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Effect of cadmium on the accumulation of arsenic in a marine green alga, *Dunaliella* sp.

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To investigate the effect of cadmium on the accumulation of arsenic by *Dunaliella* sp., the arsenic accumulated in the alga was determined as a function of time for coexistence of the algae with arsenic and cadmium, with batch methodology. Growth of *Dunaliella* sp. was affected by addition of arsenic ($Na_2HAsO_4 \cdot 7H_2O$) and cadmium ($CdCl \cdot 2.5H_2O$). Growth inhibition of *Dunaliella* sp. was accelerated by coexistence of arsenic and cadmium. The content of arsenic in *Dunaliella* sp. became a maximum at 15 h after exposure. The arsenic content in the cells was influenced by addition of cadmium to the solution; the arsenic content in the alga derived from growth in a 10 mg As dm^{-3} solution decreased from 2.7 mg g^{-1} in the absence of cadmium to 0.35 mg g^{-1} for the addition of 100 mg Cd dm^{-3}. *Dunaliella* sp. accumulated cadmium in large quantities but, in conditions of coexistence with arsenic and cadmium, the cadmium content in cells decreased with an increase in the concentration of arsenic in the growth medium. Cadmium accumulation by *Dunaliella* sp. was observed in dead cells although arsenic accumulation was not observed. About 85% of arsenic in the cells was in the water-soluble fraction. On the other hand, about 42% of cadmium in the cells was in the water-soluble fraction, and about 55% was in a fraction soluble in cold trichloroacetic acid.

Keywords: Marine green alga, *Dunaliella* sp., arsenic, cadmium, accumulation, growth inhibition, water-soluble fraction

INTRODUCTION

Arsenic concentration in seawater is generally in the range 1–3 μg dm^{-3}. Marine organisms accumulate arsenic; in particular, lower order members of the food chain often contain high arsenic concentrations although inorganic arsenic is toxic for most organisms.[1–3] Recently it has been reported that micro-algae accumulate arsenic to a high concentration,[4,5] and the marine green alga, *Dunaliella* sp., also accumulates arsenic.[6] Accumulation of arsenic by *Dunaliella* sp. was found to depend greatly on environmental factors such as temperature and light intensity.[7]

Cadmium is also toxic for most organisms at high concentrations and it is often judged to be of considerable environmental importance. There have been several studies on bioaccumulation of cadmium by micro-organisms.[8–10] The effect of arsenic and cadmium on algae has been studied for each of these toxicants acting separately, but not in combination. There is also little information on accumulation of arsenic by *Dunaliella* sp. under conditions of coexistence with arsenic and cadmium.

In this paper, we report the effect of cadmium on arsenic uptake and growth inhibition in *Dunaliella* sp. in coexistence with arsenic and cadmium. Furthermore, the intracellular distribution of arsenic accumulated in *Dunaliella* sp. cells is also described.

EXPERIMENTAL

Culture of *Dunaliella* sp.

Dunaliella sp. was obtained from Hiroshima Fisheries Experimental Station, Japan. The medium was natural sea-water, 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 illumination with fluorescent lamps at a light intensity of approximately 6000 lux. The growth of *Dunaliella* sp. was monitored by determining fluorescence intensity (*in vivo* chlorophyll) with a Turner fluorimeter. The cells were collected at the stationary growth phase by continuous centrifugation at 3000 rpm.

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Uptake of arsenic by *Dunaliella* sp. cells

Cells harvested by centrifugation were suspended in artificial sea-water (1 dm^3 distilled water; 18 g NaCl; 5 g $MgSO_4 \cdot 7H_2O$; 0.6 g KCl; 0.1 g $CaCl_2$; 1 g Tris). Arsenic ($Na_2HAsO_4 \cdot 7H_2O$) in a concentration range of 0–100 mg dm^{-3} was added to the medium and cadmium ($CdCl_2 \cdot 2.5H_2O$) was added to a concentration range of 0–100 mg dm^{-3} to the arsenic-containing medium. The solution containing 100 mg As dm^{-3} and 100 mg Cd dm^{-3} was excluded from the uptake experiment as it formed a precipitate.

The cultures were incubated under illumination by fluorescent lamps with an intensity of an approximately 5000 lux at 23 °C and pH 8. The fluorescence intensity of the algal suspension and the content of arsenic and cadmium accumulated in *Dunaliella* sp. were determined 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 lyophillized.

Intracellular distribution of arsenic and cadmium

After thawing, the cells were homogenized in a distilled water and centrifuged at 26 000*g* for 20 min to obtain a particulate fraction and a water-soluble fraction by a differential centrifugation method.[11] The particulate fraction was fractionated to a cold-trichloroacetic-acid-soluble fraction, an alcohol-soluble fraction, a hot-trichloroacetic-acid-soluble fraction and a residual-precipitate fraction using the Schmidt–Thanhauser–Schneider (STS) method.[12]

Determination of arsenic and cadmium in *Dunaliella* sp. cells

The freeze-dried cells containing arsenic and cadmium were digested with a mixed solution containing 3 cm^3 of concentrated nitric acid, 1 cm^3 of concentrated sulphuric acid and 1 cm^3 of 60% perchloric acid. The amount of arsenic was determined by a hydride-generation atomic absorption spectrometry method and that of cadmium was determined using a flame atomic absorption spectrophotometer.[13] Wavelength and lamp current were 193.7 nm and 10 mA for arsenic and 228.8 nm and 5 mA for cadmium, respectively.

RESULTS AND DISCUSSION

Effect of arsenic and cadmium on growth of *Dunaliella* sp. cells

Measurement of fluorescence intensity is a reliable index of toxicity, quantitatively measuring only viable cells.[14] the fluorescence intensity in *Dunaliella* sp. cells in solutions containing various concentrations of arsenic and cadmium after 15 h and 40 h are shown in Table 1. All data are expressed as percentages of the blank (the sample without arsenic and cadmium). On addition of cadmium, the fluorescence intensity in *Dunaliella* sp. decreased slightly at 1 mg Cd dm^{-3} and at 10 mg Cd dm^{-3}. However, at 100 mg Cd dm^{-3} the fluorescence intensity in the alga rapidly decreased with time. It was found that growth of *Dunaliella* sp. was markedly affected at a concentrtion of 100 mg Cd dm^{-3}. The growth of *Chlorella regularis* was hardly affected by cadmium ions in the concentration range 0–20 mg dm^{-3}.[15] On addition of arsenic, on the other hand, the fluorescence intensity in *Dunaliella* sp. decreased slightly at 1–100 mg As dm^{-3} at 15 h. However, the fluorescence intensity at 40 h decreased with increasing arsenic concentration. Furthermore, in coexistence with arsenic and cadmium, inhibition of

Table 1 Effect of arsenic and cadmium on fluorescence intensity in *Dunaliella* sp.

Concentration (mg dm^{-3})		Fluorescence intensity (%)[a]	
As	Cd	15 h	40 h
0	0	100	100
0	1	95	84
0	10	87	64
0	100	34	3
1	0	70	41
1	1	66	38
1	10	66	38
1	100	45	3
10	0	66	38
10	1	66	38
10	10	61	3
10	100	39	3
100	0	66	25
100	1	57	20
100	10	52	8

[a] Percentage of blank (without arsenic and cadmium).

growth in *Dunaliella* sp. was accelerated with addition of a high concentration of cadmium to the solution containing arsenic. Combination of arsenic and cadmium in solution demonstrated their synergistic toxicity to growth of *Dunaliella* sp. It was found that high concentrations of cadmium led to growth inhibition of *Dunaliella* sp., and that the alga is more sensitive to cadmium than to arsenic. Cadmium in the presence of copper or chromium affected growth of natural phytoplankton more than cadmium alone.[16]

Effect of cadmium on the accumulation of arsenic by *Dunaliella* sp.

As described above, it was found that growth of *Dunaliella* sp. is affected by the presence of arsenic and cadmium. To clarify the effect of cadmium on the accumulation of arsenic by *Dunaliella* sp., the content of arsenic in the algae was determined as a function of time under conditions of coexistence with arsenic and cadmium. Time variation of the content of arsenic in *Dunaliella* sp. after the addition of various arsenic concentrations (no cadmium) is shown in Fig. 1. The content of arsenic taken up by *Dunaliella* sp. increased rapidly with time and became a maximum within 15 h in solutions with 10 mg As dm^{-3} or 100 mg As dm^{-3}. Subsequently, the content of arsenic in *Dunaliella* sp. decreased with time. However, at 1 mg As dm^{-3}, the arsenic content is small. Boottino *et al.*[17] have also reported that rapid arsenic excretion by the marine phytoplankton alga, *Hymenomonas carterae*, was observed after the arsenic concentration in the cells had reached a maximum. The maximum contents of arsenic in cells, measured at 15 h, for all cases for the addition of cadmium *and* arsenic

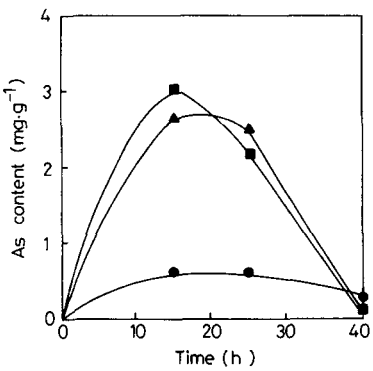

Figure 1 Time course of arsenic content in *Dunaliella* sp. on the addition of various concentrations of arsenic: ●, 1 mg As dm^{-3}; ▲, 10 mg As dm^{-3}; ■, 100 mg As dm^{-3}.

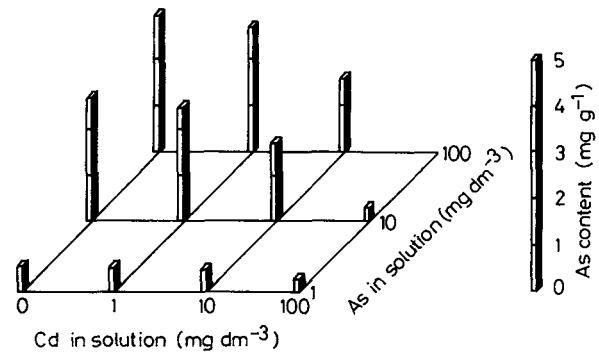

Figure 2 Content of arsenic in *Dunaliella* sp. after 15 h under conditions of coexistence of arsenic and cadmium.

are shown in Fig. 2. At 1 mg As dm^{-3} and various cadmium concentrations, the content of arsenic in cells was small and no apparent effect of cadmium on the content of arsenic in *Dunaliella* sp. was found. At 10–100 mg As dm^{-3} and various cadmium concentrations, the content of arsenic in *Dunaliella* sp. decreased with increase in the concentration of cadmium. In particular, the content of arsenic decreased by approximately 65% at 10 mg Cd dm^{-3} and by approximately 13% at 100 mg Cd dm^{-3} compared with cadmium being absent. It was found that the accumulation of arsenic by *Dunaliella* sp. in solution is greatly changed by the addition of cadmium to the solution. It has been reported that the accumulation of arsenic by *Dunaliella* sp. is affected by copper and cobalt, but unaffected by manganese and molybdenum.[18]

Effect of arsenic on the accumulation of cadmium by *Dunaliella* sp.

The time variations for the cadmium content in *Dunaliella* sp. in the solutions of various cadmium concentrations are shown in Fig. 3. Cadmium uptake by *Dunaliella* sp. increased with increase in cadmium concentration in the solution, and reached a maximum concentration within 15–40 h. After the cadmium content became a maximum in solution, the cadmium content of the algae was constant. It is found that *Dunaliella* sp. accumulated large quantities at the initial stage, not only of arsenic, but also of cadmium; however, accumulation of arsenic and cadmium by the algae differ remarkably in their bonding mechanisms. Nile-water algae for example, showed a variable ability to accumulate cadmium.[16] In conditions of coexistence of arsenic and cadmium, the content of cadmium in

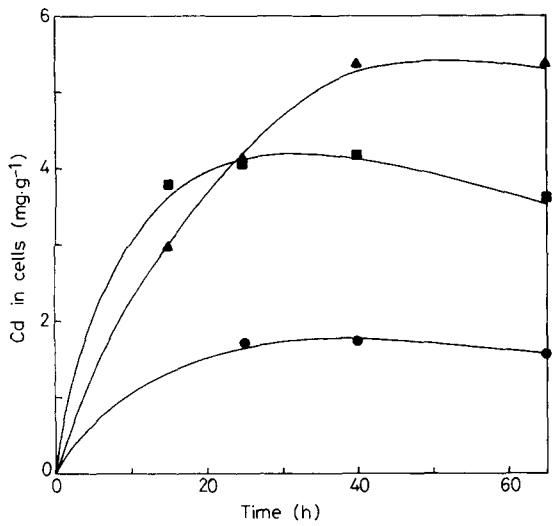

Figure 3 Time course of cadmium content in *Dunaliella* sp. on the addition of various cadmium concentrations: ●, 1 mg Cd dm^{-3}; ▲, 10 mg Cd dm^{-3}; ■, 100 mg Cd dm^{-3}.

Dunaliella sp. after 40 h at various concentrations of arsenic and cadmium are shown in Fig. 4 The cadmium content in *Dunaliella* sp. decreased stepwise with increasing concentration of arsenic in solution. For example, the cadmium content at 10 mg Cd dm^{-3} decreased from 5.5 mg g^{-1} for no arsenic present to 1.0 mg g^{-1} at 100 mg As dm^{-3}. It was found that the uptake of cadmium by *Cunaliella* sp. is greatly affected by the addition of arsenic to the solution.

Effect of treatment of cells on accumulation of arsenic and cadmium

We also examined the accumulation of arsenic and cadmium with various cell treatments. The contents of arsenic and cadmium in cells after 15 h are shown in Table 2. Arsenic content in

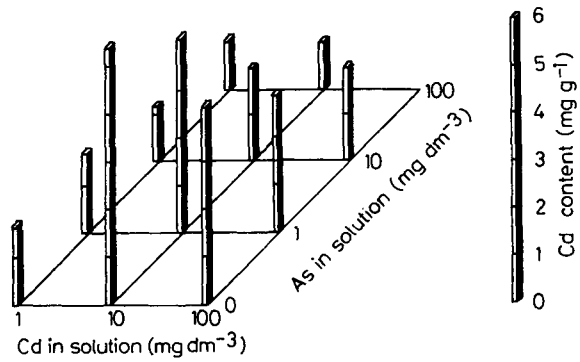

Figure 4 Content of cadmium in *Dunaliella* sp. after 15 h under conditions of coexistence of arsenic and cadmium.

Table 2 Content of arsenic and cadmium in *Dunaliella* sp. after 15 h with various treatments[a]

Treatment	As (μg g^{-1})	Cd (μg g^{-1})
Control	2100	3550
Dark	440	3250
Heated-treated	n.d.	1450
Acid-treated	n.d.	200

[a] Concentrations of arsenic and cadmium in solution were 10 mg dm^{-3} of each. Cells were treated with hot sea-water for 15 min. Cells were treated with $HClO_4$ (5×10^{-2} mol dm^{-3}). n.d., not detectable.

Dunaliella sp. decreased abruptly under conditions of darkness and it was not detected in heat-treated cells or acid-treated cells. On the other hand, there was no effect on the accumulation of cadmium in *Dunaliella* sp. in darkness compared with that of the control. The cadmium content in dead cells was reduced to 50% and 6% of the control for cells in the heat-treated and acid-treated samples, respectively. These results suggest that the accumulation of arsenic by *Dunaliella* sp. depends upon biological activity, whilst that of cadmium depends upon biological activity *and* physical adsorption. Hart and Scaife[19] studied the effect of light on the uptake of cadmium by *Chlorella pyrenoidosa*, and found that cells in the dark could not accumulate cadmium. Heat treatment of the cells is known to increase the negative charge on the cells and to increase membrane permeability for metals to bind to the intracellular organelles. For example, heat-killed *Chlorella* cells took up cadmium to a greater degree than living ones.[15] It was found here, however, that the accumulation of cadmium in *Dunaliella* sp. must be significantly different from those of the above species.

Intracellular distribution of arsenic and cadmium

Distribution of arsenic and cadmium in cellular components of *Dunaliella* sp. after 15 h after the addition of arsenic and cadmium are shown in Table 3. The fractions extracted by the STS procedure refer to the cold-trichloroacetic-acid-soluble fraction as low-molecular-weight metabolites, the ethanol-soluble fraction as lipid, the hot-trichloroacetic-acid-soluble fraction as nucleic acid and the residual-precipitate fraction as protein. The results showed that about 85% of arsenic in the cells was in the water-soluble fraction,

Table 3 Intracellular distribution of arsenic and cadmium in *Dunaliella* sp. after 15 h[a]

Fraction	As		Cd	
	(μg)	(%)	(μg)	(%)
Water-soluble	4.1	85.4	5.6	42.4
Cold-TCA-soluble[b]	0.3	6.3	7.2	54.5
Ethanol-soluble	0.1	2.1	0.2	1.5
Hot-TCA-soluble	0.1	2.1	0.1	0.8
Residual-precipitate	0.2	4.2	0.1	0.8

[a] Concentration of arsenic in solution was 10 mg dm^{-3} in each case.
[b] TCA, trichloroacetic acid.

while about 6% was in the low molecular-weight metabolites fraction. The lipid fraction, protein fraction and nucleic acid fraction contained a negligible amount of arsenic. On the other hand, the large majority of cadmium in the cells was in the water-soluble fraction and low-molecular-weight metabolites fraction, presenting about 42% and 55%, respectively. The lipid fraction, protein fraction and nucleic acid fraction contained a negligible amount of cadmium, similar to the arsenic. In the red alga, *Porphyhra yezoensis*, about 80% of the total arsenic was found in the water-soluble arsenic fraction, which was extracted with 50% aqueous methanol.[20] However, Shariatpanahi *et al.*[21] reported that a trichloroacetic-acid fractionation procedure indicated that most of the arsenic accumulated by bacterial cells was confined to the residual-protein fraction with smaller amounts found in the lipid–protein and nucleic-acid pools. The majority of zinc taken up by *Ankistrodesmus falcatus* after 24 h of exposure was found in the polysaccharide and nucleic-acid fractions.[22]

CONCLUSIONS

The marine green alga, *Dunaliella* sp., accumulated arsenic in large quantities in the initial stages after arsenic addition, although growth of the alga was affected by the presence of arsenic. In the accumulation of cadmium by *Dunaliella* sp., the content of cadmium in the cells also increased with time, and was greatly affected by the addition of arsenic to the solution. The accumulation of arsenic by *Dunaliella* sp. depended upon biological activity, but accumulation of cadmium depended upon biological activity and physical adsorption. The majority of arsenic taken up by *Dunaliella* sp. was found in the water-soluble fraction and cadmium was found in the water-soluble and acid-soluble fractions.

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