}$ stimulated the growth of *Nitzschia closterium* by 10, 13 and 16 %, respectively over the control culture after 10 days of incubation. On the other hand, zinc concentrations or 100, 200, 300, 400 and 600 $\mu g.l^{-1}$ provoked a decrease in algal growth by 8, 16, 33, 43 and 60 %, respectively. Generally, the maximum growth of *Nitzschia closterium* after 10 days of incubation was found in the cultures treated with 5 and 50 $\mu g.l^{-1}$ of copper and zinc, respectively, while the lowest growth was recorded with 300 and 600 $\mu g.l^{-1}$ copper and zinc, respectively as compared with the control culture. A significant inverse relation was recorded between both the growth and zinc concentrations. Growth = - 0.0001 (Zn conc.) + 0.15, where, $R^2 = 0.2122$, n = 48 and $p \leq 0.05$.

iii. Photosynthetic pigments

a. Chlorophyll-a: The chlorophyll-a content of *Nitzschia closterium* followed a similar pattern to that of growth in response to copper (Table 2). The low concentrations of 1 and 5 $\mu g.l^{-1}$ copper stimulated the chlorophyll-a content by 10 and 15 %, respectively over, the control culture after 10 days of incubation. Whereas, higher concentrations of 50, 100,200 and 300 $\mu g.l^{-1}$ copper reduced the content of chlorophyll-a by 6, 19, 33 and 50%, respectively below the control culture. A significant inverse relation was recorded between the chlorophyll-a and copper concentrations. Ch1.-a = - 0.0005 (Cu conc.) + 0.3851, where, $R^2 = 0.2281$, n= 30 and $p \leq 0.05$.

b. Chlorophyll-c: Compared to the control culture, lower copper concentrations of 1 and 5 $\mu g.l^{-1}$ produced a gradual increase in chlorophyll-c content in the order of 7 and 11 %, respectively over the control culture after 10 days of incubation. While, higher concentrations of 50, 100, 200 and 300 $\mu g.l^{-1}$ copper caused about 4, 16, 30 and 46 %, respectively reduction in chlorophyll-c content (Table 3). A significant inverse relation was recorded between the chlorophyll-c and copper concentrations. Chl.-c = - 0.0003 (CU conc.), + 0.2655, where, $R^2 = 0.1988$, n= 30 and $p \leq 0.05$. Application of 10, 20 and 50 $\mu g.l^{-1}$ zinc increased chlorophyll-c content by 4, 6 and 10 % respectively, while the concentrations of 100, 200, 300, 400 and 600 $\mu g.l^{-1}$ zinc reduced the chlorophyll-c content by 5, 10,26,32 and 51 % respectively after 10 days of incubation. Also a significant inverse relation between chlorophyll-c and zinc concentrations was recorded. Chl.-c = - 0.0002 (Zn conc.) + 0.266, where, $R^2 = 0.2158$, n= 40 and $p \leq 0.05$.

Table 1. Effect of different concentrations of copper and zinc (µg.l^{-1}) on the growth (measured as optical density at 750 nm) of *Nitzschia closterium* (each value is the mean of three determinations which were closely related).

Copper (µg.l^{-1})									
Day	Control	1	5	50	100	200	300	--	un
0	0.07	0.07	0.07l	0.07	0.069	0.068	0.067	--	--
2	0.092	0.095	0.098	0.08	0.077	0.076	0.073	--	--
4	0.114	0.121	0.125	0.092	0.089	0.084	0.08	--	--
6	0.144	0.157	0.161	0.122	0.099	0.102	0.089	--	--
8	0.188	0.209	0.215	0.l65	0.137	0.115	0.099	--	--
10	0.237	0.265	0.277	0.22	0.185	0.152	0.111	--	--

Zinc (µg.l^{-1})									
Day	Control	10	20	50	100	200	300	400	600
0	0.07	0.07	0.07	0.071	0.07	0.069	0.068	0.066	0.065
2	0.092	0.096	0.1	0.102	0.086	0.081	0.075	0.07	0.069
4	0.114	0.121	0.125	0.128	0.104	0.096	0.087	0.076	0.074
6	0.144	0.156	0.16	0.164	0.124	0.109	0.102	0.089	0.08
8	0.188	0.205	0.211	0.216	0.165	0.152	0.117	0.102	0.088
10	0.237	0.261	0.268	0.275	0.218	0.20	0.159	0.135	0.095

iv. Caroenoids: The accessory photosynthetic pigment carotenoid increased in the cultures treated with 1 and 5 µg.l^{-1}copper, this increase amounted to 12 and 18 %, respectively over the control culture after 10 days of incubation, Whereas, higher values of 50, 100, 200 and 300 µg.l^{-1} copper inhibited carotenoid biosynthesis by about 3.5, 16, 25 and 41 % respectively below the control culture (Table 4). A significant inverse relation between the carotenoids and copper concentrations was statistically observed in the following equation: Carotenoids = - 0.0022 (Cu conc.) + 1.8976, where, $R^2 = 0.1691$, n= 30 and $p \leq 0.05$

Table 2. Effect of different concentrations of copper and zinc (µg.l^{-1}) on the chlorophyll-a calculated as µg.ml^{-1}of *Nitzschia closterium* (each value is the mean of three determinations which were closely related).

Copper (µg.l^{-1})									
Day	Control	1	5	50	100	200	300	--	--
0	0.215	0.215	0.215	0.215	0.213	0.211	0.209	--	--
2	0.3	0.309	0.318	0.273	0.258	0.24	0.228	--	--
5	0.36	0.378	0.392	0.306	0.288	0.277	0.252	--	--
8	0.46	0.5	0.52	0.409	0.345	0.294	0.262	--	--
10	0.538	0.592	0.619	0.505	0.436	0.36	0.269	--	--

Zinc (µg.l^{-1})									
Day	Control	10	20	50	100	200	300	400	600
0	0.215	0.215	0.215	0.216	0.215	0.214	0.213	0.212	0.21
2	0.3	0.312	0.324	0.327	0.282	0.27	0.243	0.219	0.214
5	0.36	0.38	0.396	0.4	0.324	0.3	0.273	0.245	0.223
8	0.46	0.492	0.51	0.515	0.425	0.395	0.3	0.271	0.239
10	0.538	0.581	0.602	0.613	0.506	0.473	0.376	0.339	0.237

Regarding zinc lower concentrations of 10, 20 and 50 µg.l^{-1} stimulated the carotenoid synthesis by 10, 15 and 20 %, respectively as compared with higher concentrations of 100, 200, 300, 400 and 600 µg.l^{-1} zinc which reduced the carotenoids by 4, 8, 22, 30 and 46 %, respectively below the control culture after 10 days of incubation (Table 4). Also a significant inverse relation was recorded between carotenoids and zinc concentrations as observed in the following equation:

Carotenoids – 0.0014 (Zn conc.) + 1.9734, where, $R^2 = 0.2158$, n= 40 and $P \leq 0.05$

Table 3. Effect of different concentrations of copper and zinc (µg.l^{-1}) on the chlorophyll-c calculated as µg.ml^{-1} of *Nitzschia closterium* (each value is the mean of three determinations which were closely related).

Copper (µg.l^{-1})									
Day	Control	1	5	50	100	200	300	--	--
0	0.15	0.15	0.15	0.15	0.148	0.147	0.146	--	--
2	0.211	0.217	0.221	0.194	0.186	0.175	0.169	--	--
5	0.252	0.264	0.27	0.217	0.206	0.201	0.184	--	--
8	0.317	0.336	0.345	0.288	0.247	0.212	0.193	--	--
10	0.37	0.4	0.411	0.355	0.311	0.259	0.2	--	--
Zinc (µg.l^{-1})									
Day	Control	10	20	50	100	200	300	400	600
0	0.15	0.15	0.15	0.151	0.15	0.149	0.148	0.147	0.146
2	0.211	0.213	0.216	0.219	0.202	0.194	0.177	0.162	0.16
5	0.252	0.257	0.262	0.267	0.231	0.214	0.2	0.181	0.l69
8	0.317	0.326	0.333	0.342	0.298	0.279	0.219	0.2	0.18
10	0.37	0.385	0.392	0.407	0.351	0.333	0.274	0.251	0.181

v. Total soluble proteins: The total soluble proteins of *Nitzschia closterium* also increased in the culture treated with 5 and 50 µg.l^{-1} copper and zinc by about 24 and 26 %, respectively over the control culture after 10 days of incubation (Table 5). On the other hand, the concentrations of 300 and 600 µg.l^{-1} copper and zinc inhibited the total soluble proteins of the tested organism by about 26 and 31 %, respectively. Generally, in the control culture, the total soluble proteins were 8.5 µg.ml^{-1} as compared with 6.29 and 5.86 µg.ml^{-1} in the cultures treated with 300 and 600 µg.l^{-1}copper and zinc, respectively after 10 days of incubation. Statistically, a significant inverse relation existed between the total soluble proteins and copper concentrations as the following equation; Total soluble proteins = - 0.0057 (Cu conc.) + 6.3097, where, $R^2 = 0.317$, n= 30 and $p \leq 0.05$. While a low significant inverse relation was observed between the soluble proteins and zinc concentrations as recorded in the following equation: Total soluble proteins = - 0.0038 (Zn conc.) + 6.5355, where, $R^2 = 0.1408$, n= 40 and $p \leq 0.05$

vi. Photosynthetic activity and respiration: As present in Table 6, the copper concentrations of 1 and 5 µg.l^{-1} stimulated the O_2- evolution of *Nitzschia closterium* by about 2.5 and 6 %, respectively. Further increase in copper concentrations successively reduced the amount of oxygen evolved. Thus, 10, 15, 19 and 27 % reductions in O_2-evolution below the control level were recorded in response to treatment with 50, 100, 200 and 300 µg.l^{-1} copper, respectively.

The treatment of the cultures with 10, 20 and 50 µg.l^{-1} zinc stimulated the oxygen evolution of the organism by about 3, 8 and 9.5 % respectively over the control culture. On the other hand, higher zinc concentrations of 100, 200, 300, 400 and 600 µg.l^{-1} reduced the O_2-evolved by about 8, 11, 20, 26 and 32 '%, respectively below the control culture.

Table 4. Effect of different concentrations of copper and zinc (µg.l^{-1}) on the corotenoids calculated as (µg.ml^{-1}) of *Nitzschia closterium* (each value is the mean of three determinations which were closely related).

Copper (µg.l^{-1})									
Day	Control	1	5	50	100	200	300	--	--
0	1.03	1.03	1.03	1.03	1.02	1	0.98	--	--
2	1.42	1.5	1.54	1.33	1.28	1.21	1.14	--	--
5	1.78	1.92	1.99	1.56	1.51	1.46	1.33	--	--
8	2.26	2.49	2.6	2.08	1.81	1.6	1.47	--	--
10	2.6	2.91	3.07	1.51	2.18	1.95	1.53	--	--
Zinc (µg.l^{-1})									
Day	Control	10	20	50	100	200	300	400	600
0	1.03	1.03	1.03	1.05	1.03	1	0.98	0.96	0.95
2	1.42	1.48	1.47	1.5	1.38	1.33	1.21	1.12	1.06
5	1.78	1.89	1.9	1.96	1.65	1.58	1.42	1.33	1.24
8	2.26	2.44	2.51	2.6	2.15	2.05	1.63	1.5	1.38
10	2.6	2.86	3	3.12	2.5	2.39	2.03	1.82	1.4

With regard to the effect of different copper treatments on the dark respiration of the tested organism, data present in Table 6 show that values of 1 and 5 µg.l^{-1} copper stimulated the respiration of *N. closterium* by about 3 and 8 %, respectively over the control culture, while 300 µg.l^{-1} copper reduced the respiration with about 50%.

Generally, the culture treated with 10 µg.l^{-1} zinc produced the same O_2-uptake as in the control culture of *N. closterium.* Whereas, 20 and 50 µg.l^{-1} stimulated the respiratory O_2-uptake by about 5 and 7 %, respectively. On the other hand, higher zinc concentrations (100, 200, 300, 400 and 600 µg.l^{-1}) inhibited the respiration, the reduction amounting 63 % below the control level in culture treated with 600 µg.l^{-1}.

Table 5. Effect of different concentrations of copper and zinc (µg.l^{-1}) on the total soluble proteins calculated as µg.ml^{-1}of *Nitzschia closterium* (each value is the mean of three determinations which were closely related).

Copper (µg.l^{-1})									
Day	Control	1	5	50	100	200	300	--	--
0	3.41	3.41	3.42	3.41	3.4	3.39	3.38	--	--
2	4.79	5.12	5.22	4.6	4.45	4.31	4.21	--	--
5	5.75	6.32	6.55	5.35	5.18	4.77	4.66	--	--
8	7.31	8.33	8.7	6.87	6.36	5.85	5.63	--	--
10	8.5	9.94	10.54	8.16	7.56	7.1	6.29	--	--
Zinc (µg.l^{-1})									
Day	Control	10	20	50	100	200	300	400	600
0	3.41	3.41	3.42	3.44	3.42	3.41	3.4	3.39	3.38
2	4.79	4.98	5.12	5.27	4.64	4.46	4.36	4.07	3.83
5	5.75	6.1	6.44	6.61	5.35	5.12	5.06	4.66	4.37
8	7.31	7.89	8.48	8.77	6.94	6.58	6	5.55	5.34
10	8.5	9.52	10.2	10.71	8.16	7.82	7.31	6.8	5.86

Also, significant inverse relations were recorded with the statistical analysis for the different concentrations of copper and zinc against photosynthetic activity and respiration as shown in the following equations:

O_2 -evolution = - 0.0005 (Cu conc.) + 0.518, where, $R_2 = 0.8838$, n= G and $p \leq 0.05$.

O_2-uptake (respiration) = - 0.0002 (Cu conc.) + 0.1403, where, $R_2 = 0.9158$, n= 6 and $p \leq 0.05$.

O_2-evolution = - 0.0003 (Zn conc.) + 0.537, where, $R_2 = 0.8879$, n= 8 and $p \leq 0.05$.

O_2-uptake (respiration) = - 0.0002 (Zn conc.) + 0.1443, where, $R_2 = 0.9546$, n= 8 and $p \leq 0.05$

Table 6. Effect of different concentrations of copper and zinc ($\mu g.l^{-1}$) on the photosynthetic O_2-evolution and dark respiration ($\mu gmo.l^{-1}$ $O_2.m1^{-1}$) of *Nitzschia closterium* after five days of incubation (each value is the mean of three determinations which were closely related).

Copper ($\mu g.l^{-1}$)	O_2-evolution	O_2-uptake	Zinc ($\mu g.l^{-1}$)	O_2-evolution	O_2-uptake
Control	0.516	0.140	control	0.516	0.140
1	0.529	0.145	10	0.531	0.140
5	0.547	0.151	20	0.557	0.147
50	0.465	0.118	50	0.565	0.150
100	0.440	0.102	100	0.475	0.120
200	0.418	0.092	200	0.459	0.112
300	0.377	0.070	300	0.413	0.086
--	--	--	400	0.382	0.075
--	--	--	600	0.353	0.052

DISCUSSION

A clear comprehension of growth and the factors, by which it is controlled at the molecular level, should provide new approaches and a clear insight into the mechanism through which heavy metals operate. Copper concentration that affects growth in microalgae is largely variable and depends on the species assayed, cell density, composition of the medium, physical culture conditions etc., (Whitton, 1968; Stauber and Florence, 1985a). As found by Lumsden and Florence (1983), natural seawater can support the growth or *N. closterium* for at least 72 hours. The cell division rate for this species is faster in nutrient-rich modified marine medium-f, as proposed by Guillard and Ryther (1962).

The results obtained in the present study indicated the lower concentrations of 1 and 5 $\mu g.l^{-1}$ copper stimulated the growth of *Nitzschia closterium* measured as optical density by 12 and 17 %, respectively over the control culture after 10 days of incubation. On the other hand, concentrations of 50, 100, 200 and 300 $\mu g.l^{-1}$ copper inhibited the algal growth by 7, 22, 36 and 53 %, respectively (Table 1). These results coincided with that recorded by Stauber and Florence (1989), who reported that copper at concentrations higher than 200 $\mu g.l^{-1}$ inhibited the growth of *N. closterium* by 50% below the control. Also, Samuel (1976) observed 50 % reduction in the growth rate of *Nitzschia closterium* induced by 50-100 $\mu g.l^{-1}$ copper at initial cell densities of 5 x 104 $cell.ml^{-1}$.

Copper was found to be more toxic to the dinoflagellate, *Prorocentrum micans* than to the diatom, *Nitzschia closterium* as recorded by Carpene and Boni (1992). In this connection, Abalde *et al.* (1995a) indicated that 0.1 mgl^{-1} copper provoked about 50 % reduction, while 1.0 mgl^{-1} completely inhibited the growth of the marine diatom, *Phaeodactylum tricornutum.* Moreover, the toxic effect of copper on the growth of the marine green alga, *Dunaliella tertiolecta* was clearly demonstrated in the cultures treated with 12 and 16 mg Cu^{2+}.1 as recorded by Abalde *et al.*

(1995b). At the cell membrane, copper may interfere with cell permeability or the binding of essential metals (Sunda and Huntsman, 1983; Suwalsky *et al.*, 1998). Following copper transport into the cell, copper may react with -SH enzyme groups disrupting enzyme-active sites and cell division (Fisher and Jones, 1981; Stauber and Florence, 1985b; Florence and Stauber, 1986; Visviki and Rachlin, 1991 and Nies, 1999). The differences in metal toxicities could be attributed to differential affinities of the cations for sulphur complexation. Metals with high affinity for sulphur such as mercury and copper are expected to be more toxic than those which exhibited lower affinity for sulphur such as chromium and manganese (Nies, 1999).

Regarding zinc, concentrations of 10, 20 and 50 $\mu g.l^{-1}$ stimulated the growth of *N. closterium* by 10, 13 and 16 %, respectively over the control culture after 10 days of incubation. On the other hand, 100, 200, 300, 400 and 600 $\mu g.l^{-1}$ zinc reduced the algal growth by 8, 16, 33, 43 and 60 %, respectively below the control culture. Fisher and Jones (1981) reported an increase in the growth rate of *Asterionella japonica* in response to elevated levels of copper and zinc. El-Naggar (1993) indicated that higher concentrations of zinc reduced the growth of *Chlorella vulgaris* and *Scenedesmus bijuga.* Gustavson and Waengberg (1995) indicated that at lower concentration of copper (0.016 μmole), the tolerance of phytoplankton communities increased after 12 days but at higher copper value (0.24 μmole), an increased tolerance of copper was recorded in the microalgal communities even after two days. However, communities exposed to higher copper concentrations also showed increased tolerance for zinc, indicating common tolerance mechanisms for copper and zinc. Moreover, metals not only affect algal cells by interfering with the metabolic processes directly but also can influence algal growth indirectly through a change in the pH of the medium. The increase in copper concentrations resulted in a decrease in the pH of the medium of the green alga, *Chlorella vulgaris* (Lam *et al.*, 1999).

Chlorophylls are key compounds in plants for trapping light energy for photosynthesis; thus, their quantitative determination is of great importance in studies of photosynthesis, primary production and related subjects. Chlorophylls other than chlorophyll-a are distributed among the algal classes in a highly systematic way making these compounds of great value in chemotaxonomic studies (Jensen, 1978).

The chlorophyll-a content of *Nitzschia closterium* followed a similar pattern of change to that of growth in response to different concentrations of copper and zinc. Thus, 5 and 50 $\mu g.l^{-1}$ copper and zinc stimulated chlorophyll-a biosynthesis by 15 and 14 %, respectively over the control level after 10 days of incubation. Whereas, 300 and 600 $\mu g.l^{-1}$ copper and zinc reduced chlorophyll-a content by 50 and 56 %, respectively (Table 2).

Similarly, the lower concentrations of 1 and 5 $\mu g.l^{-1}$ copper stimulated the content of chlorophyll-c by 7 and 11 % respectively above the control culture after 10 days of incubation (Table 3), while concentrations of 50, 100, 200 and 300 $\mu g.l^{-1}$ copper reduced the chlorophyll-c by 4, 16, 30 and 46 %, respectively.

Zinc concentrations of 10, 20 ai1d 50 $\mu g.l^{-1}$ increased chlorophyll-c content of *Nitzschia closterium* to about 4, 6 and 10 %, respectively above the control culture after 10 days of incubation. On the other hand, concentrations of 100, 200, 300, 400 and 600 $\mu g.l^{-1}$ zinc inhibited the chlorophyll-c to about 5, 10, 26, 32 and 51 %, respectively (Table 3). In accordance with the present results, Fisher and Frood (19 m) observed reduction in the pigment content of the marine diatoms in response to excessive copper concentrations. Also, EI-Naggar (1993) indicated that low copper and zinc concentrations induced significant increase in the different pigment fractions (chl-a, chl-b and carotenoids) of the green algae, *Chlorella vulgaris* and *Scenedesmus biyga.* On the other hand, higher concentrations of CU^{2+} and Zn^{2+} suppressed the levels of the pigment fractions. Abalde *et al.* (1995a) concluded that chlorophyll-a content of the marine microalga, *Phaeodactylum tricornumtum* increased when copper concentration increased to 0.1 $mg.l^{-1}$ copper, whereas higher concentrations provoked

a decrease in chlorophyll-a. Moreover, copper inhibited the synthesis of chlorophylls (a and b) in *Chlorella vulgaris* indicating that chloroplast appears to be the primary targets for metal toxicity as recorded by Lam *et al.,* (1999).

The accessory photosynthetic pigments, carotenoids are ubiquitous polyenes studied mainly for their chemotaxonomic importance (Goodwin, 1973), although some (e.g., fucoxanthin and peridinin) are active in collecting light for photosynthesis. They share with the chlorophylls a high sensitivity to oxygen, light and acids (Jensen, 1978). In diatoms, the predominant pigments are carotenoids over the chlorophylls, fucoxanthin may account for much as 75 % of the total pigment component of the algal cells and thus is more noticeable than the chlorophylls (Bold and Wynne, 1978).

Carotenoids of *Nitzschia closterium* were also stimulated with the lower concentrations of copper and zinc, while the higher concentrations inhibited them (Table 4). Rai *et al.,* (1991) reported that CU^{2+} increased carotenoid biosynthesis compared to chlorophyll-a of *Anabaena doliolum* resulting in increased carotenoid/chlorophyll-a ratio. Also, zinc was found to increase the carotenoid/chlorophyll ratio and may enhance oxidative steps and inhibits the reductive steps in the biosynthetic pathway of these pigments in *Euglena gracilis* (De-Filippis *et al.,* 1981a). In addition, copper caused bleaching of phycocyanin in the blue-green alga, *Anacystis nidulans* (Gupta and Singhal, 1996). Moreover, El-Naggar (1993) concluded that carotenoids showed more sensitivity to high Cu^{2+}concentrations than chlorophyll-a or chlorophyll-b for *Chloroella vulgaris* and *Scenedesmus bijuga.* Abalde *et al.* (1995b) indicated that chlorophyll-a and carotenoid contents were more affected by copper than chlorophyll-b content. In the present study, the carotenoids of *N. closterium* seemed to be more resistant to copper and zinc toxicity than chlorophylls.

Regarding protein content, inclusion of 5 and 50 $\mu g.l^{-1}$ copper and zinc in the culture medium of *Nitzschia closterium* stimulated the protein content by 24 and 26 %, respectively over the control culture. The higher concentrations of 300 mg 600 $\mu g.l^{-1}$ copper and zinc inhibited the biosynthesis or soluble proteins by 26 and 31 %, respectively (Table 5). The increased protein content with low concentrations of heavy metals may be attributed to inhibition of amino acids export out of cells by heavy metals as reported by De-Filippis *et al.* (1981a), who observed that sublethal concentrations of $ZnCl_2$, $CdCl_2$ and $HgCl_2$ increased the total protein content of *Euglena gracilis.* Similar observations were reported by De-Filippis and Pallaghy (1976) and EI-Naggar (1993). However it is known that the accumulation of protein al low heavy metal concentrations may be one of the ways through which the algae can abolish their toxic effect, and or may be due to increased respiration leading to the utilization of carbohydrate in increased nitrogen metabolism. On the other hand, inhibition of protein accumulation induced by higher concentrations of heavy metals may be attributed to the toxic action of these heavy metals on the enzymatic reactions responsible for protein biosynthesis and the extent of such inhibition was concentration and pH dependent (Hart and Scaife, 1977; Kobbia *et al.,* 1985; Rai *et al.,* 1991 and EI-Naggar, 1993).

Copper may also exert its toxicity in subcellular organelles interfering with photosynthesis in the chloroplasts (Overnell, 1975) and ATP (adenosine triphosphate) production (Viarengo *et al.,* 1981). As for growth, copper concentration that affects photosynthesis depends on the species assayed (Abalde *et al.,* 1995a, b).

The photosynthetic oxygen evolution of *N. closterium* was stimulated in the cultures treated with 5 and 50 $\mu g.l^{-1}$ copper and zinc by about 6 and 9.5 %, respectively over the control culture. While, concentrations of 300 and 600 $\mu g.l^{-1}$copper and zinc inhibited the O_2-evolved by about 27 and 32 %, respectively (Table 6). In this connection, Stauber and Florence (1990) reported that most of the zinc associated with the cells of *Nitzschia closterium* was bound to the cell surface, with only 3 to 4 % of the extracellular zinc penetrating the cell membrane. Once inside the cell, zinc exerted its toxicity at a number of

sites. They reported that increased adenosine triphosphate (ATP) production and electron transport system (ETS) activity were observed in zinc-treated cells. Zinc also enhanced the production of cellular thiols (SH) and total glutathione. Wong and Chang (1991) have observed that 0.1 mg $Cu.l^{-1}$ produced a slight inhibition on photosynthesis of *Chlorella pyrenoidosa,* while 0.25 mg $Cu.l^{-1}$ produced total inhibition. EI-Naggar (1993) indicated that the toxic effect of heavy metals on photosynthesis of *Chlorella vulgaris* and *Scenedesfmus bijuga* could be arranged as $Cd^{2+} > CU^{2+} > Zn^{2+}$. Abalde *et al.,* (l995a) concluded that the photosynthetic rate of *Phaeodactylum tricornutum* decreased when copper concentration increased (0.5 mg $Cu.l^{-1}$ reduced the photosynthetic rate by 50 %) and the growth was more affected by copper than photosynthesis. This uncoupling between division rate and photosynthesis can be due to copper inhibiting the process of cell division independently of any effect on the production of new cell material (Stauber and Florence, 1987). The inhibitory concentration for the photosynthsis rate of the green alga, *Dunaliella tertiolecta* was between 8 and 12 mg $Cu.l^{-1}$ (Abalde *et al.,* 1995b). Moreover, Osman *et al.,* (1996) showed that copper concentrations higher than 0.4 μM in *Anabaena cylindrical* and > 1.0 μM in *Nostoc muscorum* decreased the photosynthetic 0_2-evolution of both organisms.

As regards the effects of heavy metals on respiration, the obtained results showed that 5 and 50 $\mu g.l^{-1}$ copper and zinc had a stimulatory effect on respiration of *N. closterium,* but the values of 300 and 600 $\mu g.l^{-1}$ copper and zinc, respectively caused a significant reduction in respiratory O_2-uptake below the control culture (taking into consideration that culture treated with 10 $\mu g.l^{-1}$ Zinc produced the same O_2-uptake as in the control culture). These results coincided with those obtained by Gupta and Arora (1978) and El-Naggar (1993), who recorded an increase in the respiratory rate of the cultures treated with low concentrations of copper, whereas higher concentrations of copper inhibited the O_2-uptake. On the other hand, De-Filippis *et al.* (1981b) and El-Naggar (1993) observed that higher concentrations of zinc caused a significant reduction in O_2-uptake. However, they concluded that the effect of heavy metals on photosynthesis and respiration is metal concentration and species dependent. Furthermore, Elizabeth and Gadd (1996) stated that zinc stimulated the respiration of the seaweed, *Ulva lactuca* with maximum stimulation at 1.5 mM, whereas above 5 mM respiration was inhibited by about 50%.

In conclusions, low concentrations of copper (ranges of 1 to 5 $\mu g.l^{-1}$) and zinc (ranges of 10 to 50 $\mu g.l^{-1}$) showed an increase in the growth, different pigment fractions, total soluble proteins as well as O_2-evolution in photosynthesis and dark respiration (O_2uptake) of the diatom, *Nitzschia closterium* as compared with the control culture. Whereas, higher concentrations of these metals (50 to 300 $\mu g.l^{-1}$ copper and 100 to 600 $\mu g.l^{-1}$ zinc) inhibited the above metabolic processes of the organism, where the concentrations of copper above 300 $\mu g.l^{-1}$ and zinc above 600 $\mu g.l^{-1}$were the lethal values of the organism (complete inhibition). These results supported with statistical analysis of linear regression equations; where they recorded a significant reverse correlation between them.

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