Effects of Various Elements on Arsenic Accumulation of the Alga *Dunaliella salina*

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The effect of 23 various elements (nitrogen, manganese, magnesium, molybdenum, zinc, selenium, gallium, nickel, cobalt, lithium, strontium, vanadium, tin, antimony, bismuth, cadmium, chromium, lead, iron, silver, copper, potassium and calcium) in water on growth and arsenic accumulation in *Dunaliella saline* was investigated. The order of growth inhibition of *D. salina* by these elements was Ag > Cd > Co > Ni > Cu > Zn > Fe > Sb > Ga > Cr > Bi > Sr > Mn > Sn > Se > Pb > V > Ca, Mg, Mo, K, Li.

Arsenic accumulation in *D. salina* was unaffected by an increase in calcium and chromium. Also, the arsenic content in *D. salina* decreased at a potassium concentration of 100 mg dm^{-3}, and was also reduced by the addition of cadmium and nitrogen; however, it was increased by the addition of lithium at 100 mg dm^{-3}, tin, gallium, bismuth, strontium, vanadium, iron and manganese at 10 mg md^{-3}, lead, antimony, zinc, copper cobalt and nickel at 1 mg dm^{-3}, selenium at 0.1 mg dm^{-3}, and silver at 0.005 mg dm^{-3}, respectively. These results imply that arsenic accumulation by *D. salina* depends upon biological activity and physical adsorption.

Keywords: Arsenic, *Dunaliella salina*, microalgae, elements, accumulation

INTRODUCTION

The use of marine organisms for the removal of toxic and valuable elements from wastewater has continued to attract considerable attention in recent years, particularly because they are available at little or no cost and are capable of accumulating the elements by incorporation, adsorption, chelation and ion exchange.[1] The microalgae (*Chlorella*,[2] *Dunaliella*,[3] *Phoridinium*[4]) are capable of accumulating arsenic to concentrations several orders of magnitude higher than in the surrounding medium. In fact, microalgae can be employed for the purification of arsenic-contaminated water. In recent work we have shown that *D. salina* accumulated arsenic at rates as high as those of other algae.[5] This high accumulation capacity can even be used for the enrichment or recycling of arsenic. Because this observation could have a significant impact on arsenic recovery, we have initiated a thorough investigation of arsenic accumulation by *D. salina*. No information has been reported on the effects of various elements on arsenic accumulation by *D. salina*. There is also little information on the accumulation of arsenic by *Dunaliella* sp. (*D. sp*) over eight hours under conditions of co-existence with arsenic, and phosphorus, chlorine, molybdenum, managanese, copper and cadmium.[6] This report describes the effects of various elements (nitrogen, manganese, magnesium, molybdenum, zinc, selenium, gallium, nickel, cobalt, lithium, strontium, vanadium, tin, antimony, bismuth, cadmium, chromium, lead, iron, silver, copper, potassium and calcium) on arsenic accumulation and growth inhibition of *D. salina* in coexistence with arsenic and various elements.

MATERIAL AND METHODS

Microalgae

D. salina 19/30 (Chlorophyceae) was obtained from the Culture Collection of Algae and Protozoa (University of Cambridge, UK) and was used throughout the experiments.

Cultures of algae

D. salina was grown at 23 °C in an agitated and aerated filtered sterilized seawater medium containing mg dm^{-3}: $H_3AsO_4$10; KNO_3 72; KH_2PO_4 4.5; Fe–EDTA 1; and the various elements (each at 0, 0.5, 1, 5, 10, 50 and 100 mg dm^{-3}). The pH of each sample was adjusted by addition of dilute hydrochloric acid or sodium hydroxide to a pH 6.9. The density of *D. salina* in the medium before incubation was adjusted to 10^3 cells cm^{-3}

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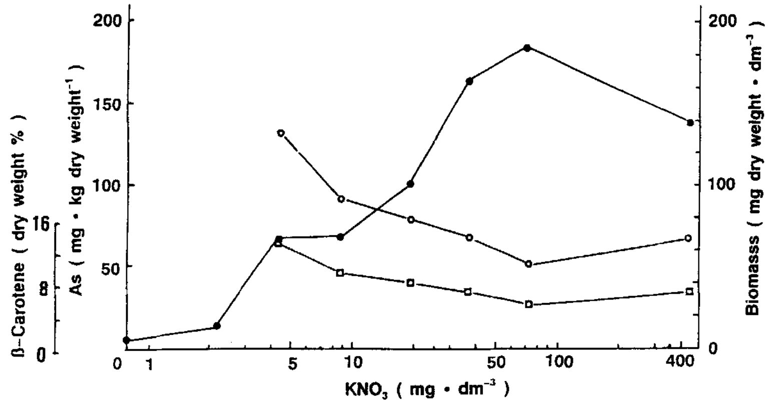

Figure 1 Effect of nitrogen (KNO_3) on growth and accumulation of arsenic and β-carotene of *Dunaliella salina*; ●, growth; ○, arsenic; □, β-carotene.

and the culture was kept at 23 °C under constant aeration ($1\,dm^3\,min^{-1}$) and illumination (15 000 lx).

The cells were harvested by drying by lyophilization after cooling centrifugation (5 °C, 2500 rpm) and the content of arsenic and the various elements in the centrifuged cells was determined. The elements in the culture medium were added as to the following chemical forms: $CoCl_2$, $NiCl_2$, LiCl, $Ga(NO_3)_3$, $Sr(NO_3)$, $(NH_4)Mo_7O_4$, NH_4VO_3, $SbCl_3$, $Bi(NO_3)_3$, $CdCl_2$, $K_2Cr_2O_7$, $Pb(NO_3)_2$, $MgCl_2$, $MnCl_2$, Fe–EDTA, $Zn(NO_3)_2$, SeO_2, $AgNO_3$, $CuCl_2$, KCl, $CaCl_2$, $SnCl_2$.

The optical density (at 630 nm) of the living cell suspension was found to be proportional to the cell density, so measurement of the growth of the cells (g dry weight of cell per dm^3 of medium) was obtained by determination of the optical density of the culture.

Determination of arsenic and the various elements in algae

The freeze-dried algae containing arsenic and other elements were digested with a mixed solution containing 3 cm^3 of concentrated nitric acid, 1 cm^3 of concentrated sulfuric acid and 1 cm^3 of perchloric acid (60%).[7] The arsenic content in algae was determined by a hydride-generation atomic absorption spectrometry method. Wavelength and lamp current were 193.7 nm and 10 mA for arsenic. The amount of the other elements present in *D. salina* was determined by flame atomic absorption spectrometry.[8]

RESULTS AND DISCUSSION

Effect of nitrogen concentration on arsenic accumulation

If *D. salina* was grown in seawater containing an appropriate mixture of nitrogen, phosphorus, iron and arsenic, it accumulated a higher content of arsenic in the cells.[5] Arsenic content in *D. salina* was greatly affected by addition of phosphorus.[5] In order to demonstrate the effects of nitrogen on arsenic accumulation, *D. salina* was cultured in a medium containing various levels of nitrogen (KNO_3) and 10 mg dm^{-3} of H_3AsO_4, and growth and arsenic accumulation were measured (Fig. 1). The growth of *D. salina* increased with an increase in the nitrogen concentration in the medium and became a maximum at 72 mg dm^{-3} nitrogen. The amount of β-carotene decreased with an increase in nitrogen concentration in the medium. *D. salina* was recently shown to accumulate β-carotene to at least 8% of its dry weight when grown under defined growth conditions such as high light intensity, high salt concentration, extreme temperatures or nitrate deficiency.[9] Arsenic content in *D. salina* gradually decreased with an increase in the nitrogen level in the medium. This result suggests that arsenic accumulation was inhibited to some degree by nitrogen at nitrogen levels higher than 5 mg dm^{-3}.

Effect of iron concentration on arsenic accumulation

Iron is an essential trace element for the growth of microalgae. In order to demonstrate the effects of iron on arsenic accumulation, *D. salina* was cultured in a medium containing various levels of iron (0–20 mg dm^{-3}), 10 mg dm^{-3} of H_3AsO_4 and 4.5 mg dm^{-3} of KH_2PO_4. The growth and iron and arsenic contents of *D. salina* are shown in Table 1. The growth of *D. salina* became a maximum with 1.0 mg Fe dm^{-3} and inhibition of growth in *D. salina* was accelerated, with a maximum a 10 mg Fe dm^{-3}. Arsenic content in *D. salina* increased with an increase of iron concentration to 20 mg dm^{-3} and growth was at a maximum at an iron concentration of 1.0 mg dm^{-3}. Maeda *et al.*[10] have recognized, for *Chlorella vulgaris*, a tendency similar to our experimental result. On the other hand, iron content in *D. salina* increased abruptly with an increase of the iron concentration in the medium. Iron accumulates in microalgae in proportion to their concentration in the medium.[11] Also, adsorption of arsenic occurs on iron oxyhydroxides formed diagenetically in natural water.[12] These results, obtained from batch culture experiments, clearly indicate that only arsenic(V) is associated with iron oxyhydroxides in cells. In conclusion, accumulation of arsenic by *D. salina* was accomplished by the common uptake pathway for arsenic and the adsorption of arsenic on iron oxyhydroxides.

Table 1 Effect of various elements on the growth and arsenic accumulation of *Dunaliella salina*

Element	Concn of element in medium (mg dm^{-3})	Algal biomass (mg dm^{-3})	*Concn of* element in alga (mg kg^{-1})	
			As	Fe
Iron	0	20	114	91
	1	125	148	910
	10	18	237	1331
	20	16	396	2127
			As	Mn
Manganese	0	23	107	93
	1	90	126	192
	10	109	131	424
	20	73	274	526
			As	Ag
Silver	0	118	145	Trace
	0.001	105	142	7
	0.005	31	290	12
			As	Cu
Copper	0	127	144	29
	0.01	133	189	294
	0.1	129	323	348
	1	43	379	718
			As	Ni
Nickel	0	122	136	25
	0.1	92	146	75
	1	74	246	143
	10	Dead	—	—
			As	Co
Cobalt	0	125	136	10
	0.1	53	205	104
	1	47	240	216
	10	Dead	—	—
			As	Zn
Zinc	0	128	176	87
	0.1	138	175	98
	1	68	413	104
	10	14	617	221
			As	Cd
Cadmium	0	122	176	29
	0.001	145	113	32
	0.01	73	92	65
	0.1	48	89	107

Note: For analytical method, see ref. 8. The medium contained: KNO_3, 72 mg dm^{-3}; KH_2PO_4, 4.5 mg dm^{-3}; H_3AsO_4, 10 mg dm^{-3}.

Effect of manganese concentration on arsenic accumulation

Manganese, like iron, is an essential trace element for microalgae. In order to demonstrate the effect of manganese arsenic accumulation, *D. salina* was cultured in a medium containing various levels of manganese (0–20 mg dm^{-3}), 10 mg dm^{-3} of H_3AsO_4 and 4.5 mg dm^{-3} of KH_2PO_4. The growth, and manganese and arsenic contents, of *D. salina* after four days of culture are shown in Table 1. The growth of *D. salina* reached a maximum with 1–10 mg Mn dm^{-3}, and decreased at 20 mg Mn dm^{-3}. Arsenic and manganese contents in *D. salina* increased with an increase in the manganese concentration in the medium. In the case of *Chlorella vurgaris*,[10] increasing concentrations of manganese increased the accumulation of arsenic in cells. These results suggest that arsenic accumulation was accelerated to some degree by manganese at manganase levels higher than 10 mg dm^{-3}.

Effects of cadmium concentration on arsenic accumulation

The growth, and cadmium and arsenic contents, of *D. salina* in a medium containing various concentrations of cadmium are shown in Table 1. The growth of *D. salina* is unaffected by cadmium concentrations of 0.001 mg dm^{-3}. *D. salina* growth inhibition was observed at cadmium concentrations exceeding 0.01 mg dm^{-3}. The arsenic content in *D. salina* decreased with an increase of cadmium concentration in the medium. At a cadmium concentration of 0.1 mg dm^{-3}, the arsenic content was lower in the cadmium containing medium than in the cadmium-free medium. On the other hand, the cadmium content in *D. salina* increased with an increase of cadmium concentration. Generally, the cadmium accumulation in algae is not affected by the temperature or by inhibition of metabolism; it is mediated by two processes (first, cadmium adsorption on the surfaces of cell walls; second, energy-dependent transport) and increases with an increase of cadmium concentration in the culture medium.[13] Chloroplasts of *D. minuta* cells exposed to cadmium were significantly smaller than controls, indicating that cadmium stress caused a decrease in the photosynthetic potential of this alga.[14] These results suggest that arsenic accumulation by *D. salina* is inhibited by cadmium. Also, arsenic accumulation by *D. salina* depends upon biological activity, but accumulation of cadmium depends upon biological and physical adsorption.

Effects of cobalt and nickel concentrations on arsenic accumulation

The growth, and cobalt, nickel and arsenic contents, in *D. salina* in a medium containing various concentrations of cobalt and nickel are shown in Table 1. Growth of *D. salina* decreased with an increase in nickel and cobalt concentrations in the medium. The additon of 10 mg dm^{-3} of nickel and cobalt inhibited the algal growth. In the case of *Scenedesmus*, increasing concentrations of nickel were toxic.[15] This phenomenon was also recognized in the experiments using copper, zinc and cadmium. In general, nickel toxicity to green algae is lower than that to blue–green algae and nickel accumulation reaches a maximum at pH 8.[15]

Arsenic content in *D. salina* increased with an increase in cobalt and nickel concentrations, and showed a rapid increase at cobalt and nickel concentrations of 1 mg dm^{-3}. At this concentration cobalt content in the alga increased with an increase of cobalt concentration in the medium. In the case of *D. sp.*, arsenic accumulation was affected by cobalt over eight hours. Cobalt has been reported to be more toxic than manganese, and less toxic than zinc, to *Nitzschia closterium*[16]). It was found here that arsenic accumulation by *D. salina* is unaffected by the addition of low concentrations of nickel and cobalt to the solution and accelerated with a high concentration of cobalt and nickel.

Effect of copper concentration on arsenic accumulation

The growth, and copper and arsenic contents, in *D. salina* in a medium containing various concentrations of copper are shown in Table 1. The growth of *D. salina* reached a maximum at 0.01 mg Cu dm^{-3}. Decreased growth was recorded at higher concentrations (1 mg Cu dm^{-3}). Arsenic content in *D. salina* increased with an increase of copper concentration in the medium, and increased rapidly at a copper concentration of 0.1 mg dm^{-3}. Copper content in *D. salina* increased with an increase of copper concentration in the medium. This result suggests that arsenic accumulation is accelerated to some degree by copper at copper levels higher than 0.01 mg dm^{-3}.

Effect of zinc on concentration on arsenic accumulation

Zinc is one of the essential elements for the growth of microalgae. The growth, and zinc and arsenic contents, in *D. salina* in a medium containing various concentrations of zinc are shown in Table 1. The growth of *D. salina* at a zinc concentration of 0.1 mg dm^{-3} was increased slightly over a zinc-free medium. The growth of *D. salina* decreased with an increase to a zinc concentration of 1 mg dm^{-3}. Generally, zinc is regarded as a toxin against phytoplankton.[15] the content in *D. salina* increased with an increase of zinc concentration in the culture medium, and accumulated on the cell walls.[17] The arsenic content in *D. salina* increased with an increase of zinc concentration. This increase of arsenic content indicated the existence of high accumulation of zinc in cells. It was found that arsenic accumulation by *D. salina* was accelerated by the addition of zinc to the medium.

Effect of antimony concentration on arsenic accumulation

The growth and arsenic content in *D. salina* in a medium containing various concentrations of antimony are shown in Table 2. Growth of *D. salina* decreased with increases of 0.1–10 mg Sb dm^{-3} in the medium. The growth of

Table 2 Effect of various elements on the growth and arsenic accumulation of *Dunaliella salina*

Element	Concn of element in medium (mg dm^{-3})	Algal biomass (mg dm^{-3})	Arsenic concn in alga (mg · kg^{-1})	Element	Concn of element in medium (mg dm^{-3})	Algal biomass (mg dm^{-3})	Arsenic concn in alga (mg · kg^{-1})
Gallium	0	124	138	Chromium	0	112	171
	0.1	162	218		0.1	108	216
	1	159	289		1	115	171
	10	Dead	—		10	42	169
Lead	0	131	142	Tin	0	124	149
	0.1	139	131		0.1	124	161
	1	112	480		1	112	172
	10	112	519		10	77	266
Molybdenum	0	135	149	Magnesium	0	135	143
	1	134	153		1	139	257
	10	126	255		10	139	221
	100	135	342		100	128	175
Selenium	0	112	147	Vanadium	0	122	138
	0.1	117	706		0.1	122	120
	1	167	168		1	120	117
	10	101	—		10	135	475
	100	16	—		100	15	—
Potassium	0	129	149	Lithium	0	129	149
	1	143	117		1	103	156
	10	84	102		10	101	174
	100	120	108		100	120	266
Strontium	0	108	124	Antimony	0	108	124
	0.1	124	124		0.1	124	122
	1	147	119		1	93	352
	10	69	146		10	35	334
Bismuth	0	133	145	Calcium	0	128	122
	0.1	128	168		1	123	108
	1	134	239		10	123	112
	10	45	485		100	114	115

Note: For analytical method, see ref. 8. The medium contained: KNO_3, 72 mg dm^{-3}; KH_2PO_4, 4.5 mg dm^{-3}; H_3AsO_4, 10 mg dm^{-3}.

D. salina was unchanged at 0.1 mg Sb dm^{-3} in the medium. Arsenic accumulation in *D. salina* is accelerated by antimony at concentrations exceeding 10 mg Sb dm^{-3} in the culture medium.

Effects of magnesium, lithium, potassium, calcium and molybdenum concentrations on arsenic accumulation

The microalgae contained 1 g kg^{-1} of magnesium. Growth and arsenic content of *D. salina* in a medium containing various concentrations of magnesium, lithium, potassium, calcium and molybdenum are shown in Table 2. The growth of *D. salina* is unchanged with an increase of magnesium, lithium, potassium, calcium and molybdenum concentrations in the medium. These results indicate that the growth of *D. salina* was unaffected by magnesium, lithium, potassium, calcium and molybdenum.

The arsenic content in *D. salina* was higher in a medium containing magnesium (1–10 mg dm^{-3}) than in the magnesium-free medium. This indicates that arsenic accumulation was slightly accelerated by magnesium. Matsutoh *et al.*[4] have found, for *Phormidium*, a positive correlation between arsenic accumulation and magnesium concentration in the medium.

The arsenic content in *D. salina* was unchanged with increases to 0–100 mg dm^{-3} of calcium and potassium concentrations in the medium. On the other hand, the arsenic content in *D. salina* increased slightly with an increase to 0–100 mg dm^{-3} of molybdenum and lithium in the medium. These results suggested that the growth and arsenic accumulation of *D. salina* are unaffected by calcium and potassium copncentration, but arsenic accumulation is accelerated by molybdenum and lithium.

Effect of vanadium concentration on arsenic accumulation

The growth and arsenic content in *D. salina* in a medium containing various concentrations of strontium and gallium are shown in Table 2. The growth of *D. salina* slightly increased with increases of 0–10 mg dm^{-3} of vanadium in the medium. The arsenic content in *D. salina* was unaffected at increases of 0–1 mg dm^{-3} of vanadium, but arsenic accumulation increased by the addition of 10 mg dm^{-3}. *D. marina* grows in environments containing vanadium at 0.5 mg dm^{-3} and accumulates vanadium at 4.95–185 μg V (g dry weight)$^{-1}$.[18] As in the case of other heavy metals (iron, manganese, etc.), arsenic accumulation is accelerated by vanadium addition at concentrations over 10 mg dm^{-3}.

Effect of strontium and gallium concentrations on arsenic accumulation

The growth and arsenic content in *D. salina* in a medium containing various concentrations of strontium and gallium are shown in Table 2. The growth of *D. salina* slightly increased with increases of 0–1 mg dm^{-3} of strontium and gallium, but the addition of 10 mg dm^{-3} reduced growth. Arsenic content in *D. salina* was unaffected by the increase to 0–10 mg Sr dm^{-3}. On the other hand, the arsenic content in *D. salina* increased with an increase in the gallium concentration. In the experiment using *D.sp.*, gallium existed on the cell wall. As in the case of iron, arsenic accumulation was increased by the addition of gallium.

Effect of bismuth and tin concentrations on arsenic accumulation

The growth and arsenic content in *D. salina* in a medium containing various concentrations of bismuth and tin are shown in Table 2. The growth of *D. salina* was unaffected by the addition of 0–1 mg dm^{-3} of bismuth and tin, but the addition of 10 mg dm^{-3} decreased growth. The arsenic content in *D. salina* increased with an increase to 0–10 mg dm^{-3} of bismuth and tin concentrations. In the experiment using *D.sp.*, bismuth clearly existed on the cell wall.[19]

Effect of silver concentration on arsenic accumulation

The growth and arsenic content in *D. salina* in a medium containing various concentrations of silver are shown in Table 1. The growth of *D. salina* decreased slightly with an increase to 0–0.005 mg Ag dm^{-3} in the medium. A decreased growth was recorded at relatively low concentrations (0.005 mg Ag dm^{-3}). Although no marked changes in arsenic content were observed at silver concentrations of 0–0.001 mg dm^{-3}, the arsenic content increased abruptly at a silver concentration of 0.005 mg dm^{-3}. These results suggest that growth and arsenic accumulation of *D. salina* are affected by trace amounts of silver.

Effect of chromium and lead concentration on arsenic accumulation

The growth and arsenic content in *D. salina* in a medium containing various concentrations of chromium and lead are shown in Table 2. The growth of *D. salina* was unaffected by addition of 0–1 mg dm^{-3} of chromium and 0–0.1 mg dm^{-3} of lead, but the addition of 10 mg dm^{-3} of chromium and 1–10 mg dm^{-3} of lead decreased growth. The arsenic content in *D. salina* was unaffected by the increase of chromium. On the other hand, the arsenic content in *D. salina* increased with an increase to 1–10 mg Pb dm^{-3}. Therefore chromium had no effect on arsenic accumulation. These results suggest that arsenic accumulation is unaffected by chromium, but accelerated by lead to some degree.

Effect of selenium concentration on arsenic accumulation

The growth and arsenic content of *D. salina* in a medium containing various concentrations of selenium are shown in Table 2. The growth of *D. salina* increased with an increase to 0–1 mg dm^{-3} of selenium in the medium. A decrease in growth was found with 10 mg Se dm^{-3}. The arsenic content in *D. salina* reached a maximum at a selenium concentration of 0.1 mg Se dm^{-3}, but was decreased by the addition of 1 mg Se dm^{-3}.

Yamaoka *et al.* have observed, for *D.sp.*, a tendency similar to this experimental result.[19]

CONCLUSION

The effect of 23 elements on the growth and arsenic accumulation of the green alga *D. salina* was examined. The growth of *D. salina* was unaffected by increases of molybdenum, magnesium, calcium, potassium and lithium to 100 mg dm^{-3}. The growth of *D. salina* decreased with addition of selenium and vanadium at 100 mg dm^{-3}, manganese at 20 mg dm^{-3}, iron, strontium, bismuth, gallium, tin and chromium at 10 mg dm^{-3}, copper, zinc, antimony and lead at 1 mg dm^{-3}, nickel and cobalt at 0.1 mg dm^{-3}, cadmium at 0.01 mg dm^{-3}, or silver at 0.005 mg dm^{-3}. Although it is known that the toxicities of these metals depend on their bond strengths regarding coordination (normal bond strength: Hg > Cu > Pb > Cd > Zn > Ni), the results obtained in the experiments using *D. salina* were different from this order. The order of growth inhibition of *D. salina* by elements was Ag > Cd > Co > Ni > Cu > Zn > Fe > Sb > Ga > Cr > Bi > Sr > Mn > Sn > Se > Pb > V > Ca, Mg, Mo, K, Li.

Arsenic accumulation of *D. salina* was unaffected by an increase in calcium and chromium. The arsenic content of *D. salina* decreased at a potassium concentration of 100 mg dm^{-3}, and decreased on addition of calcium and nitrogen. However, it was increased by the addition of lithium at 100 mg dm^{-3}, tin, gallium, bismuth, strontium, vanadium, iron and manganese at 10 mg dm^{-3}, lead, antimony, zinc, copper, cobalt and nickel at 1 mg dm^{-3}, selenium at 0.1 mg dm^{-3}, and silver at 0.005 mg dm^{-3}. Arsenic accumulation by *D. salina* therefore depended both upon biological activity and physical adsorption.

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