Effect of NaCl concentration on cadmium uptake by the halophilic alga *Dunaliella salina*

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ABSTRACT: The effect of NaCl concentration on cadmium uptake by *Dunaliella salina* was investigated. Algae were grown in the presence of 5 mg l^{-1} cadmium at NaCl concentrations varying from 0.5 M to 4 M. The response of cell-specific growth rate, pigment content, and cadmium uptake was studied. Results show maximum cadmium uptake at NaCl concentrations between 0.5 and 1.0 M and considerably lower cadmium uptake at NaCl concentrations above 1.0 M. It is suggested that of the Cd species existing under the experimental conditions, the positively charged Cd^{+2}, $CdCl^+$ and possibly the uncharged $CdCl_2(aq)$ play a major role in cadmium uptake processes.

INTRODUCTION

The toxicological response of eukaryotic algae to cadmium has been investigated in many studies by 2 different approaches: (1) as a factor in environmental pollution in relation to the importance of eukaryotic algae as the base for aquatic productivity; (2) biochemical-physiological studies aimed at establishing the responses of microalgae to heavy metals and at identifying the mode of toxicity to the algal cell.

Cadmium is regarded as one of the potentially toxic metals with cumulative toxicity to humans (Vallee & Ulmer 1972) and various aquatic organisms (Bryan 1971, Nomiyama 1975, Prosi 1981). Cadmium was found to exert toxic action on various algae such as *Chlorella* (Rosko & Rachlin 1975, Gipps & Coller 1980, Rebhun & Ben-Amotz 1984), *Euglena* (De Filippis et al. 1981), diatoms (Rachlin et al. 1982, 1983), *Dunaliella* (Jennings & Rainbow 1979), and *Monochrysis, Isochrysis,* and *Phaeodactylum* (Wikfors & Ukeles 1982). The importance of these algae as the base of the aquatic productivity cycle and the continuous contamination of the aquatic environment by cadmium and other heavy metals has been summarized recently by Davies (1978).

The toxic effect of cadmium on algae may be attributed to membrane phenomena or to intracellular accumulation. Cadmium uptake has been shown in

Dunaliella (Jennings & Rainbow 1979) and in *Chlorella* (Rebhun & Ben-Amotz 1984). Rachlin et al. (1984), using X-ray energy dispersive analysis, identified intracellular cadmium-polyphosphate bodies and cytoplasmic cadmium in *Anabaena.* However, the question of the exact location of cadmium in algae is still unresolved.

Most of the dissolved cadmium in sea water is present in the form of chloride complexes (Rebhun & Ben-
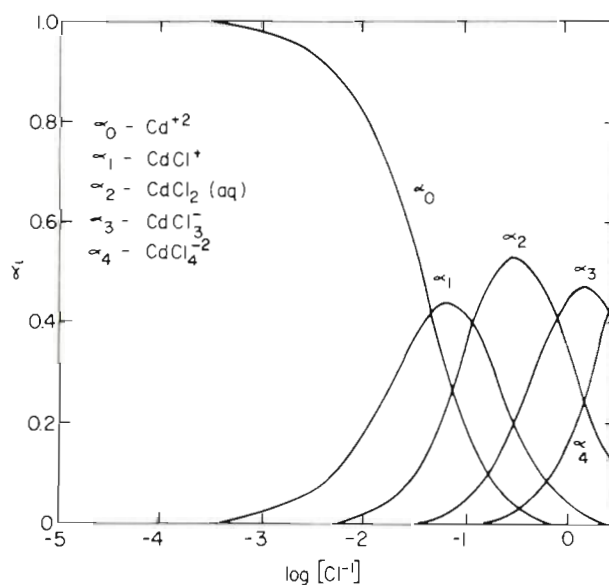

Fig. 1. Distribution diagram of Cd chloride complexes (Posselt & Weber 1974). α: mole fraction of Cd

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Amotz 1984). At the investigated salinities, all the following 4 species were present: $CdCl^+$, $CdCl_2(aq)$, $CdCl_3^-$, $CdCl_4^{-2}$, in addition to free Cd^{+2} ions (Fig. 1; Posselt & Weber 1974).

The halophilic alga *Dunaliella salina* can grow in very saline media and adapt itself to changes in NaCl concentrations (Ben-Amotz & Avron 1981). The purpose of this work is to study the effect of sodium chloride concentrations on *Dunaliella salina* and on the distribution of the dissolved cadmium between the medium and the alga at elevated salinities.

MATERIALS AND METHODS

Solubility of Cd in culture medium. Preliminary studies were undertaken to determine the solubility of Cd in the culture medium at NaCl concentrations of 0.5 to 5.0M. $Cd(NO_3)_2$ atomic absorption (AA) spectrometry grade was added to samples of the sterilized medium containing NaCl at the investigated levels. After 4 to 5 d, portions of the samples were filtered, and Cd was determined in the filtrate and in the non-filtered samples of culture medium.

Medium for Dunaliella salina. The medium for *D. salina* cultures consisted of sea water (about 0.6M salinity) filtered through a 0.45 μm Millipore filter and enriched with the following nutrients: KNO_3, 2 mM;

KH_2PO_4, 0.2 mM; $NaHCO_3$, 5 mM; Na_2EDTA, 30 μM; $FeCl_3$, 1.5 μM; traces of Cu, Zn, Mn, Co, Mo; tris-Cl 20 mM, final pH 8.0; and NaCl concentration as indicated.

$Cd(NO_3)_2$ AA spectrometry grade was introduced into samples prepared for investigation of Cd uptake.

Dunaliella salina cultures. Cultures of *D. salina* were grown with shaking at 23 °C under constant illumination from cool white fluorescent lamps, at a light intensity of approximately 4 W m^{-2}. Initial cell concentration was approximately 0.2×10^5 cells ml^{-1}. Portions were withdrawn at 1 to 3 d intervals throughout the log phase of growth. The number of cells was determined with a Coulter Counter, Model Zb. Chlorophyll was determined spectrophotometrically following extraction with 90 % acetone (Jensen 1978).

Cd content in the *Dunaliella salina* cells at the end of the log phase was determined with an AA spectrophotometer, Model IL 453, operating with air-acetylene flame. Cultures (~250 ml) containing about 10^6 cells ml^{-1} were centrifuged; the unwashed pellets were transferred by using a small volume of distilled water to a pre-weighed crucible, dried at 90 °C and ashed at 480 °C. The ash, containing $CdCl_2$ and NaCl from the algae and the residual medium supernatant, was dissolved in acidified (HCl) distilled water and the volume made up in a volumetric flask of 25 ml for determination of Cd.

Residual Cd in the algal-free supernatant was determined after appropriate dilution.

RESULTS

Solubility of Cd in culture medium

Due to the presence of nutrients, EDTA, tris-Cl and NaCl, it was imperative to make sure that no precipitation of Cd occurs under the specific experimental conditions. Determination of dissolved Cd in filtered and unfiltered medium at different NaCl concentrations showed that no precipitation of Cd occurs. The Cd added remains dissolved in the form of chloride complexes and Cd^{+2} which inter-reacted with *Dunaliella salina* cells (Fig. 1).

Effect of NaCl concentrations on algal growth

Fig. 2 shows growth curves of *Dunaliella salina* in sea water augmented with different NaCl concentrations. Maximum growth rate and yield were obtained at approximately 0.6M NaCl. A significant inhibitory effect of NaCl on algal growth was observed at concentrations above 2M, similar to the response of *D. salina* grown in artificial medium (Ben-Amotz & Avron 1981).
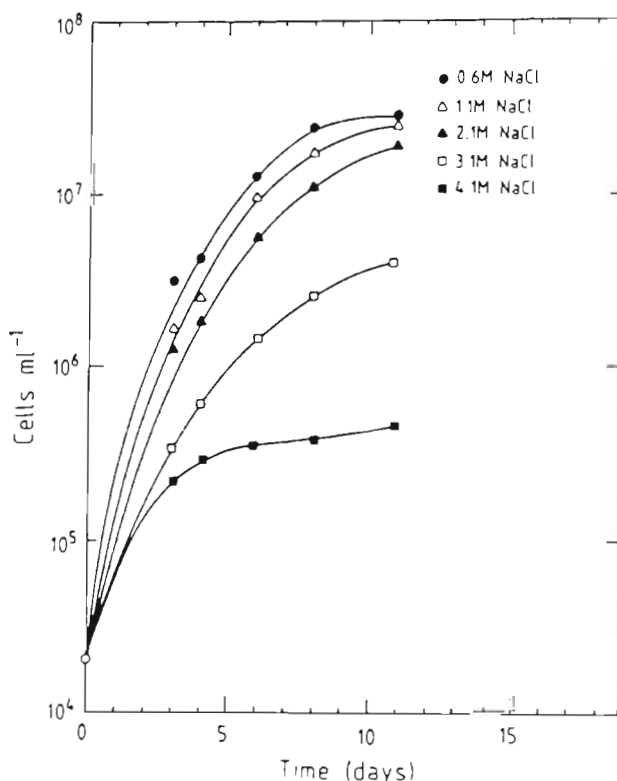

Fig. 2. Effect of NaCl on growth of *Dunaliella salina*

Effect of Cd on *Dunaliella salina*

The effect of Cd on *Dunaliella salina* was studied at NaCl concentrations which promote maximal growth (Fig. 3). Cadmium inhibited cell division of *D. salina* at concentrations as low as 1 mg l^{-1} with about 50 % inhibition at 5 mg Cd l^{-1}. A standard concentration of

5 mg Cd l^{-1} was, therefore, chosen for determination of the effect of NaCl on cadmium toxicity.

Fig. 4 shows the effect of NaCl on growth of *Dunaliella salina* in the presence of 5 mg Cd l^{-1}. At NaCl concentrations between 0.6 and 2.1M, Cd exerts a consistent reproducible inhibitory effect on algal growth. The number of cells per ml is lower as compared to the cultures not exposed to Cd (Fig. 2). At NaCl concentrations above 2M, Cd seems to have an insignificant effect on algal growth. This may be due to the inhibition of Cd toxicity by the higher NaCl concentrations.

Cd uptake

Fig. 5 shows considerable Cd uptake at 0.5 to 0.8M NaCl with a maximum of $0.4 \, \mu \text{g Cd(mg algae)}^{-1}$. Above 1.0M NaCl, Cd uptake decreases sharply to a
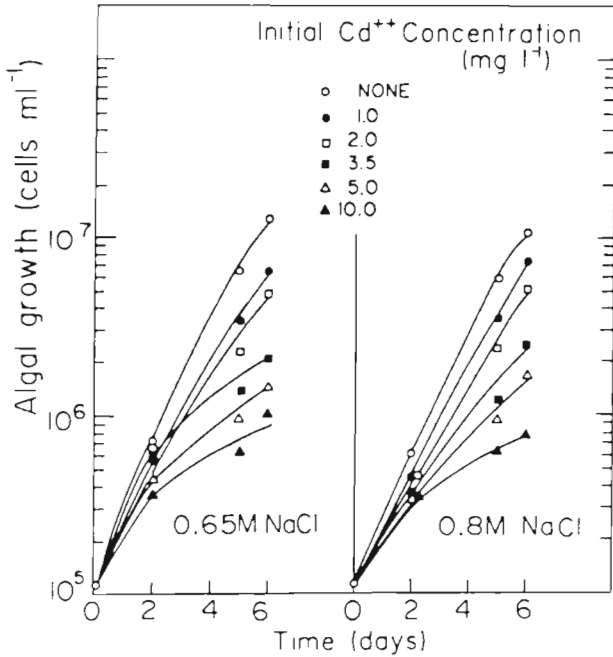
Fig. 3. Effect of Cd on growth of *Dunaliella salina* at NaCl concentrations which promote maximum growth

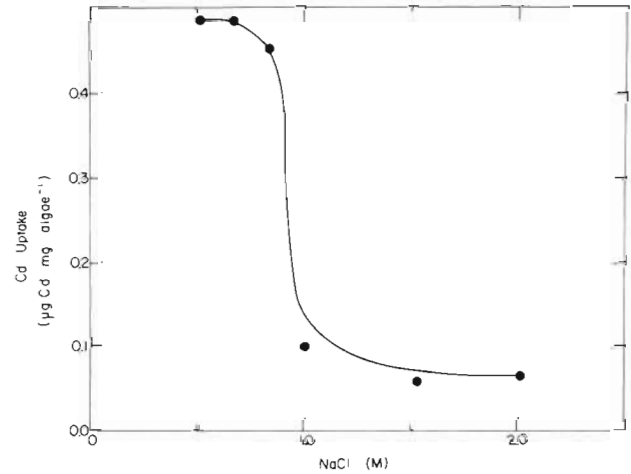Fig. 5. Uptake of Cd by *Dunaliella salina* at different NaCl concentrations

Fig. 4. Effect of NaCl on growth of *Dunaliella salina* in the presence of 5 mg Cd l^{-1}
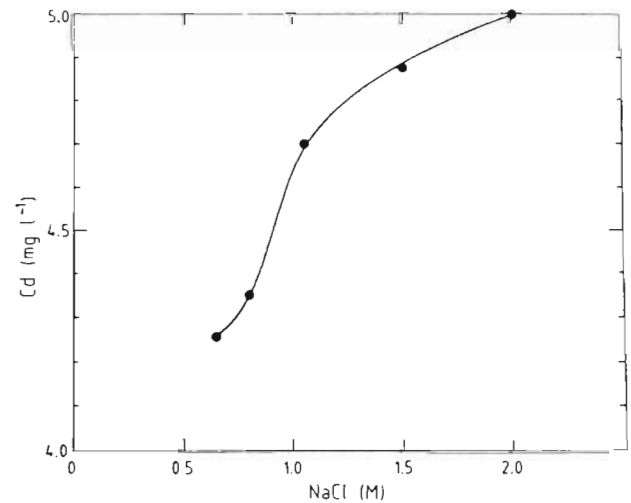
Fig. 6. Residual Cd concentration in medium at different NaCl concentrations

minimum of 0.1 μg Cd (mg algae)$^{-1}$. Fig. 6 shows the residual Cd concentration in the medium versus Cd uptake. Cd concentration in the algal free supernatant decreased from 5 mg l^{-1} to a minimum of 4.2 mg l^{-1} at 0.6M NaCl and returned to approximately 5 mg l^{-1} at higher NaCl levels, in agreement with the corresponding uptake of Cd (Fig. 5).

DISCUSSION

Our explanation of the observed results is based to a great extent on the chemistry of Cd in saline media. The NaCl concentration determines the Cd species present in the culture medium. The distribution diagram for Cd chloride complexes (Fig. 1; Posselt & Weber 1974) shows the mole fraction (α_i) of each Cd species at different chloride concentrations. The diagram indicates that under our experimental conditions (sea water medium at NaCl concentrations between 0.5 and 2.0M), Cd exists mainly in the form of chloride complexes: $CdCl^+$, $CdCl_2(aq)$, $CdCl_3^-$ and $CdCl_4^{-2}$. Calculated values using instability constants (Sillen & Martell 1964) show that the fraction of the free Cd^{+2} ion is low and decreases from 0.012 at 0.5M NaCl (~1.2 % of total Cd) to 0.003 at 2.0M NaCl. Its concentration in μg Cd l^{-1}, however, is significant because it is of the same order of magnitude as the amount of Cd sorbed from 1 l of the algal culture. The fraction of

Fig. 7. Relation between (a) Cd sorbed and concentrations of (b) Cd^{+2} and (c) $CdCl^+$ at different salinities

$CdCl^+$ is 10 times as high as that of the Cd^{+2} ion at 0.5M NaCl and decreases from 0.126 to 0.014 at 2M NaCl (a decrease of approximately 90 %). The decrease in the concentration of the uncharged $CdCl_2(aq)$ is only 2-fold, from 0.5 to 0.23, while its overall concentration remains fairly high. There is a gradual increase in the fraction of the negatively charged $CdCl_3^-$ from 0.3 to 0.48 and an increase in the $CdCl_4^{-2}$ fraction from a low of 0.05 to 0.34.

Our results indicate that the decrease in the concentrations of the Cd^{+2} ion and of the $CdCl^+$ and $CdCl_2(aq)$ complexes is accompanied by a decrease in the Cd uptake. It is suggested, therefore, that Cd^{+2} and $CdCl^+$ are the main species participating in the uptake processes.

In order to better evaluate the results, Fig. 7 was drawn. It shows the relations between (a) the amount of Cd sorbed (μg Cd[l culture]$^{-1}$), (b) the concentration of the Cd^{+2} ion (μg Cd[l culture]$^{-1}$), and (c) the $CdCl^+$ complex (μg Cd [l culture]$^{-1}$), at different salinities. It can be observed that the pattern of Cd sorbed at different salinities runs parallel to the change in the concentration of Cd^{+2}. The change in the concentration of $CdCl^+$ has a similar trend, but the overall concentration range of the $CdCl^+$ species is significantly higher. These facts might suggest that of the 2 main active species, the positively charged Cd^{+2} and $CdCl^+$, it is the free Cd^{+2} ion which plays a major role in the sorption phenomena. These species (and possibly the uncharged $CdCl_2[aq]$) have a high affinity to the *Dunaliella salina* cells, thus explaining the decrease in Cd uptake with the increase in salinity. $CdCl_3^-$ seems to play a rather passive role or to be involved, together with $CdCl_4^{-2}$, in blocking the sorption of other cadmium species by preventing their approach to *D. salina* by virtue of their negative charge.

Preliminary experimental data from our laboratory on the determination of cadmium adsorption on the *Dunaliella salina* surface coat versus cadmium penetration into the cell, using a new technique of cell separation (Pick et al. unpubl.), clearly support the assumption of cadmium adsorption to the cell surface coat. This conclusion is in accordance with the suggestion made by Davies (1978) that the cellular surface of microalgae consists of a mosaic of cationic and anionic exchange sites acting as ion exchangers for anionic and cationic species in the medium. The amount of Cd bound to the cell surface coat at equilibrium should be a function of the affinities of the binding sites for the metal, the chemical form of the metal, and its concentration in the medium.

Binding of Cd to the surface coat is of considerable importance as it may block passive or active passage of other micronutrients essential for growth and reproduction of the algae (Gipps & Coller 1980). Cadmium
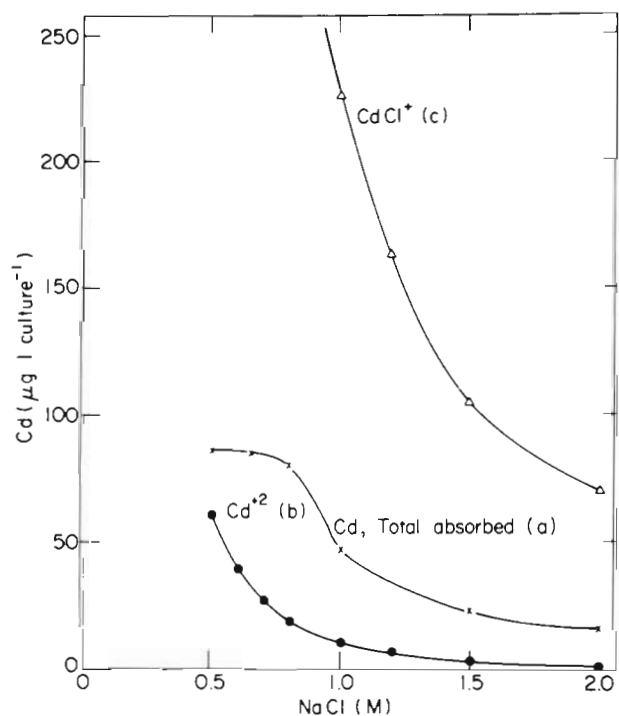
interferes with the uptake of zinc, calcium and manganese in some microalgae in a complex competitive relation controlled by pH, phosphate concentration and the presence of chelators (Hart & Scaife 1977, Hart et al. 1979). The antagonistic effect of NaCl to cadmium toxicity in *Dunaliella salina*, which is similar to the same phenomenon observed in the halophilic bacterium *Pseudomonas* sp. (Onishi et al. 1984), further supports the hypothesis that cadmium toxicity in algae and bacteria is a membrane phenomenon. Thus cadmium toxicity could be affected by any variable which will alter the properties of the cell surface coat facing the medium and its constituents.

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