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Effect of metal ions on accumulation of arsenic in marine green algae, *Dunaliella* sp.

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To investigate the effect of metal ions such as cobalt(II), copper(II), molybdenum(VI) and manganese(II) on the accumulation of arsenic in *Dunaliella* sp., the amount of arsenic accumulated in these algae was determined under coexistence of arsenic and the other metals by the hydride-generation atomic absorption spectrometry method. Survival of *Dunaliella* sp. was inhibited by the addition of copper and cobalt in a solution containing arsenic. Copper and cobalt in the medium also inhibited the accumulation of arsenic in *Dunaliella* sp., and the amount of arsenic in the algae decreased with increase in the concentration of copper and cobalt. On the other hand, the amount of arsenic accumulated in *Dunaliella* sp. was found to be unaffected by manganese and molybdenum in the solution.

Keywords: Accumulation, arsenic, *Dunaliella* sp., metal ions,

INTRODUCTION

In general, arsenic is an essential trace bioelement, but it is toxic for most organisms at high concentrations. Recently it has been reported that micro-organisms accumulate arsenic to a high extent,[1,2] and the marine green algae *Dunaliella* sp. also accumulates arsenic.[3] Accumulation was found to depend greatly on environmental factors (pH, temperature, light intensity, etc.).[4] It was also found that micro-organisms can take up other dissolved metals from their surroundings as well as arsenic.[5–8] However, there is little information on accumulation of arsenic in *Dunaliella* sp. in coexistence with arsenic and other metals.

In this paper, we describe the effect of various metal ions on the accumulation of arsenic in *Dunaliella* sp.

MATERIALS AND METHODS

Culture of *Dunaliella* sp.

Dunaliella sp. was obtained from the Hiroshima Fisheries Experimental Station, Japan. The medium was natural seawater, which was collected from inshore and filtered (0.22 μm) to remove particulate materials. Nitrate (KNO_3; 72 mg dm^{-3}) and phosphate (KH_2PO_4; 4.5 mg dm^{-3}) were added to the medium to promote growth.

Dunaliella sp. was incubated in the aerated medium at 23°C under the illumination of cool-white fluorescence lamps at a light intensity of approximately 6000 lux. The growth of *Dunaliella* sp. was monitored by a Turner fluorimeter. The cells were collected at the stationary growth phase by continuous centrifugation at 3000 rpm.

Uptake of arsenic by *Dunaliella* sp. cells

The cells harvested by centrifugation were suspended in an artificial seawater (1 dm^{-3} distilled water; 18 g NaCl; 5 mg $MgSO_4 \cdot 7H_2O$; 0.6 g KCl; 0.1 g $CaCl_2$; 1 g Tris). Arsenic ($Na_2HAsO_4 \cdot 7H_2O$) with a concentration range of 0–100 mg dm^{-3} was added to the medium and metals were added with concentration ranges of 0–10 mg dm^{-3} to the arsenic-containing medium. Metal compounds used were $CoCl_2 \cdot 6H_2O$, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, $MnCl_2 \cdot 2H_2O$ and $CuCl_2 \cdot 2H_2O$.

The cultures were incubated under the illumination of fluorescence lamps with an intensity of an approximately 5000 lux at 23°C and pH 8. The amounts of arsenic and metals accumulated in *Dunaliella* sp. were measured as a function of time using a batch method. After an appropriate time, the cells were collected by centrifugation at

3000 rpm for 5 min, washed three times with an arsenic-free artificial medium, and then lyophilized.

Determination of arsenic and other metals in *Dunaliella* sp.

The freeze-dried cells were digested with a solution of concentrated nitric acid, concentrated sulphuric acid and perchloric acid (60%). The amount of arsenic in *Dunaliella* sp. was determined by the hydride-generation atomic absorption spectrometry method.[9] The amount of a metal in *Dunaliella* sp. was determined by an atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

Arsenic uptake by *Dunaliella* sp.

The time variation in the amount of arsenic and the relative fluorescence (*in vivo* chlorophyll) in *Dunaliella* sp. in solutions containing different arsenic concentrations are shown in Fig. 1. The amount of arsenic taken up by *Dunaliella* sp. increased with time and became a maximum within 15–25 h except for 1 mg As dm^{-3}. Relative fluorescence in *Dunaliella* sp. slightly decreased with time.

We examined the effect on uptake of arsenic using living cells and dead cells treated with hot seawater for 10 min. The effect of dead and living cells on arsenic uptake by *Dunaliella* sp. is summarized in Table 1. In the living cells, the amount of arsenic accumulated in cells rapidly increases with time. On the other hand, the amount of arsenic taken up by the cells does not increase with time in the dead cells. These results suggest

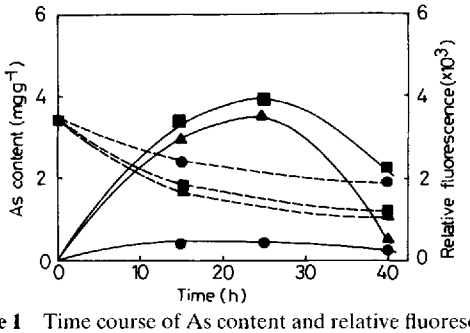

Figure 1 Time course of As content and relative fluorescence (– – –) in *Dunaliella* sp.: ●, 1 mg As dm^{-3}; ▲, 10 mg As dm^{-3}; ■, 100 mg As dm^{-3}.

Table 1 Effect of living cells and dead cells on arsenic uptake by *Dunaliella* sp.

	As content in *Dunaliella* sp. ($\mu g\ g^{-1}$)	
Uptake time (h)	Living cells	Dead cells
1	334	36
2	469	37
4	571	34

Arsenic concentration 10 mg dm^{-3}; pH 8.0; Light intensity 5000 lux

that the uptake of arsenic by *Dunaliella* sp. depends upon biological activity. Horikoshi *et al.*[10] studied the effect of living cells and dead cells on the uptake of uranium by *Chlorella regularis* and obtained higher accumulation of uranium by dead cells than by living cells; therefore in this case the uptake of uranium by *C. regularis* depended upon physical adsorption on the cell surface. There is a significant difference in the mechanism of accumulation of element by algae in these two cases.

Tolerance in *Dunaliella* sp. cells with various concentrations of arsenic and metal ions

Cobalt is a component of Vitamin B_{12}. Copper is also a component of several metalloenzymes including ascorbic acid oxidase, phenolase and cytochrome oxidase. Molybdenum and manganese are essential elements for algal growth. At the level of concentration in the sea, these metals are apparently not toxic to algae. However, they inhibit the growth of marine algae at high concentrations. The effect of metal ions on the survival of *Dunaliella* sp. in solutions containing arsenic and metal was examined here. Relative fluorescence of *Dunaliella* sp. (a sign of vitality) in solutions containing various concentration of copper from 0 to 10 mg dm^{-3} and various arsenic concentrations are shown in Fig. 2. Survival of *Dunaliella* sp. was inhibited by arsenic; furthermore, inhibition of survival was accelerated with added copper. In particular the relative fluorescence of *Dunaliella* sp. was markedly reduced in solution with 1–100 mg As dm^{-3} and 10 mg Cu dm^{-3}. An apparent affect of molybdenum and manganese on the survival of *Dunaliella* sp. was not found for the concentrations examined (Fig. 3). Copper inhibited the growth of

Dunaliella sp. cells at high concentration. It is shown that relative fluorescence decreased consequently (Fig. 2).

Effect of various metal ions on the uptake of arsenic by *Dunaliella* sp.

As described above, it was found that survival of *Dunaliella* sp. cells is markedly affected by the presence of metal ions. To clarify the effect of various metal ions on the uptake of arsenic by *Dunaliella* sp., the amount of arsenic in *Dunaliella* sp. was determined under coexistence of arsenic and metals.

The amount of arsenic taken up by *Dunaliella* sp. in solutions containing arsenic was greatly changed by the addition of metal ions to the solution. The accumulation of arsenic in *Dunaliella* sp. was influenced by addition of cobalt. Cobalt at 1 mg dm^{-3} has no effect on arsenic uptake by *Dunaliella* sp., but cobalt at 10 mg dm^{-3} inhibited arsenic accumulation in a concentration-dependent manner. The maximum amount of arsenic in cells, showing a maximum value at 25 h, for all cases in the addition of cobalt and arsenic are shown in Fig. 4. Results of copper and manganese addition are shown in Figs 5 and 6, respectively. In the case of copper addition, the amount of arsenic increased with increased arsenic concentration at 1 mg Cu dm^{-3} solution; however, at 10 mg Cu dm^{-3}, *Dunaliella* sp. only slightly accumulated arsenic. It is found that the amount of arsenic taken up by *Dunaliella* sp. rapidly decreased with increase in the concentrations of cobalt and copper in arsenic solution. On the other hand, with addition of manganese, the amount of arsenic in cells showed increase with increase in the concentration of arsenic, without

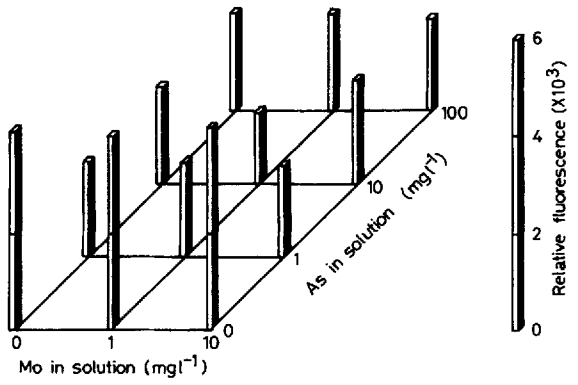

Figure 3 Effect of molybdenum and arsenic concentration in solution on relative fluorescence in *Dunaliella* sp.

showing any sudden variation with increase in the concentration of manganese. Relative fluorescence of *Dunaliella* sp. decreased in solution containing 10 mg dm^{-3} of copper and cobalt, respectively. When concentrations of copper and cobalt in solution were at toxic levels, as a result the accumulation of arsenic decreased to a minimum uptake. In relation to the result that dead cells do not accumulate arsenic (Table 1), it is supposed that the large majority of *Dunaliella* sp. are killed in solutions containing high concentrations of copper and cobalt.

The amount of copper and manganese in the cells after 25 h are shown in Tables 2 and 3, respectively. The high level of copper was noted in 10 mg Cu dm^{-3} solution in spite of the large majority of *Dunaliella* sp. cells being dead. The high concentration of manganese in the cells was also recognized in solutions containing 10 mg Mn dm^{-3}. Furthermore, accumulation of

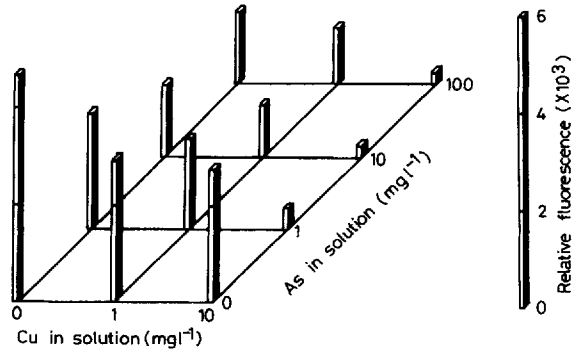

Figure 2 Effect of copper and arsenic concentration in solution on relative fluorescence in *Dunaliella* sp.

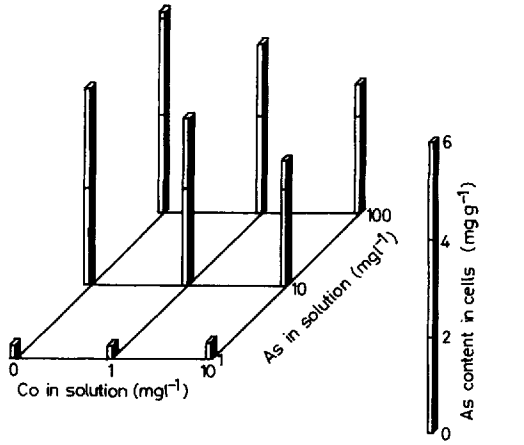

Figure 4 The amount of arsenic in *Dunaliella* sp. under coexistence of arsenic and cobalt.

Table 2 The amount of copper in *Dunaliella* sp. under coexistence of arsenic and copper in coexistence.

As in solution (mg dm^{-3})	Cu content in *Dunaliella* sp. (mg g^{-1})	
	Cu in solution 1 mg dm^{-3}	10 mg dm^{-3}
0	1.16	1.40
1	1.10	3.64
10	1.06	3.74
100	1.14	2.89

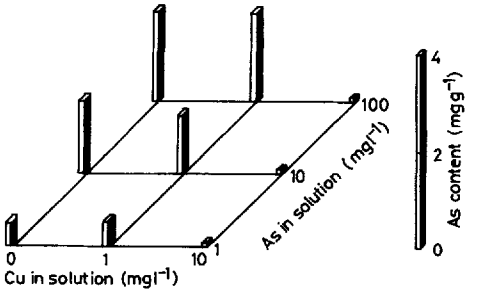

Figure 5 The amount of arsenic in *Dunaliella* sp. under coexistence of arsenic and copper.

metal ions by *Dunaliella* sp. was not influenced by arsenic addition to 10 mg As dm^{-3}. *Chlorella regularis* accumulates a large amount of copper[11] and manganese[12] and it was suggested that the uptake of these metal ions by *Chlorella regularis* mostly depends on physicochemical adsorption at the cell surface. Noro[13] has shown that a manganese concentration of 0.1–0.5 mg dm^{-3} is optimal for growth of *Dunaliella tertiolecta,* with lesser

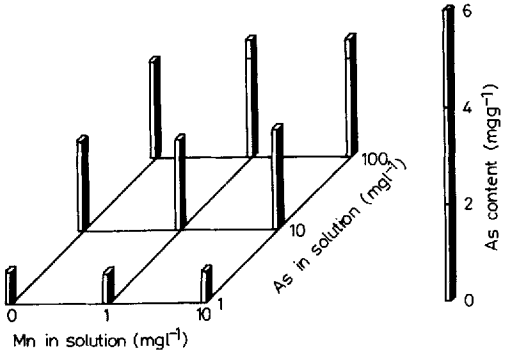

Figure 6 The amount of arsenic in *Dunaliella* sp. under coexistence of arsenic and manganese.

Table 3 The amount of manganese in *Dunaliella* sp. under arsenic and manganese.

As in solution (mg dm^{-3})	Mn content in *Dunaliella* sp. (mg g^{-1})	
	Mn in solution 1 mg dm^{-3}	10 mg dm^{-3}
0	1.06	5.35
1	1.24	4.81
10	1.19	3.82
100	1.27	3.78

concentrations inhibiting growth, and levels greater than 10 mg dm^{-3} being toxic. *Dunaliella* sp. cells accumulated not only arsenic, but also metal ions; however arsenic and metal ions differ remarkably in their uptake mechanisms. It was found that the mechanism of accumulation of metallic elements in *Dunaliella* sp. is significantly different from those of the above species.

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