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Adsorption of zinc ions by *Scenedesmus obliquus* and *S. quadricauda* and its effect on growth and metabolism

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Abstract

Zinc adsorption by two green algae, *Scenedesmus obliquus* and *Scenedesmus quadricauda*, was investigated. The maximum adsorption capability of zinc ion obtained from the Langmuir adsorption isotherms was higher for *S. obliquus* (6.67) than for *S. quadricauda* (5.03), and *S. obliquus* was more tolerant to zinc phytotoxicity than *S. quadricauda*. Lower concentrations of zinc increased dry mass, chlorophylls *a* and *b*, carotenoids, and total amino acid contents in both algae. On the other hand, higher concentrations of zinc were inhibitory for growth and the other metabolic activities in a concentration dependent manner.

Additional words: amino acids, green algae, growth, metal biosorption, phosphatase.

Introduction

The adsorption (physical adsorption, ion exchange, or chemisorption) of heavy metal ions is a very beneficial property of most of algae (Pappas *et al.* 1990, Scott and Palmer 1990). The kinetics of metal uptake took place in two stages (Nourbakhsh *et al.* 1994). The first stage, physical adsorption or ion exchange at the cell surface, is very rapid and occurs in a short time after the alga comes into contact with the metal. The subsequent stage, related to metabolie activity, is slower and called chemisorption. Cellular distribution analysis showed that relatively large amounts of metal ions were bound to the cell wall, and to an intracellular insoluble fraction (Garnham *et al.* 1992), namely, polyphosphates (Jensen *et al.* 1982), and/or phytochelation (Robinson 1988). There are some aquatic organisms that can accumulate heavy metals without marked toxic efiects. For example, *Euglena gracilis* could accumulate Zn^{2+} ions until 5 mg g^{-1}(d.m.) (Fukami 1988). Zinc is essential micronutrient for algae. Its deficiency leads to poor growth and low dry mass (Shrotri *et al.* 1981). Algal growth is stimulated by lower concentrations of the metals and totally inhibited by higher concentrations (Whitton 1970). Moreover, higher concentrations of Zn^{2+} decreased cell division, movement, total chlorophyll and ATP contents (De Filippis *et al.* 1981), and carotenoids/chlorophyll ratio (Rai *et al.* 1981). The goal of this study was to determine the effect of zinc on the biosynthesis of pigments, amino acid contents, and acid and alkaline phosphatases as well as the range of zinc adsorption capabilities by two green algae, *Scenedesmus obliguus* and *S. quadricauda*.

Materials and methods

Algae and growth conditions: The green algae *Scenedesmus obliquus* (Turpin) Kutz. and *Scenedesmus quadricauda* (Turpin) Br. were obtained from Dr. Y. El-Ayouty, Algal Laboratory, Botany Department, Zagazig University. The cells were grown photoautotrophically in sterilized nutrient basal medium (Starr 1971) containing different concentrations of zinc (0.0, 0.5, 1.5, 4.5, and 8.0 mg dm^{-3}). The cultures were illuminated continuously with fluorescent tubes (irradiance of 134 W m^{-2}) and incubated at temperature of 25 °C. The cultures were

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harvested towards the end of exponential phase (ten days after inoculation). Three replicates were set up for each treatment. Growth of the cultures was monitored by determination of the dry mass according to American Public Health Association (1985). The contents of chlorophyll (Chl) *a*, Chl *b*, and carotenoids (Car) were determined according to Metzner *et al.* (1965).

Total amino acid contents: The protocol used by Moore *et al.* (1958) was followed: 0.05 g of each dried algal sample was transferred to an analysis tube, then 10 cm^3 6 M HCl containing 5 cm^3 dm^{-3}(acid) mercaptoethanol were added. The tubes was sealed off, then hydrolyzed at 110 °C for 24 h. After hydrolysis, the tube was cooled at room temperature, then cracked by a tube cutter. The hydrolyzed sample was diluted to 25 cm^3 with distilled water and injected in a syringe fitted with *Swinex* filter. The sample was then dried by slow evacuation in a desiccator heated to 60 °C in the presence of KOH. The dried residue was dissolved in 0.2 M sodium citrate buffer (pH 2.2) and run in a *Beckman* amino acid analyzer *Model 119 Cl.* The quantity of different amino acids was calculated as the percentage composition of total amino acids.

Estimation of phosphatases: Phosphatases were extracted according to Weimberg (1975). For enzyme essay, algal cells were collected and extracted with 0.1 M Tris-HCl (pH 8.0), 0.01 M EDTA, 0.1 % (β-mercapto-ethanol. Enzyme activities were determined by measuring the released Pi from enzyme substrate according to Ames (1966). The activity of acid phosphatase was determined using the following reaction mixture: 1 M acetate buffer (pH 5.5), 0.05 M sodium pyrophosphate (pH 5.5), and crude enzyme. The activity of alkaline phosphatase was estimated using the reaction mixture: 1 M Bicine buffer [16.32 g N,N-bis (2-hydroxyethyl) glycine was dissolved in 100 cm^3 bidistilled water, and the solution was adjusted to pH 8.0], 0.05 M sodium pyrophosphate {pH 8.0), 0.1 M Mg C12, and crude enzyme. The essay mixture of ATPase contained 5 mM ATP/Tris (pH 6.5), 5 mM $MgSO_4$, 30 mM Tris (pH 6.5), and crude extract.

Zinc adsorption was analyzed using an atomic absorption spectrophotometer *Perkin Elmer Model 3100* (Zagazig University). An equivalent biomass of fresh algal cells (50 mg d.m.) were added to the different zinc concentrations (0.5, 1.5, and 4.5 mg dm^{-3}) and incubated in a shaker for 24 h then the algal cells were washed with EDTA (1 mg dm^{-3}) according to Fayed *et al.* (1983). The algal suspensions were filtered through *GF/A* filter disk using venturic pump. The tiltrate was collected in glass vials and immediately analyzed to measure the concentration of Zn^{2+} adsorbed on the surface of algal cells. The mean values from triplicate runs of both initial and final metal concentrations in the solution, were used to calculate the following parameters: *1*) Percentage of Zn^{2+} removed by algae, and *2*) adsorption capability (mg of metal adsorbed/initial mass of algae). A linearized form of the Langmuir adsorption isotherm equation was obtained: C/Y = C/Ym + I/KYm where, Ym = the maximum adsorption, K = equilibrium constant, related to the affinity of binding site, Y = adsorption at residual metal concentration, C = residual metal concentration. From a plot of C/Y *vs.* C, the slope (S = l/Ym), gives Ym, the maximum adsorption capability, and the intercept (I = 1/KYm) gives K, the affinity constant of metal on the biomass unit.

Results and discussion

Growth and photosynthetic pigments: The present study indicated that low zinc concentrations (0.5 and 1.5 mg dm^{-3}) induced increase in growth of *S. obliquus* and (0.5 mg dm^{-3}) in *S. quadricauda*, whereas application of high Zn^{2+} concentrations suppressed the growth of both algae (Fig. 1). The results clarified that *S. quadricauda* is more sensitive to high zinc concentrations than *S. obliquus*. In accordance with the above mentioned results, Bariaud and Mestre (1984) reported that growth rate decrease as Zn^{2+} concentration increased in the culture media of *Euglena gracilis*. Visviki and Rachlin (1991) attributed the reduction of algal growth by heavy metals to the inhibition of normal cell division due to binding of metal to sulfhydryl groups which are important in regulating plant cell division. Low zinc concentrations (1.5 mg dm^{-3} for *S. obliquus*, and 0.5 mg dm^{-3} for *S. quadricauda*) induced an increase in pigment contents. These results were in agreement with Fisher and Jones (1981), who reported that low Zn^{2+} concentrations

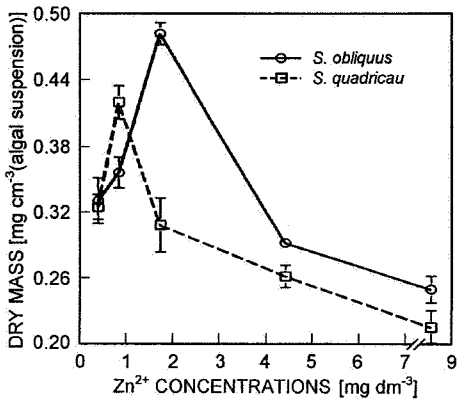

Fig. 1. Effect of different zinc concentrations on the dry mass of *Scenedesmus obliquus* and *S. quadricauda*.

enhanced the total chlorophyll content in *Asterionella japonica*. However, higher zinc concentrations were associated with a progressive reduction in the contents of pigments. Zn^{2+} showed more inhibitory effect on Chl *b* as compared to Chl *a* for both algae. Rai *et al.* (1991) also found that zinc induced a drastic effect on Chl *b*. In addition, the obtained results showed that Car were more sensitive to zinc than Chl in *S. obliquus*. In contrast, Car were more resistant to zinc toxicity in *S. quadricauda* which suggested that zinc may enhanced oxidative steps and inhibit the reductive steps in the biosynthesis pathway of these pigments. Similar observations have been reported by Sharma and Chopra (1987). Clijsters and Van Assche (1985) and De Filippis and Ziegler (1993) found that heavy metals generally inhibit overall physiological processes with marked effects on Chl *a* and *b*.

Table 1. Effects of different zinc concentrations [mg dm^{-3}] on the pigment contents [mg g^{-1}(d.m.)] of *Scenedesmus obliquus* and *S. quadricauda* (means ± SE of three replicate experiments).

Algae	Zn^{2+}	Chl *a*	Chl *b*	Car	Chl *a*/*b*	Car/Chl *a*+*b*
S. obliquus	0.0	1.43 ± 0.16	1.23 ± 0.21	0.45 ± 0.11	1.16	0.17
	0.5	1.70 ± 0.00	1.24 ± 0.17	0.48 ± 0.13	1.37	0.16
	1.5	2.49 ± 0.18	1.27 ± 0.04	0.59 ± 0.21	1.96	0.16
	4.5	1.66 ± 0.03	0.87 ± 0.05	0.29 ± 0.00	1.91	0.11
	8.0	0.90 ± 0.00	0.75 ± 0.11	0.15 ± 0.03	1.20	0.09
S. quadricauda	0.0	1.30 ± 0.21	1.20 ± 0.13	0.68 ± 0.15	1.08	0.27
	0.5	1.49 ± 0.35	1.33 ± 0.17	0.83 ± 1.12	1.12	0.29
	1.5	1.26 ± 0.21	0.80 ± 0.22	0.61 ± 0.05	1.58	0.30
	4.5	1.10 ± 0.05	0.66 ± 0.00	0.42 ± 0.12	1.67	0.24
	8.0	0.65 ± 0.00	0.26 ± 0.03	0.23 ± 0.00	2.50	0.25

Table 2. Effect of different zinc concentrations [mg dm^{-3}] on the amino acid contents. The percentage composition (by mass) of total amino acid from *Scenedesmus obliquus* and *S. quadricauda* after 10 d of incubation.

Amino acid	*S. obliquus*			*S. quadricauda*		
	0.0	1.5	8.0	0.0	0.5	8.0
Glutamate	10.8	12.5	8.2	11.2	12.1	8.4
Arginine	6.4	7.6	6.9	6.3	7.1	6.8
Proline	4.1	5.2	4.6	4.5	5.2	4.9
Histidine	2.9	3.1	1.8	2.4	2.8	1.8
Aspartate	9.9	11.8	8.8	9.3	10.1	7.4
Threonine	3.2	3.4	2.7	4.1	4.3	3.2
Lysine	7.4	8.3	6.5	6.5	7.2	4.8
Isoleucine	4.1	4.8	3.4	4.3	4.6	3.7
Methionine	2.2	2.4	1.8	2.6	2.8	1.4
Glycine	5.8	6.4	4.1	6.1	7.3	5.2
Serine	3.2	3.8	2.7	3.3	3.5	2.8
Cystine	0.6	1.1	0.8	0.5	0.8	0.7
Alanine	4.8	5.3	4.0	5.7	6.1	3.8
Valine	3.2	3.9	2.8	4.1	4.4	3.2
Leucine	8.5	9.4	7.5	8.4	9.0	6.3
Phenylalanine	6.5	7.4	5.6	6.2	7.2	4.1
Tyrosine	3.1	3.3	2.3	3.4	4.3	2.6

Total amino acids: The composition of amino acid of both algae exhibited resemblance under different concentrations of zinc (Table 2). Zinc at low concentrations (1.5 and 0.5 mg dm^{-3} a for *S. obliquus* and *S. quadricauda*, respectively) increased total amino acid contents (15 and 11 %, respectively), however, decreased them at high concentration (8.0 mg dm^{-3}). The present study showed an increase of arginine, proline, and cystine at high concentrations of zinc. In this connection, Kobbia *et al.* (1985) reported an accumulation of cystine, and arginine in *Chlorella fusca* in response to Ni^{2+} treatment. The increase in proline content may be attributed to promotion of proline synthesis from glutamate, decrease in the rate of proline oxidation, and/or inhibition of

incorporation of proline into protein. This finding was confirmed by the report of Buhl and Stewart (1983). Thus, lower Zn^{2+} concentrations had pronounced effect on rising the proportion of amino acids in both tested organisms (glutamate, aspartate, lysine, glycine, alanine, leucine, and phenyialanine). The sharp decrease in the content of total amino acids at high concentrations of Zn may be related to the drastic effect of the metal on the content of several amino acids.

Phosphatases: It is clear that the influence of zinc greatly differed according to the alga (Fig. 2). The maximum specific activity of acid phosphatase in *S. obliguus* was in control sample, while the highest activity of the same enzyme by *S. quadricauda* was at high concentration of Zn^{2+} (4.5 mg dm^{-3}). *S. quadricauda* is capable of synthesizing metal binding protein as a detoxification mechanism (Ting *et al.* 1991). Any increase in zinc concentration led to gradual decrease in its phosphatases activities by *S. obliquus* and *vice versa* for *S. quadricauda*. Shaaban-Dessouki *et al.* (1991) observed that Zn^{2+} at moderate concentration 300 μg dm^{-3} stimulated the activity of acid phosphatase in the *Scenedesmus*. Moreover, elevation of zinc to 500 μg dm^{-3} led to slight inhibition in the activity of acid phosphatase, while the previous concentration led to drastic decrease in the activity of this enzyme in *Anabaena*. Regarding to the influence of zine on alkaline phosphatase and ATPase, zinc had a more or less similar effect on the activities of either in *S. obliquus* or *S. quadricauda*. The maximum activity of both enzymes were recorded at 1.5 mg dm^{-3} zinc. It could be stated that zinc can act either in a stimulatory mode (Norbert 1978) or in an inhibitory one, depending on its concentration (Engel *et al.* 1981). The stimulatory action of Zn^{2+} on ATPase was explained indirectly by Rachlin *et al.* (1984). They observed that Zn^{2+} caused a reduction in the volume of thylakoid space of blue green algae and the photosynthetic activity under metal stress needs more ATP to derive metabolic machinery. In addition, Zn^{2+} activates many other enzymes, catalyzes the release of phosphorous (Bowen 1966), and eliminates carbon dioxide (Keilin and Mann 1944).

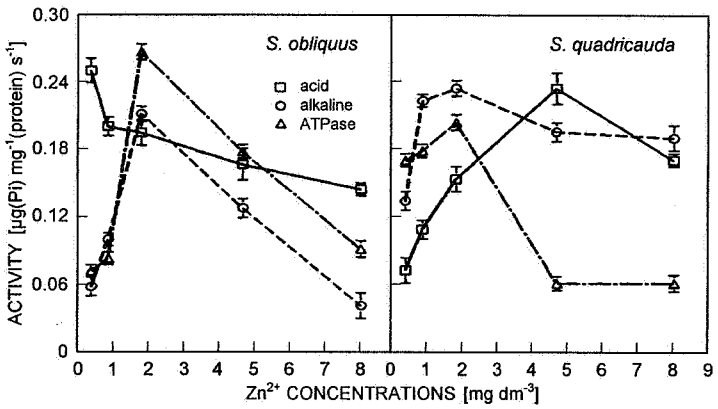

Fig. 2. Effect of different zinc concentrations on the phosphatases activities of *S. obliquus* and *S. quadricauda*.

Metal adsorption: Adsorption isotherms have commonly been used to describe experimental results for the uptake of metal ions by microorganisms, since the initial rapid uptake is believed to be due to binding of the metal ions onto the cell wall. Many studies have shown that at low metal ion concentrations, the amount of the metal ion accumulated (per unit of cell mass) is directly proportional to the concentration of the ion in the solution (Ting *et al.* 1991). From the linearized Langmuir adsorption isotherm the maximum specific adsorption capacity (Ym) was 6.67 in *S. obliquus* and 5.03 in *S. quadricauda*, indicating that the former alga is more efficient than the later one for removing of zinc ions (Table 3). On the other hand, the affinity of binding site (K) to Zn^{2+} ions were 0.19 and 0.27 for *S. obliquus* and *S. quadricauda*, respectively. The data revealed that

Table 3. The adsorption at residual metal concentration (Y), residual metal concentration (C) and their ratio of *S. obliquus* and *S. quadricauda* obtained from Langmuir isotherms (means ± SE of three replicate experiments).

Algae	Initial Zn^{2+} conc.	Y	C	C/Y
S. obliquus	0.5	0.294 ± 0.02	0.206 ± 0.01	0.70
	1.5	0.772 ± 0.04	0.728 ± 0.02	0.94
	4.5	2.016 ± 0.08	2.484 ± 0.10	1.23
	8.0	3.300 ± 0.10	4.700 ± 0.15	1.42
S. quadricauda	0.5	0.303 ± 0.02	0.197 ± 0.02	0.65
	1.5	0.740 ± 0.03	0.760 ± 0.05	1.03
	4.5	1.920 ± 0.07	2.580 ± 0.13	1.34
	8.0	2.950 ± 0.09	5.050 ± 0.18	1.71

S. obliquus is more resistant to the toxicity of zinc than *S. quadricauda*. Premuzic *et al.* (1991) established that chemical and structural characteristics of cell membranes vary with species and should therefore influence the capacity for uptake of metals by different micro-organisms. Wang *et al.* (1995) reported that *Phormidium* has a good potential activity for bioremoval of heavy metals and has a wide range of metal biosorption capacity. In addition, Wang *et al.* (1998) found that the specific adsorption capacity of *Phormidium* was 13.6 mg kg^{-1} for Pb and 10.1 mg kg^{-1} for Cu and the alga could reduce metal concentration to very low residual levels (0.01 mg cm^{-3}) within 60 min. Muramoto and Ohi (1983), and Wilde and Benemann (1993) indicated that bioremoval exhibits two stages: an initial fast (reversible) metal binding process (biosorption) and following slow process which can occur via complixation, coordination, chelation and ion exchange.

In conclusion, the application of biosorption in the purification of waste water offers a high potential for large-scale exploitation. The potential of natural, abundant and cheap algal biomass can be used successfully in removal of metal ions from solutions.

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