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Effect of Aluminum and Zinc on Enzyme Activities in the Green Alga *Selenastrum capricornutum*

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Acid rain produced by atmospheric pollution may decrease the pH value of water and increase the availability and potential toxicity of metals in water which have detrimental effects on aquatic organisms, including algae, the important component of the primary production, and, thus, the entire aquatic food chain. Recent reviews of the effects of acid rain on freshwater ecosystems have emphasized research interest in soluble trivalent aluminum, although Al is rated low among trace metals in biological importance (Winchester 1992). On the other hand, zinc is an important trace element for the growth of phytoplankton and the cofactor of some enzymes. The growth response and tolerance of different species of algae to Al and Zn have been reported by Whitton (1970) who showed that algal growth would be stimulated by lower levels of the metals and totally inhibited by higher levels. There is little information, however, on the effect of Al on biochemical processes in aquatic organisms.

This study investigates the influence of aluminum and zinc on several physiological processes in *S. capricornutum*, a common species of green alga in lake water. Algal growth (dry weight), ATP levels and the activities of several enzymes in the algal cells were measured after the treatment with various concentrations of Al and Zn in culture medium. Special attention is given to the relation between the enzymatic response and algal growth.

MATERIALS AND METHODS

S. capricornutum UTEX 1648 was obtained from University of Texas Culture Collection (USA), cultured in 80 ml liquid HB-4 medium (Li 1959) in 250 ml flasks kept on a rotatory shaker at 25 °C, and illuminated with cool-white fluorescent lights.

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The metals were introduced into the culture medium sterilized at 121 °C, 15 lb in^{-2} for 30 min at the beginning of the experiment. The theoretical concentrations were at 0.0, 0.02, 0.04, 0.10, 0.16 and 0.20 mg l^{-1} for Al^{3+} [as $Al_2(SO_4)_3$] and 0.0, 0.12, 0.30, 0.48, 0.60, and 0.96 mg l^{-1} for Zn^{2+} (as $ZnSO_4$). The stock solutions of $Al_2(SO_4)_3 \cdot 18H_2O$ and $ZnSO_4 \cdot 7H_2O$ were sterilized by filtering the solution through a 0.45 μm membrane. The pH of all solutions was adjusted to 5.5 with 1.0 M HCl. Cell density was 3.0-5.0 × 10^6 cells per ml. Algal cells were treated with metals for 96 hr. Three replicates were set up for each treatment and the experiments were repeated twice.

At the end of experiment, the dry weight per unit volume algal suspension was determined by filtering the algal suspension through filter papers, followed by a wash with distilled water and drying to constant weight at 105 °C (APHA et al. 1985).

The algal cells treated with metals were collected by centrifugation. For enzyme assays, the algal cells were extracted with 1 ml Tris/Borate (0.1 M/0.3 M, pH 7.5, 5 mM EDTA, and 7 mM β-mercaptoethanol) buffer on ice for 10 min, centrifuged at 10,000 × g for 10 min at 4 °C, the extracts were stored at -58 °C until enzyme measurement; For adenosine triphosphate (ATP) analysis, the algal cells were extracted with 1 ml 0.5 M Tris/HCl buffer, pH 8.1, at 95 °C for 3 min, centrifuged and stored by the method described previously.

The activities of glucose-6-phosphate dehydrogenase (G6PDH, EC. 1.1.1.49), acid phosphatase (EC. 3.1.3.2) and nitrate reductase (EC. 1.6.6.2) were determined as described earlier (Hammond 1985; Boller and Kende 1979; Hageman and Reed 1980). ATP level in algal cells was measured according to the method of David and Osmund (1978). Protein concentration in the samples was determined by the method of Lowry et al.(1951) and with bovine serum albumin (BSA) as the standard.

All biochemical compounds used in this experiment were from Sigma (USA).

RESULTS AND DISCUSSION

The growth of *S. capricornutum* was significantly inhibited by Al and Zn over the range of concentrations tested in this experiment. The dry weight of algal cells per unit volume of culture suspension declined with the increase of metal concentration in the culture medium (Figs. 1a and b).

However, the soluble protein concentration (mg soluble protein per

mg dry weight algal cell) increased after Al and Zn treatment (Figs. 1c and d). For Al, the concentration of 0.20 mg l^{-1} increased the protein content by 65% compared with the control. Similarly, at 0.96 mg l^{-1} Zn, the protein concentration in algal cells was about eightfold that in the control. These results indicated that the protein synthesis in the algal cells was not suppressed by the metals at the tested concentrations. Previous reports about the effect of metals on protein synthesis in fungi and plants vary. The present result was contrary to the conclusion obtained by Olelb-Farivar (1985) who demonstrated the suppression of protein synthesis in fungi exposed to Al, but the experimental data of Assche et al. (1988) showed that protein concentration increased in roots and leaves of the plant *Phaseolus vulgaris* after Zn and Cd treatment.

The enzymatic responses in the algal cells to metals are shown in Figs. 2a and b. The activity of acid phosphatase was significantly inhibited by the treatment of Al and Zn at all test concentrations. The correlation coefficients were highly significant ($r = -0.93$ and -0.96, respectively). For G6PDH, the activity decreased when the Al concentration was higher than 0.02 mg l^{-1} in the culture medium; when Zn was present, this enzyme activity was stimulated at 0.3 mg l^{-1}, but inhibited at higher concentrations. Likewise, the activity of nitrate reductase was stimulated by specified concentrations of Al (0.04-0.16 mg l^{-1}), but it was inversely related to all the tested concentrations of Zn. The measurements of ATP levels in algal cells are shown in Figs. 2c and d. ATP levels were lower after Al and Zn were applied compared with the control. The correlation between the Al or Zn concentration and ATP level (μg per ml algal culture suspension) was significant indeed ($r = -0.86$ and -0.96, respectively).

In previous reports, it was demonstrated that the activity of G6PDH in several plant species was stimulated by Zn (Matthys 1975) and Cd (Assche et al. 1988). The present results confirmed these data, but it was restricted to one Zn concentration (0.3 mg l^{-1}). Moreover, this enzyme activity was strongly inhibited at all the tested concentrations of Al. It appears that the effect of metals on G6PDH was dependent on the species and amount of metal.

The question about the possible physiological meaning of variation of enzyme activity by metals is still open. Among the enzymes studied in this investigation, G6PDH catalyzes NADPH-producing reactions (Matthys 1975). Zn is the trace element that plays an important role in many physiological processes of algal metabolism, and can stimulate some enzyme activities in the appropriate amount, especially those enzymes which depend on the NAD or NADP. The concentration factor (CF) of Zn in algal cell is up to 1-4·

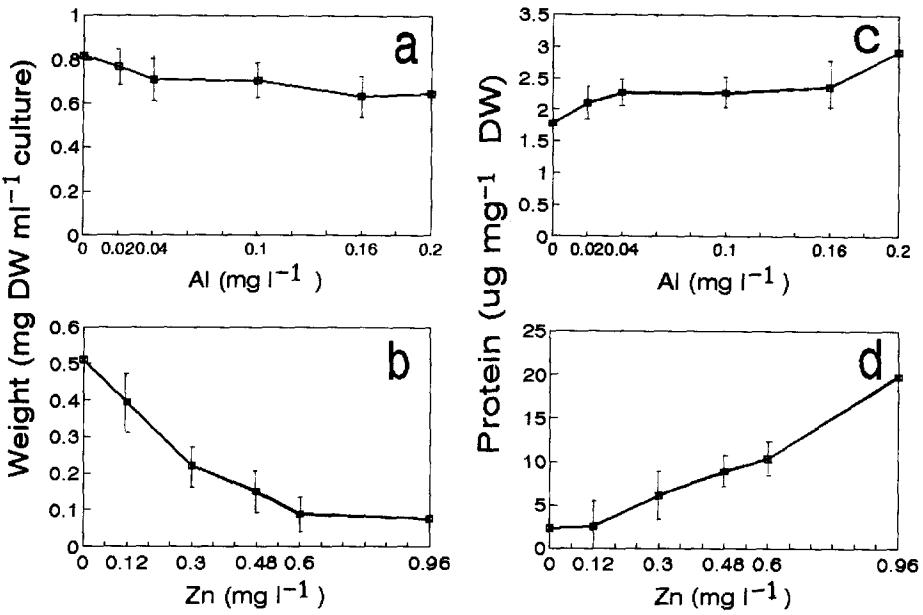

Figure 1. Dry weight and protein content in algal cells of *S. capricornutum* treated with (a,c) Al and (b,d) Zn in the culture medium.

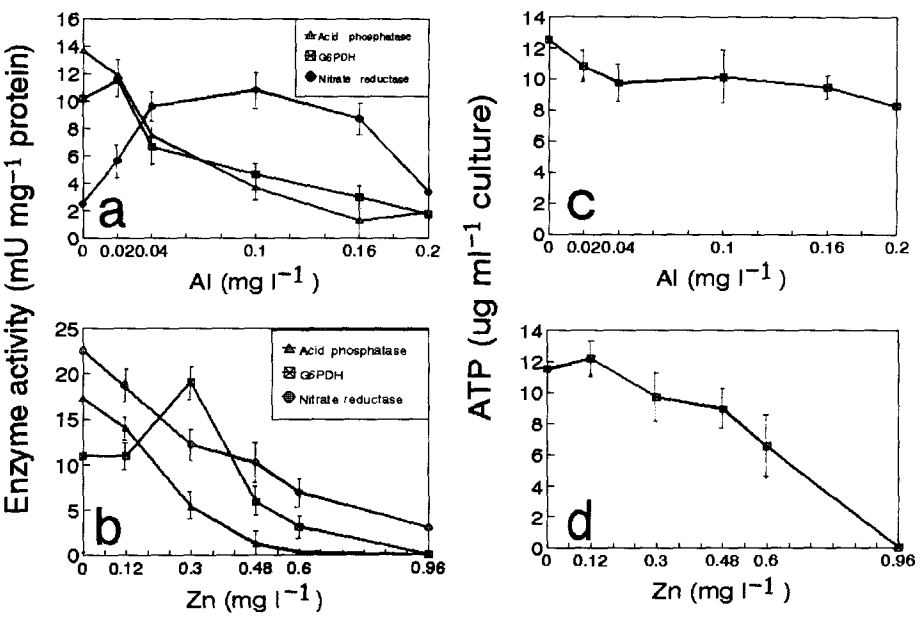

Figure 2. Enzyme activity (mU per mg protein) and ATP level in algal cells of *S. capricornutum* treated with (a,c) Al and (b,d) Zn in the culture medium. (each point is the mean of the results of two independent experiments, n=6)

10^3 in natural aquatic systems, but higher Zn concentrations will decrease the chlorophyll content in plant cells and inhibit chloroplast NADPH production (Assche and Clijsters 1986). Assche et al.(1988) suggested that the higher activity of G6PDH stimulated by Zn could compensate for a possible shortage of reducing power in the cell. The solubility and chemical speciation of aluminum are pH-dependent. Below pH 4.5, Al^{3+} predominates, whereas between pH 4.5-6.3 $Al(OH)_3$ becomes dominant. In other words, the presence of specified amounts of Al may readjust the ratio between H^+ and OH^- and maintain the pH of the solution. This is probably the way in which Al influences the activity of nitrate reductase, because this enzyme is especially pH-sensitive. Acid phosphatase plays a role in decomposing organic phosphate into free phosphate and orthophosphate and its activity contributes significantly to phosphate availability by algal cells in aquatic ecosystems. A significant decrease in phosphate uptake was found in phytoplankton at 50 $\mu g\ l^{-1}$ Al. This physiological process was more affected at pH 5.2 to 6.9 than at pH 4.5 (Stokes et al. 1992). The results of enzyme measurement in his investigation correlated with the relationship between Al and the decrease of available P concentration, especially over the pH 5.0-6.0 range. Apparently, the influence of metals on *S. capricornutum* might relate to the uptake and utilization of phosphate and nitrogen by the algal cell. A direct competition between metal and nutrient ions for uptake has been reported in blue-green algae (Singh and Yadav 1983). It is possible that the balance between the availability of phosphate and nitrogen that is optimum for algal growth is disturbed when some metals are present. This hypothesis will be the basis for our future work.

The experimental data were transformed as presented in Table 1 to

Table 1. Correlation coefficients of the relationship between dry weight of algal cells and enzyme activity, ATP and protein in *S. capricornutum* treated with various concentrations of Al and Zn in the culture medium.

Dry weight of algal cells		
	Al	Zn
Acid phosphatase	0.96 **	0.97 **
G6PDH	0.90 **	0.39
Nitrate reductase	-0.27	0.99 **
ATP	0.98 **	0.85 **
Protein content	-0.88 **	-0.85 **

** Significantly correlated at the 99% confidence level

show the correlation between the response of growth and activity of enzymes and ATP level to metals. It was clear that the relationships between dry weight versus acid phosphatase, G6PDH and ATP for Al and acid phosphatase, nitrate reductase and ATP for Zn were particularly close.

Under controlled environmental conditions, these enzyme activities and ATP level in algal cells might provide a useful biochemical criterion for the evaluation of the physiological toxicity of water contaminated with Al or Zn.

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