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INTERACTION OF ZINC WITH *ASTERIONELLA JAPONICA*

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ABSTRACT

Asterionella japonica cultures grown in the presence of 0, 0.5, 1.0 and 2.0 mg of Zn/L were capable of concentrating zinc and the amount accumulated is directly proportional to the concentration of metal present initially and is dependent upon the pH of the medium. No accumulation occur in the dark, at 4°C, or in dead or in dead cells. 10^{-3} EDTA was found to restore the growth rate to normal.

INTRODUCTION

THE REMARKABLE accumulation of various metals by organisms existing in the marine environment is a well known phenomenon (Goldberg, 1965; Bowen *et al.*, 1971). Among these organisms, plankton (mainly phytoplankton) is the representative species, as Nichols *et al.* (1959) suggested that for any given chemical element they will eventually be found at least one planktonic species capable of spectacularly concentrating it. From the ecological point of view, the metal accumulated in plankton could be further concentrated in the body of various trophic organisms throughout the marine pre-predator system. The aim of this research has been to determine the effects of zinc on the growth and the degree to which zinc can be concentrated by these organisms under different growth conditions.

MATERIAL AND METHODS

All glasswares used in this study was soaked in 50% nitric acid (V/V) and then rinsed thoroughly in distilled deionized water.

Culture Techniques

The culture of *Asterionella japonica* used in this study was isolated from Khor-Al-Zubair (Fig. 1). The experiments were performed using seawater medium described by Sverdrup *et al.* (1942). All cultures, unless otherwise stated were grown in 100 ml conical flask at a constant temperature of 18°C. The flasks were illuminated from above at a light intensity of 4,000 lux supplied by 'day light' flourescent tubes. pH measurement of cultures was made by using a portable pH meter. NHCl and N NaOH were used to adjust the pH of cultures when this was required.

Growth determination

Cell counts: The samples drawn from culture flasks were enumerated by the haemo cytometer, algal samples were counted in five groups of 16 small squares. When the count exceed 10^6 cells ml^{-1}, the samples were approximately diluted and counted.

of I part perchloric acid and 5 parts concentrated nitric acid until only perchloric acid remained, as evidenced by the appearance of dense, white fumes. The clear solutions resulting from the oxidation process were then diluted with distilled deionized water. Zinc analysis were performed with the atomic absorption. The zinc concentration present in the oxidized cell pellet or

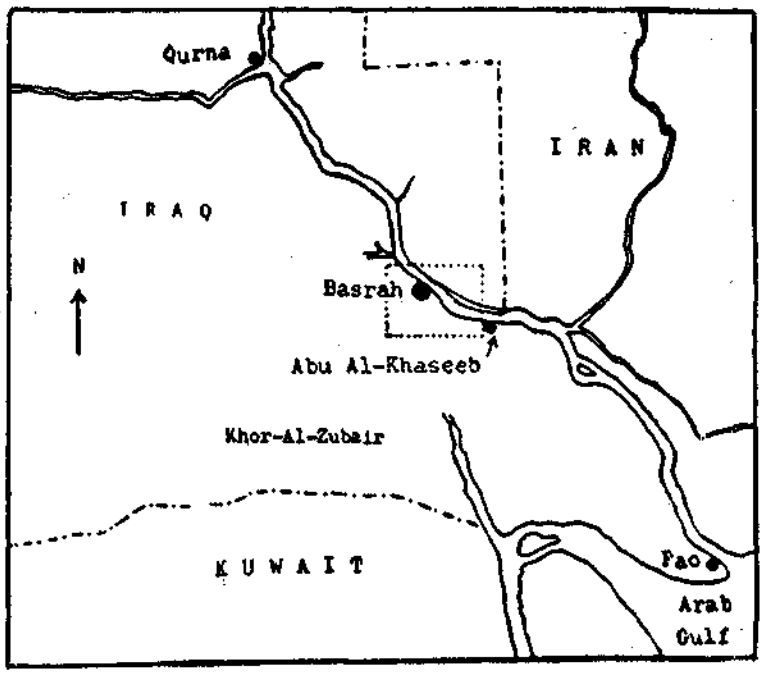

Fig. 1. Sampling area.

Chlorophyll analysis: At intervals during a growth experiment, 5ml aliquots were removed from the cultures and centrifuged for 10 minutes at 30,000 g. Following centrifugation the pellets were extracted with 90% acetone at 4°C. The chlorophyll content was calculated using the Unicam Sp 200 Spectrophotometer.

Zinc analysis

50 ml aliquots of the culture were washed three times with the growth medium by repeated resuspension and centrifugations (30,000 g for 10 minutes at 4°C). The washed pellets were then oxidized by gentle refluxing in the presence

culture supernatant fluids were calculated from a standard curve prepared each time the analysis were performed. It was experimentally shown that the culture medium did not interfere with the zinc determinations. Cellular zinc was expressed as milligram per 10^6 cells.

Results

Effect of zinc on the growth of A. japonica

The growth of *A. japonica* in the control culture and at zinc concentrations of 0.5, 1.0 and 2.0 mg/L is shown in Fig 2. Cell number after 8 days of growth in seawater medium have

been expressed as a percentage of the control culture and plotted against respective zinc concentrations (Fig. 3).

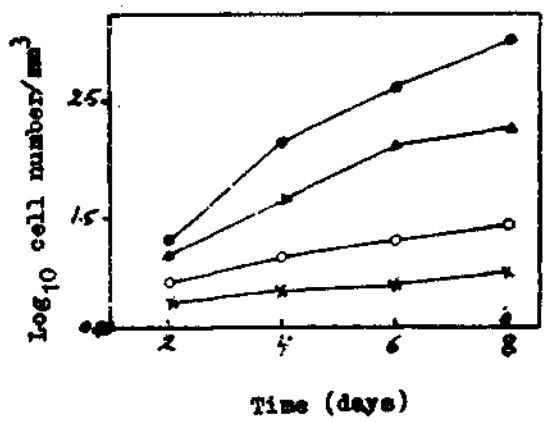

Fig. 2. Effect of zinc on the growth of *A. japonica*; on Zn (●); 0.5 mg of Zn/l (▲); 1.0 mg of Zn/l (o); 2.0 mg of Zn/l (x).

Concentrations of zinc as low as 0.5 mg/L caused a reduction in the cell number. From the toxicity curve (Fig. 2) the concentration of zinc which might be expected to cause a 50% reduction in the cell number (LD_{50}) would be approximately 0.9 mg/L. An identical inhibitory pattern was observed when growth was monitored by chlorophyll determinations (Fig. 4).

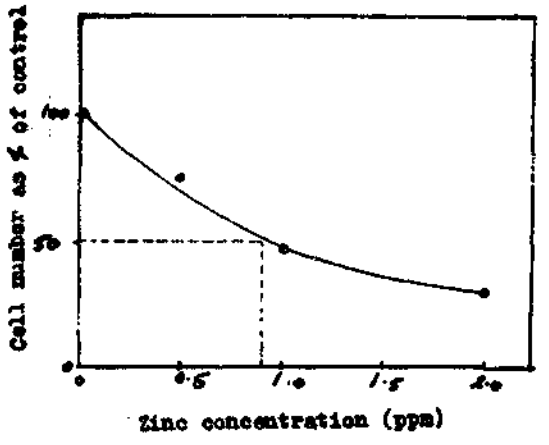

Fig. 3. The growth of *A. japonica* as a percentage of control, after 8 days growth in seawater medium containing zinc.

Zinc accumulation in A. japonica *cultures*

Effect of metabolic state of cells: The results of investigation into the accumulation of zinc by living and killed cells of *A. japonica* after 24 hours shown in Table 1. A suspension of cells was divided into two portions, one portion of cells was maintained at 18°C whilst the other was heated to 45° for 20 minutes. Cells which had been heated in this manner were assumed to be dead, since pigments were lost from the cells.

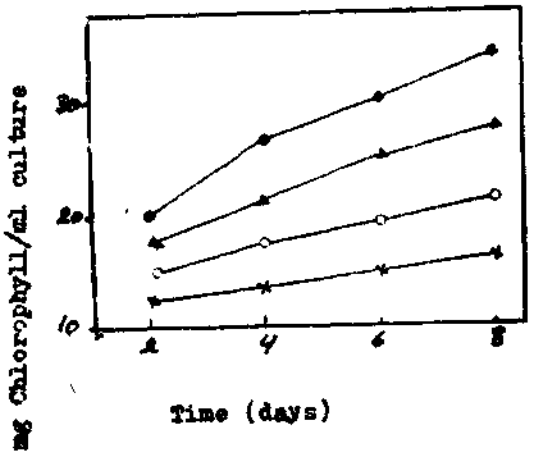

Fig. 4. Effect of zinc on cellular chlorophyll, on Zn(●); 0.5 mg Zn/l (▲); 1.0 mg of Zn/l (o); 2.0 mg of Zn/l (x).

After the heat treated cells has been cooled to 18°C, the accumulation of zinc both by living and killed cells was followed at this temperature in seawater medium. The accumulation of cadmium by living cells showed more uptake than the killed cells reaching 0.175 mg zinc bound per 10^6 cells. The final concentration of cadmium bound by killed cells was 0.04 mg per 10^6 cells. Very little zinc was detected in cells placed in the cold (4°C) or in the dark

during exposure (Table 1); the amount of zinc present in these cells was less than 20% and 1.14% of the control respectively.

TABLE 1. *Zinc accumulation in metabolic active, inactive or dead cells*

Metabolic state of cells	Incubation condition		(mg of Zn/ 10^6 cells)	Control (%)
	Lighting	Temperature (°C)		
Living	Light	18	1.75	100
Living	Dark	18	0.35	20
Living	Light	4	0.02	1.14
Dead	Light	18	0.42	24

Asterionella japonica cultures were incubated in medium containing 1.0 mg Zn/l for a period of 24 hours. Cell density is 10^6 cells /ml.

Effect of pH of the medium: The decreased sensitivity to zinc shown by cultures grown at more alkaline pH conditions (Fig. 5) suggested that the removal of zinc from the culture medium and its simultaneous deposition within the cells might be a pH dependent process. Approximately 1 - 5 times more zinc is accumulated by cells grown at pH 7 than by cells grown at pH 8.

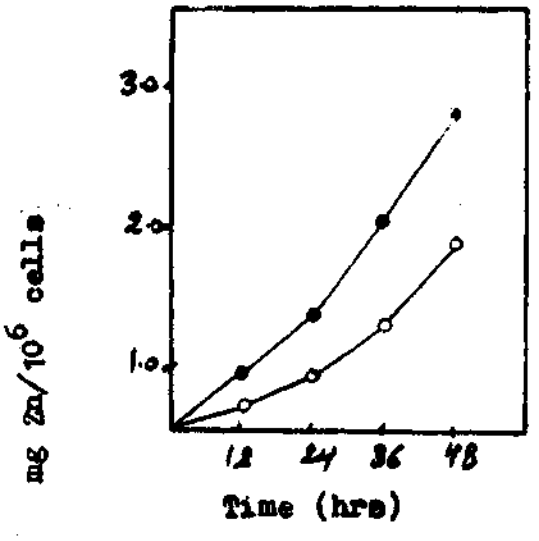

Fig. 5. Zinc accumulation by *A. japonica* cultures grown at pH 7 (●) and ph 8 (o) in the presence of 1.0 mg Zn/l, cell number 10^6 cells/ml.

The cells grown at pH 7 accumulated approximately 2.8 mg Zn/10^6 cells at the end of 48 hour incubation period.

The relationship between zinc cellular content and its concentration

Cells of *A. japonica* were incubated in the presence of zinc at four concentrations for 48 hours. The results of this experiment show that the intracellular zinc content is directly proportional to the concentration of zinc in the medium (Fig. 6). The zinc content of *A. japonica* grown in the presence of 2.0 mg Zn/Lwere twofold the zinc content of the same cells in the presence of 0.5 mg Zn/L in the growth medium.

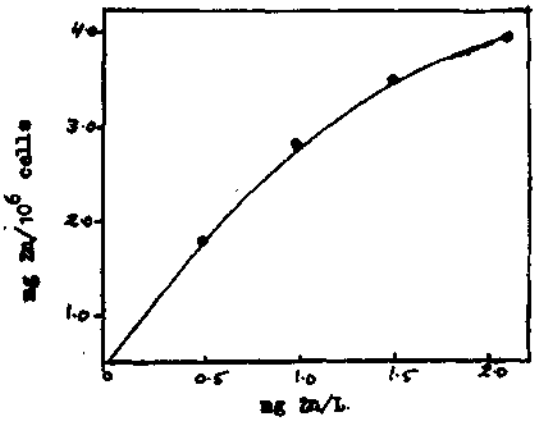

Fig. 6. Zinc accumulation in *A. japonica* cultures grown in 48 hours in the presence of 0, 0.5, 1.0 and 2.0 mg of Zn/l. Cell density 10^6 cells/ml.

The effect of EDTA on intercellular zinc content

The effect of 10^{-3} M EDTA on intercellular zinc content of cells of *A. japonica* is shown in Fig. 6. Loss of zinc may be seen to have occured almost instantaneously falling from 4.15 mg Zn/10^6 cells at time zero, to 1.10 mg Zn/10^6 cells after 5 hours of exposure. Little loss of zinc occured after this time. After 20 hours of exposure to EDTA, 0.6 mg Zn remained per 10^6 cells.

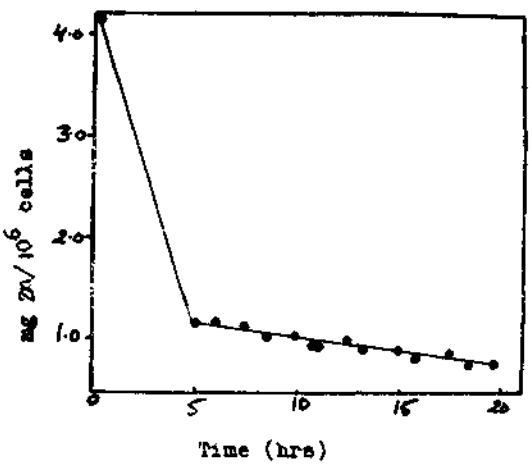

Fig. 7. The loss of zinc from cells of *A. japonica* on exposure to 10^{-3} M EDTA. Cells had been incubated in seawater medium for 48 hours at Zinc concentration of 1.0 mg. Zn/l. Cell density 10^6 cells/ml.

Discussion

This investigation has demonstrated that zinc concentrations of 0.5 mg/L and higher inhibition on the growth rate of logarithmically growing cultures of *Asterionella japonica*. The concentration of zinc which reduced cell numbers to 50% of the control, after 8 days growth, was found to lie in the range of 0.9 ppm (Fig. 4), while the growth of the green algae, *Nanochloris oculata* grown in ASP_2 medium is reduced to 50% of the control by 0.025 mg Zn/ L (Ibrahim, 1980). In contrast, Raclin and Farran (1974) have shown that the growth of *Chlorella vulgaris*, in a defined medium is reduced to 50% by 2.4 ppm Zn^{2+}. Bryan (1969) reported that 515 ppb (μg/l) of zinc in sea water medium inhibited the growth of sections from lamina of *Laminaria digitata*, and that lower concentrations of 100 ppb zinc inhibited the growth of very rapidly growing sections of sea weed. It is therefore apparent that estimates of the toxicity of a heavy metal may be much affected by the experimental conditions.

A. japonica grown in the light in seawater medium, can be removed zinc from the culture medium in a rapid and complete manner. The light depend nature of zinc accumulation provides additional support for the existence of an active process requiring ATP derived from photophosphorylation. Moreover, the fact that zinc accumulation is greatly reduced at 4°C may indicate that an enzymatic process is involved.

Zinc accumulation is influenced by the pH of the medium. Cultures exposed to 1.0 mg of Zn/L at pH 7 accumulated approximately 1 - 5 times as much as zinc as cells which have been grown at pH 8. In nature, zinc and other heavy metals are known to be predominantly associated with particle. The i e of these particles apparently cannot be transformed and accumulated by *A. japonica*.

Approximately 71.5% of intercellular zinc removed from cells of *A. japonica* during the first 5 hours of exposure to 10^{-3} EDTA. This pattern of a very rapid loss of bound metal on exposure to a chelating agent would appear to be common to a number of organisms and metals. Thus, Davies has shown that EDTA will remove bound iron from *Dunaliella tetriolecta* (1970) and bound zinc from *Phaeodactylum tricornutum* (1973). Cossa (1976) found that 2×10^{-3} M cysteine removed 97% of the cadmium bound by *Phaeodactylum tricornutum* after 2 hours. Schulz-Blades and Levlin (1976) were able to remove 40 - 80% of bound lead from the same organism with 2×10^{-2} M EDTA. 10^{-3} were found to remove 50% of bound zinc from cells of *Nanochloris oculata* during the first minute of the exposure (Ibrahim, 1980)

The effect of EDTA may be more complex than removal of metal bound outside of the plasmalemma, possibly due to the alteration

in the equilibrium between the metal bound by the cells and the free ionic metal in the loathing medium.

In conclusion, the results in this work show that the accumulation of zinc by *A. japonica* occurs mainly by active processes.

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