Pb$^{2+}$ interactions with the marine phytoplankton
*Dunaliella tertiolecta*

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Abstract

Metal ions become adsorbed to algal surface groups and complexed to organic material released by algae at all growth stages influencing their distribution in the seawater. In this study, the differential pulse anodic stripping voltammetry (DPASV) technique was used to evaluate the adsorption and the interaction between the exudates excreted by the marine algae *Dunaliella tertiolecta* and Pb$^{2+}$. The adsorption process has been studied in seawater as a function of pH (2 to 8), temperature (5 to 45°C), salinity (5 to 36), biomass and the presence of a second metal, copper. The rate of adsorption was found to occur in two steps: first a relatively fast adsorption step (10 min) and second, a slow, diffusion-controlled uptake into the cells. A two-site model which considers the presence of two major functional groups, namely: high-affinity binding and low-affinity binding was used to fit the experimental data. The values for the stability constants of Pb$^{2+}$ on these two groups were log $K_{b1} = 8.40 \pm 0.18$ and log $K_{b2} = 7.25 \pm 0.31$. The high-affinity constant is similar to the complexing capacity of the exudates (log $K_{b_{ex}} = 8.40 \pm 0.05$) produced by the alga showing that extracellular ligands play an important role in buffering the concentration of the free metal concentration. A decrease in the temperature and salinity result in lower adsorption of Pb$^{2+}$ to the algae and a higher complexation of lead with the exudate. The presence of copper decreases the lead complexation to the algal surface by 70%.

1. Introduction

The distribution and chemical speciation of trace metals in the oceans is determined by the interactions between the metal ions, particulate matter, and dissolved organic matter present in the sea (Whitfield and Turner, 1987). Biologically produced particulate matter and organic ligands have important implications for the geochemical cycling, activity and environmental toxic effects of trace metals (Coale and Bruland, 1988; Gonçalves and Lopes da Conceição, 1989; Bruland et al. 1991; Xue and Sigg, 1993; Gonzalez-Davila, 1995). An understanding of the interaction between the metals (Cu$^{2+}$, Pb$^{2+}$, Cd$^{2+}$, Zn$^{2+}$, Fe$^{3+}$, Mn$^{2+}$, etc.) and natural particles such as algae, bacteria, yeasts, organic macromolecules and exopolymers from algae or bacteria is indispensable for determining the behaviour of these cations in aqueous environments (Xue and Sigg, 1990; Huang et al., 1991; Crist et al., 1992).

Phytoplankton cells can regulate the concentration of dissolved metals on the water column by surface reactions. The large surface area of
these cells contains various functional groups, such as the N-terminal NH₂-groups, the C-terminal COO⁻ groups, the S-terminal SH-groups and the functional side chain of amino-acid residues that are major potential binding sites (Sigg, 1987; Huang et al., 1991). Adsorption of metals ions to cell surface groups is followed by a slow transport step across the cell membrane and into the cytoplasm (Sunda, 1988/1989; Garnham et al., 1992). Moreover, metal ions in the solution equilibrate with the ligands produced and excreted by the alga at all growth stages. The presence of these organic ligands regulates the bioavailability, bio-accumulation, toxicity and transport of trace metals through biological membranes (Sunda and Guillard, 1976; Jackson and Morgan, 1978; Anderson and Morel, 1982). Organic complexation of metals can markedly influence their transport and cycling by decreasing or increasing its adsorption onto the suspended inorganic and organic particles (Florence, 1982; Donat and Bruland, 1990). Many studies have been carried out on the determination of metal complexation in seawater (e.g. Coale and Bruland, 1988, 1990; Donat and Bruland, 1990; Bruland, 1992; Donat and van den Berg, 1992; Donat et al., 1994). Both aspects, that is adsorption on algae and complexation by dissolved organic ligands in seawater, have only been studied for copper (Xue and Sigg, 1990; Gonzalez-Davila et al., 1994) and zinc (Muller and Kester, 1991). All of these studies have shown that the binding of metal ions to algal exudates has a more significant effect on metal speciation than the binding to the algal surfaces. The conditional complexation constants for the algal surfaces for copper (log $K_{\text{ads}}$ 8.4–10) were always lower than the complexation constants of the exudates (log $K_{\text{ex}}$ 9.0–11.0). Muller and Kester (1991) found that zinc was one or two orders of magnitude more strongly bound to the exudates present in Narragansett Bay than to surfaces of the particulate matter.

In this work, the binding of lead to algal cell surfaces and to the ligands produced and excreted by the alga has been studied. Lead has no known physiological requirement and, thus, can affect growth only adversely (Maeda and Sakaguchi, 1990; Sunda, 1988/1989). Lead may displace nutrient metals from coordination sites of biological molecules, such as enzymes, and thereby alter normal metabolic function. Due to the nonspecificity shown by most of the coordination sites, there may be competition between metal ions in binding to biological ligands. The effects of pH, temperature, salinity, and algal biomass on the adsorption and complexation of lead by the alga Dunaliella tertiolecta in seawater have been studied by voltammetry in the presence and absence of copper.

2. Experimental

The alga used in this study was the unicellular green alga Dunaliella tertiolecta supplied by Dr. Aristegui from the University of Las Palmas. A f/2 medium (Guillard and Ryther, 1962) was continuously fed to keep a continuous culture in quasi steady-state conditions (Brand et al., 1983). Axenic conditions were maintained at 19 ± 2 K under 24 h light and 27 μmol photon m⁻² s⁻¹ (photon flux density) in a growth chamber. All chemicals were reagent grade or the highest obtainable grade.

Ellipsoid-shaped cells, with diameters of 8.25 ± 1.25 μm and 6.41 ± 1.5 μm could be observed through the microscope. A cell number of 2.42 × 10⁷ cell l⁻¹ (optically counted with a hemacytometer) in a suspension of 38 mg l⁻¹ (dry weight) were determined. From the apparent geometry of the cells an approximate surface of c. 166.24 μm² per cell and a specific surface area of 0.18 m² g⁻¹ can be estimated.

When the cell concentration was sufficiently high (10⁹ cell l⁻¹), 200 ml of the cultures were centrifuged at 4000 rpm for 15 min, then washed 4 times with 0.45 μm filtered seawater (Millipore acid-washed filters). This cell content was diluted to get 1 l of sample with a cell number in the range 1–3 × 10⁷ cell l⁻¹. The sample was stored in refrigerator for 24 h before use. The seawater used in the preparation of the cultures and for all experiments was collected northwest of Gran Canaria (Islas Canarias), one mile off the coast from a depth of ~ 10 m.

Differential pulse anodic stripping voltammetry (DPASV) was used for all the metal determin-
ations. The measurements were performed with the PAR 303 static mercury drop electrode (SMDE) in the hanging mercury drop electrode (HMDE) mode, using the PAR Model 384B polarographic analyzer system connected to a DMP-40 plotter. A polyethylene cell was employed. An Ag/AgCl electrode was used as the reference electrode and a coiled platinum wire as the auxiliary electrode. The reduction potential was -0.9, -0.8 and -0.6 V, respectively, for Cd$^{2+}$, Pb$^{2+}$ and Cu$^{2+}$. In all cases, the scan rate was 2 mV/s; the pulse height, 50 mV; and the deposition time 2 min. For most of the studies, the measurements were made in triplicate.

Each experimental point records a measurement of an individual batch after equilibration time of 10 min at the desired temperature in a shaker bath. All the samples were equilibrated at a given pH, temperature and salinity studied. After filtration (HA Millipore acid-washed 0.45 μm filters), the electrochemically labile metal concentration was determined in the untreated sample. The total dissolved metal concentration was determined after acidification (pH = 2) with 2 M HCl and microwave treatment (CEM-MDS-81D, 630 W, 20 min). By a mass law, the amount of metal adsorbed by the cell was determined. The sensitivity of the determination was determined in each experimental condition to analyze the metal concentration. The concentration of adsorbed metals calculated in this way were in good agreement with the values measured on filtered and acid digested (HNO$_3$ + HClO$_4$) algal cells.

3. Results and discussion

3.1 Uptake kinetics of Pb$^{2+}$

The kinetics of uptake of 0.7 μM Pb$^{2+}$ are shown in Fig. 1. The process follows a two step-sorption kinetics. The Pb$^{2+}$ adsorbed to the cells initially increases rapidly for a few minutes taking up 50% ($6.90 \times 10^{-15}$ mol cell$^{-1}$) of the total amount of Pb$^{2+}$ ($1.43 \times 10^{-14}$ mol cell$^{-1}$) assimilated by the alga. Pb$^{2+}$ uptake continues even until the end of the experiment (600 min) (data point not shown). This implies that the extracellular

![Fig. 1](image)

Fig. 1. Kinetics of Pb$^{2+}$ uptake to the alga *D. tertiolecta* in seawater (pH = 8.2, 25°C). The changes in the complexed and labile lead concentration as a function of time are also included. Initial concentration of lead is $7 \times 10^{-7}$ M.
association (surface binding) occurs first and takes place rather fast. Further Pb\(^{2+}\) uptake by the live cells takes place more slowly and may be controlled by the diffusion process through the cell wall or regulated by intracellular metabolic processes. The rate of Pb\(^{2+}\) uptake was analyzed according to Crank (1976). After 10 min, the uptake of Pb\(^{2+}\) by the live phytoplankton conformed well to the parabolic diffusional model \([\text{Pb}^{2+}]_{\text{adsorbed}} = 1.33(\pm 0.04) \times 10^{-7} + 0.067(\pm 0.003) \times 10^{-7} \sqrt{t}\) suggesting that intracellular diffusion may be the rate limiting step. After 6 h equilibration time, 41% of the initial Pb\(^{2+}\) was assimilated by the cells. At the same experimental conditions, the amount of Pb\(^{2+}\) assimilated by the algae is lower than that of Cu\(^{2+}\) (Gonzalez-Davila et al., 1994). This order, Cu\(^{2+}\) > Pb\(^{2+}\), shows a correlation with the second ionization potential, as exhibited by other adsorbents (Gonzalez-Davila et al., 1990). In all the studies, 10 min equilibration time was used in order to obtain a pseudo-equilibrium with the algal surface. No attempts were made to study the assimilation of lead by the algae.

### 3.2 Adsorption isotherms

In Fig. 2, a typical determination of the complexing capacity of seawater is shown by curves 1 and 2 for Pb\(^{2+}\). Using a van den Berg–Ruzic plot (van den Berg, 1982) the natural complexing capacity of seawater can be evaluated (Fig. 3).

\[
\frac{[\text{Me}']}{[\text{MeL}]} = \frac{[\text{Me}']}{C_L} + \frac{1}{K'_{\text{cond}([\text{Me}'])}C_L}
\]

where \([\text{MeL}]\) is the concentration of organically complexed metal, \(C_L\) is the total ligand concentration in seawater and \(K'_{\text{cond}([\text{Me}'])}\) is the conditional stability constant determined with respect to \([\text{Me}']\), the concentration of the metal ion in all inorganic forms. In order to compare the different metal complexing capacity values of seawater, an attempt was done to study the cadmium capacity of seawater in the absence and in the presence of algae (Fig. 3). The values obtained for the Gran Canaria (Islas Canarias) seawater for Pb\(^{2+}\), Cd\(^{2+}\) and Cu\(^{2+}\), are shown in Table 1. The conditional stability
constants of the form $K'_{\text{cond}(\text{Me}^{2+})}$ have been determined with respect to the free Me$^{2+}$

$$K'_{\text{cond}(\text{Me}^{2+})} = \alpha^{-1} K'_{\text{cond}(\text{Me}')}$$

where $\alpha$ is the inorganic side reaction coefficient of metals in seawater.

Although variability in the accepted inorganic side reaction coefficients for metals has been reported, values of 0.039, 0.028 and 0.087 (Millero and Hawke, 1992) were chosen to normalize our conditional stability constant in Table 1 with respect to free metal for Cu$^{2+}$, Pb$^{2+}$ and Cd$^{2+}$, respectively. The highest ligand concentration is exhibited by Cu$^{2+}$, and the lowest by Cd$^{2+}$. The normalized stability constants show similar values for all three metal complexes. Cadmium complexing ligands exist at the lowest concentrations, but are stronger than those formed by Cu$^{2+}$ and Pb$^{2+}$. Other studies (Coale and Bruland, 1990; Bruland, 1992; Donat et al., 1994) have shown that Cu forms the strongest complexes with natural organic ligands, Cd the next strongest and Pb the weakest. In our case, copper and

Table 1
<table>
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<tr>
<th>Metal</th>
<th>Natural seawater</th>
<th>Seawater + exudates</th>
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<tr>
<td></td>
<td>$C_L$ (nM)</td>
<td>log $K_{\text{Me}^{2+}}$</td>
</tr>
<tr>
<td>Pb(II)</td>
<td>120 ± 3</td>
<td>6.81 ± 0.05</td>
</tr>
<tr>
<td>Cd(II)</td>
<td>29 ± 3</td>
<td>7.72 ± 0.09</td>
</tr>
<tr>
<td>Cu(II)$^a$</td>
<td>214 ± 2</td>
<td>7.19 ± 0.03</td>
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$^a$Gonzalez-Davila et al., 1994.
cadmium form complexes with similar strength. The treatment used assumes that metal complexation can be adequately represented by a single ligand and complexes having a 1:1 stoichiometry. The presence of two ligands or sites with sufficient affinity for the metal ions have been shown to cause the non-linear behaviour of the plots at lower concentrations (van den Berg, 1984). In our studies, only the linear relationship has been analyzed due to the limited measurements made at low concentrations. Thus, our conditional stability constant of Cu corresponds with the weaker ligand of two Cu-complexing ligands usually found in seawater (van den Berg, 1982). Different analytical methods determine metal-complexing organic ligands of different strengths and concentrations in seawater, both because a variety of ligands exist and because the different methods have different detection windows. Determination of organic complexation at any single detection window may give only partial information on the true concentration of organically complexed and other forms of metals in seawater (Donat et al., 1994). Thus, one must keep in mind that this simple treatment of metal-organic interaction does not necessarily prove that only one or two ligand sites are present, and only an average stability constant will be obtained by this technique.

The addition of phytoplankton cells to the seawater will change the complexing capacity of the seawater due to the release of exudates from the cells. In order to determine the complexation capacity changes, algal suspensions were filtered after 22,000 cells ml\(^{-1}\) were kept in equilibrium with seawater for 24 h without the addition of metals. Fig. 3 shows the changes produced in the Pb(II) complexation. No appreciable changes were observed for the conditional stability constants of Cd\(^{2+}\) and Pb\(^{2+}\) (Table 1). Only a slight increase in the ligand concentration was observed in the filtrates of the algae. The same behaviour for the \(C_L\) and \(K'\) values has been observed by Seritti et al. (1986) for Dunaliella salina, and by Stolzberg and Rosin (1977) for the marine diatom Skeletonema. This seems to show that at the beginning of the culture, the presence of cells does not affect the value of complexation capacity of Pb\(^{2+}\) and Cd\(^{2+}\). Only the conditional stability constant for the Cu complexes is clearly affected (Gonzalez-Davila et al., 1994).

Fig. 2 shows the changes of the complexing capacity of seawater (curves 3 and 4) as a consequence of both the adsorption of lead on the cell walls and the complexation by the exudates produced and excreted by the algal cells. When the same study was carried out for cadmium, it was found that cadmium stays in the solution as organically (non-labile) and inorganically (labile) complexed cadmium and does not appreciably adsorb onto the cell surface groups. Curve 4 represents the concentration of labile lead in the filtered sample after 10 min of equilibrium at natural pH. For the determination of total dissolved lead in the presence of algae (curve 3), the samples were acidified to pH = 2 after filtration and equilibrated for 3 h. Our analytical procedure allows us to determine the amount adsorbed as the difference between curve 1 (total metal added) and curve 3 (total dissolved metal). The amount of metal complexed with the non-labile ligands present in the seawater is determined taking into account curves 3 and 4 (labile metal) (Gonçalves et al., 1987). The peak potential shifts when Pb\(^{2+}\) is added to the algal suspension or to the solution of the algae after centrifugation at different pH values correspond well to the hydrolysis of the metal. It shows that labile organic lead complexes are not formed in addition to the non-labile lead complexes.

The extent of binding of metal ion onto biological surfaces may be described by mass law equations of the following type of surface complex formation equilibria:

\[ \equiv \text{SOH} + \text{Me}^{2+} \leftrightarrow \equiv \text{SOMe}^{2+} + \text{H}^+; \ K_1^\equiv \] (3)

\[ 2 \equiv \text{SOH} + \text{Me}^{2+} \leftrightarrow (\equiv \text{SO})_2 \text{Me}^{2+} + 2\text{H}^+; \ \beta_2^\equiv \] (4)

The surface binding may also be generalized by the mass law equations at pH values of natural seawater considering the algal surface as a poly-functional macromolecule:

\[ \equiv \text{S}_j \text{H} + \text{Pb}^{2+} \rightarrow \text{S}_j \text{Pb}^+ + \text{H}^+; \ K_{\text{Pb},j}^\equiv \] (5)
where \( \equiv S \), designates a (deprotonated) bidentate surface chelating site, such as an amino, thio, hydroxyl or carboxylic functional groups.

The algal surface acid–base properties may be characterized by:

\[
\equiv \text{SH}^+ \leftrightarrow \text{SH} + \text{H}^+; \quad \ast K_{\alpha}, 1
\]

\[\equiv \text{SH} \leftrightarrow \text{S}^- + \text{H}^+; \quad \ast K_{\alpha}, 2 \quad (6)
\]

Because the functional groups on the surface are not identical, \( \ast K_{\alpha,j} \) may be interpreted as an average constant \( \ast K_{\alpha} \); it is obtained by applying the law of mass action to a mixture of surface chelating sites:

\[
\equiv \text{SH} + \text{Pb}^{2+} \leftrightarrow \equiv \text{SPb}^+ + \text{H}^+; \quad \ast K_{\beta}^S \quad (7)
\]

which is a function of both the hydrogen ion concentration and the free metal concentration. The free metal concentration of lead as a function of total lead in the seawater suspension is affected by the production of extracellular organic matter with metal complexing properties coming from the algae. For quantitative study of metal adsorption to algal surfaces, binding to exudates must be considered. In the presence of algae, the total Pb(II) added is

\[ [\text{Pb}^{2+}]_T = [\text{Pb}^{2+}]_{\text{inorg}} + \sum_i \{\equiv S_i - \text{Pb}\} + \sum_n [\text{PbL}_n] = \alpha^{-1} [\text{Pb}^{2+}]_{\text{free}} \]

\[ + \sum_i \{\equiv S_i - \text{Pb}\} + \sum_n [\text{PbL}_n] \quad (8)
\]

where \([\text{Pb}^{2+}]_{\text{inorg}}\) expresses the concentration of Pb\(^{2+}\) in all the inorganic forms (labile lead), \(\alpha\) is the fraction of free metal, and \(\sum_i \{\equiv S_i - \text{Pb}\} + \sum_n [\text{PbL}_n]\) expresses the concentration of lead adsorbed in the algal surface and binding with a soluble exudate, respectively. In all our studies, the binding of lead to the exudates were considered in order to determine the true free concentration of lead in equilibrium with the metal adsorbed.

The equilibrium constant for Eq. (8) may be defined for a given pH, in terms of a conditional constant (omitting the charges) as:

\[
\ast K_{\beta}^S = \frac{\{\equiv \text{SPb}\}}{[\equiv \text{SH}][\text{Pb}^{2+}]} \quad (10)
\]

This equilibrium constant depends somewhat on the charge of the surface which in turn depends on the extent of surface binding of metal ions and protons (Stumm and Morgan, 1981; Sposito, 1984). Thus, the equilibrium constants, \( \ast K_{\beta}^S \), evaluated from low surface coverage or from low \([\text{Me}^{2+}]\) are of most general interest to seawater studies.

Eq. (10) can also be interpreted in terms of a Langmuir isotherm:

\[ \Gamma_{\text{Pb}} = \frac{\Gamma_{\text{max}}[\text{Pb}^{2+}]}{(\ast K_{\beta}^S)^{-1} + [\text{Pb}^{2+}]} \quad (11)
\]

\[ \{\equiv \text{Pb}\} = \frac{\{S_T\}[\text{Pb}^{2+}]}{(\ast K_{\beta}^S)^{-1} + [\text{Pb}^{2+}]} \quad (12)
\]

where \(\{\equiv \text{SPb}\}\) and \(\{S_T\}\) correspond to \(\Gamma_{\text{Pb}}\) and \(\Gamma_{\text{max}}\), respectively, and are the amount of metal ion adsorbed in mol cell\(^{-1}\), and the maximum value of the metal ion adsorption capacity (complexing capacity), respectively. Fig. 4 shows the usual forms of Langmuir isotherms (Eq. 12) for the adsorption of lead on two different amounts of Dunaliella tertiolecta cells. From the experimental data we may derive the equilibrium constants \( \ast K_{\beta}^S \), as defined by Eq. (12) by transforming Eq. (12) into:

\[ \equiv \text{SPb} = \left\{ \frac{1}{\Gamma_{\text{max}}} \right\} + \ast K_{\beta}^S \{S_T\} [\text{Pb}^{2+}] \quad (13)
\]

and by plotting \(\{\equiv \text{SPb}\}\) vs. \([\text{Pb}^{2+}]\), the values of \(\{S_T\}\) and \(\ast K_{\beta}^S\) can be evaluated. Fig. 5 illustrates such a plot for the binding of Pb\(^{2+}\) to the alga at pH = 8.2 in seawater, \(S = 36.2\) and 25°C. At high surface coverage considerable deviation from a linear relationship is observed. This indicates that Pb\(^{2+}\) ions bind first to the highest affinity surface ligands and subsequently to those of less affinity. The values obtained for the amount adsorbed \(\{S_T\}\) and for the conditional constant \( \ast K_{\beta}^S \) or Langmuir parameters are given in Table 2, where the value was taken as 0.028 (Millero and Hawke, 1992). Using a \( \ast K_{\beta}^S \) values of 8.5 for the adsorption of Cu\(^{2+}\) (Gonzalez-Davila et al., 1994), appear to be
Fig. 4. Adsorption isotherms of Pb\(^{2+}\) and algae in natural seawater according to the Langmuir equation (Eq. 12) at two different algae concentrations.

Fig. 5. Langmuir plot (Eq. 13) for the binding of Pb\(^{2+}\) from titration data (Fig. 4).
Table 2

Lead adsorption constants determined in accordance with the one-site Langmuir equation (Eq. (12)) and with the two-site Langmuir equation (Eq. (14))

<table>
<thead>
<tr>
<th>One-site model</th>
<th>Two-site model</th>
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<tbody>
<tr>
<td>Cell $^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$\Gamma_{\text{max},1} 10^{15}$ mol cell$^{-1}$</td>
<td>$\Gamma_{\text{max},1} 10^{15}$</td>
</tr>
<tr>
<td>2.83 $10^{7}$</td>
<td>5.21 $\pm$ 2.71</td>
</tr>
<tr>
<td>1.75 $10^{7}$</td>
<td>4.38 $\pm$ 3.21</td>
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more strongly adsorbed on cell surface groups than lead.

When the data are plotted using the linear Scatchard plot, $\{\equiv \text{SMB}/\text{Me}\}$ vs. $\{\equiv \text{SMe}\}$ (Fig. 6), a curvilinear relationship occurs. Thus, the possibility of at least two sites with a significantly different metal affinity is physically reasonable. Those sites with the greatest affinity for the metal ion will be occupied first, and subsequently the lower affinity site will be occupied. This behaviour is interpreted assuming a two-site model, which classified various sites into two groups (high affinity and low affinity) with two different binding capacities, $\Gamma_{\text{max},1,2}$, with corresponding binding constant $\ast K_{H,1}^{S}$ (Sposito, 1984; Stroes-Gascoyne et al., 1987):

$$
\Gamma = \{\equiv \text{SMB}\} = \frac{\Gamma_{\text{max},1}[\text{Pb}^{2+}]}{\left(\ast K_{H,1}^{S}\right)^{-1} + [\text{Pb}^{2+}]} + \frac{\Gamma_{\text{max},2}[\text{Pb}^{2+}]}{\left(\ast K_{H,2}^{S}\right)^{-1} + [\text{Pb}^{2+}]}$$  (14)

![Fig. 6. Scatchard plot for the data presented in Fig. 4. Two-site Langmuir isotherms (Eq. 14) can be applied to the linear isotherm regions for each site.](image)

log $K_{H,1}^{S} = 8.36 \pm 0.10$
log $K_{H,2}^{S} = 7.22 \pm 0.05$
$\Gamma_{\text{max},1} = 6.21 \pm 2.71 \times 10^{-15}$ mol cell$^{-1}$
$\Gamma_{\text{max},2} = 21.5 \pm 3.1 \times 10^{-15}$ mol cell$^{-1}$
The values for the four adjustable parameters of Eq. (14) are determined by using a non-linear regression (Sposito, 1984) and the results are given in Table 2. Using the constants determined in such a way, Fig. 4 demonstrates that a two-site Langmuir isotherm with four adjustable constants can reproduce the experimental data very well (Hunston, 1975). No significant differences were observed for studies carried out at two different concentrations of cells. The data given in Figs. 2 and 4 and Table 2 illustrate that the functional groups of algal surfaces coordinately bind metal ions in a way similar to that of soluble ligands. The curves with lead are smooth and follow a straight line parallel to the calibration plot of metal concentration in excess of the complexing surface sites. The tendency to form surface complexes decreases with increasing metal loading of the algal surface. The reliability of the two-site adsorption model is understandable because the metal ions bind first to the surface functional groups having the highest affinity, e.g. amino-acid groups, and subsequently to those groups having a lower affinity. The data presented in Tables 1 and 2 clearly demonstrate the important role of ligands produced and excreted by the algae in the complexation of metal ions. The equilibrium constants and complexation capacities for the binding of Pb\(^{2+}\) with algal surfaces and exudates represent average conditional formation constants and are only valid for the specified conditions. However, when we compare the values obtained at pH = 8.2 and S = 36.2 (Tables 1 and 2), it is clearly observed that for the same order of magnitude of maximum binding capacity of algal exudates and algal surface groups (\(C_L\) in mol cell\(^{-1}\) \(\approx \Gamma_{\text{max}}\)), the complexation constant of the exudates \(K_{\text{MC}}^{2+}\) is higher than the conditional constant for the algal surfaces, \(K_{\text{SH}}\), in the homogeneous model. As the complexation constant \(K_{\text{MC}}^{2+}\) represents an average stability constant for a set of ligands with broadly similar stability constants, comparison with the two-site model must be done with caution. Nevertheless, our data show that the complexation constant of the algal exudates are at least of the same strength as the adsorption constant for the high affinity sites on the algal surface groups. The adsorption and binding of Pb\(^{2+}\) reported here show that algae influence the residual concentration of lead in seawater by binding to algal surfaces (and subsequent assimilation into the inside of the cells), and by production of strong ligands that affect the concentration of free ions. This lead-induced production of complexing exudates keeps the lead concentrations at low levels and prevents the accumulation of lead in the cells.

The conditional stability constants \(K_{\text{H,I}}\) are a function of the proton concentration, e.g.:

\[
K_{\text{H,I}} = \frac{K_{\text{S,I}}}{1 + K_{\text{a,I}}[H^+]}
\]

The term \(1 + K_{\text{a,I}}[H^+]\) is called the proton competition coefficient. When metal adsorption occurs at a constant pH value, \(K_{\text{a,I}}\) may be replaced by an apparent constant \(K_{\text{a}}\) which can be estimated from the gradient of the proton titration curve at a given proton concentration (Huang et al. 1991). Using a value of \(\log K_a = 7.47 \pm 0.32\) (pH = 8.2, NaCl 0.7 m), determined from a proton titration curve (Gonzalez-Davila et al., 1994) intrinsic equilibrium constants have been computed for the homogeneous and two-site models. Table 2 shows these intrinsic values which are independent of pH and can be applied at any pH where the changes in the lead speciation can be determined. The proton effect on metal adsorption to the algae is insignificant near pH = 8.2.

When the pH of the seawater is lowered by addition of HCl (Fig. 7), the amount of Pb\(^{2+}\) adsorbed decreases at pH values lower than 6. The adsorption of metal ions at the algal surface–water interface is strongly pH-dependent, because the properties of both the algal surface groups and the solution composition (metal ion speciation) change with the pH. The adsorption of Pb(II) on the algae as a function of pH has been determined by measuring the total and labile solute concentration both in the presence and in absence of algae to take into account any effect of the plastic walls of the experimental container and in the voltammetric signal due to changes in the metal speciation with pH (Gonçalves et al., 1985). In accordance with previous work (Gonçalves et al., 1985; Gonzalez-Davila et al., 1990) the inorganic complexes of lead, such as PbOH\(^+\), are fully labile. At pH values lower than 5, no adsorption of lead
Fig. 7. Effect of pH on the adsorption of 0.7\( \mu \)M Pb\(^{2+}\) on 2.56 \times 10^7 cell\(^{-1}\). The pH of seawater was adjusted by addition of NaOH or HCl.

by the algal cells was observed; at pH values above 6, a nearly constant amount of Pb\(^{2+}\) was adsorbed. At pH values between 5 and 6, the amount of Pb(II) adsorbed increased until more than 30% of the initial Pb(II) concentration in solution was adsorbed. Neglecting the activity coefficients, the mass law expression for the reaction (8) is:

\[
K^S_B = \frac{[\equiv SPb][H^+]^{n}}{[\equiv SH_n][Pb^{2+}]} \tag{16}
\]

Upon rearrangement we have:

\[
\log \frac{[\equiv SPb]}{[Pb^{2+}]} = Z + npH \tag{17}
\]

where \(Z = \log K^S_B + \log ([\equiv SH_n])\). If \(Z\) is considered a constant, Eq. (17) becomes:

\[
\log \frac{[Pb^{2+}]_{ads}}{[Pb^{2+}]} = Z + npH \tag{18}
\]

Eq. (18) predicts a slope of 2 for the adsorption of divalent metals to a ligand which reacts with the metal to form a complex with the release of 2 protons (bidentate or monodentate complexes):

\[
2SH_n + Pb^{2+} \leftrightarrow (SH_n-1)_2Pb + 2H^+ \tag{19}
\]

or a slope of 1 for the adsorption to a ligand with the release of 1 proton:

\[
SH_n + Pb^{2+} \leftrightarrow SH_n-Pb + H^+ \tag{21}
\]

where SH\(_n\) in Eqs. (19-21) are not necessarily the same groups. The adsorption process may also take place on deprotonated sites \(\equiv S^-\), thus there are no protons released. Several binding sites on the algal surfaces have been reported. These sites include the NH\(_2\)-groups, the COO-groups, and the side chain functionalities of amino-acid residues. Thus, for example, amino and/or carboxylic groups can be bidentate ligand with metal ions, whereas the surface complexes of phosphate and metal ions are monodentate (Beveridge and Murray, 1980).

The slope determined from Eq. (18) (Fig. 7), and taking into account the changes in the speciation of Pb\(^{2+}\) with the pH (Millero and Hawke, 1992), is 1.57 \(\pm\) 0.27. This value can be attributed to the co-ordination of Pb\(^{2+}\) to groups that have a macroscopic proton value of 1 and another with a value of 2. However, the difference between the slope and the ratio may be also due to the different values of \(Z\) in Eq. (18), which depend on the acid–base properties of the surface sites. The values obtained
for the amphoteric properties of the surface of *D. tertiolecta* in 0.7 m NaCl were \( pK_a,1 = 4.92 \), \( pK_a,2 = 6.28 \) and \( pK_a,3 = 10.06 \) (Gonzalez-Davila et al., 1994). Thus, the number of \( \equiv \text{SH}_i \) sites in the algal surface changes with the pH and will affect the value of \( Z \). Values of pH\(_{P_{zc}}\) of 5.60 and 8.17 were determined for the alga. At pH values close to the pH\(_{P_{zc}}\) of the alga, changes in the charge of the surface from positive to neutral to negative are expected. Moreover, the adsorption of anions, directly or indirectly as counterions affects the expected ratio. Thus, there is no reason to assume the observed exchange ratio should be an exact number. At pH values lower than pH\(_{P_{zc}}\), the surface will be positively charged and the adsorption of Pb\(^{2+}\) ions will be unfavoured.

### 3.3 Effect of temperature and salinity

In order to understand the effect that changes in the temperature of seawater have on the adsorption of Pb\(^{2+}\) on the cell surface, we carried out experiments at 6 different temperatures, between 5 and 45°C. We carried out additional kinetics studies at these temperatures to take into account any effect temperature had on the rate of the adsorption. Again, a pseudo-equilibrium was observed after 8–10 min of equilibration. The specific adsorption energy \( E \) for the adsorption of Pb\(^{2+}\) was calculated using:

\[
\ln K_p = A - \frac{E}{RT}
\]

(22)

where \( A \) is the frequency factor and \( K_p \) is the partition coefficient of Pb\(^{2+}\) on the alga. The linear fit of the values of \( \ln K_p \) vs. \( 1/T \) (K) yields a value of \( E_{\text{Pb}} = 6.51 \pm 0.97 \) kJ mol\(^{-1}\). This low positive specific adsorption energy of Pb\(^{2+}\) may be interpreted as the heat of hydration of Pb\(^{2+}\) being higher than its heat of adsorption. A decrease in temperature from 45°C to 5°C decreases the amount of lead adsorbed by 28%. As a result of the increase in the dissolved lead concentration at low temperatures, a slight increase (8–10%) in the amount of complexed lead was found. The increase in the amount of dissolved lead (also observed for Cu\(^{2+}\)) may decrease the low ratio of [Fe(III)]:[other trace metals] usually found in natural seawaters (Brueland et al., 1991). This possibility should be considered in future work. More studies are needed on the effect of temperature on the adsorption and assimilation of nutrient and toxic heavy metals both in laboratory and field experiments.

When the seawater solution is diluted with MilliQ deionized seawater, the changes in the salinity of the solution may be considered to account for the effects of changes in the ionic strength. The ionic strength influences adsorption by affecting the activities of the metal ions in solution, as well as affecting surface charge and double-layer capacitance of the hydrated cells. The corresponding variation of the concentrations of dissolved and labile Pb\(^{2+}\) with the salinity of the solution corrected by changes in the voltammetric signal is shown in Fig. 8. The labile Pb\(^{2+}\) decreases linearly with \( S^{1/2} \), being 50% less at \( S = 15 \) than at \( S = 36 \). The amount of Pb\(^{2+}\) adsorbed, determined as the difference between the lead added and the total dissolved lead, increases at low salinity values and 43% more lead is adsorbed at low salinities than at seawater salinity. Also, at low salinities a small increase in the complexed amount of Pb\(^{2+}\) is observed. Similar behaviour of the adsorption of Pb\(^{2+}\) by *Rhizoclonium* has been shown by Crist et al. (1992) where the adsorption in seawater is about half that of deionized water. The difference may be due to competition of sites on the algae for metals such as Mg\(^{2+}\) and Ca\(^{2+}\).

### 3.4 Competition with other metals

Despite the fact that a single toxic metabolic species rarely exists in natural seawater, and that the presence of a multiplicity of metal ions often gives rise to interactive effects, insufficient attention seems to have been paid to this problem. The interactive effects of a mixture of metals on an aquatic organism is extremely complex. We have demonstrated that metal uptake can be affected by environmental conditions such as pH, temperature and salinity. However, the expression of the effect on algae also depends on species of algae, metal combination, and levels of metal concentration. To examine the effect of competing metals we have focused on the effect of Cu(II) (at the same concentration) on the adsorption of the toxic metal
Pb$^{2+}$. Cu$^{2+}$ has been described as both a toxic and nutrient trace metal for algae, depending on its concentration (Sunda, 1988/1989; Bruland et al., 1991). The addition of copper has been found to increase both, the labile and total dissolved lead in the solution, and as a direct consequence, a decrease in the adsorbed and complexed amount of lead. This effect is observed both at low and high concentrations showing that the highest and lowest affinity surface ligands of the algal surface are rapidly occupied by the stronger adsorbed Cu$^{2+}$ and a lower concentration of Pb$^{2+}$ is adsorbed. Using the transformed Langmuir equation (Eq. 10) a value of $7.57 \pm 0.85 \times 10^{-15}$ mol cell$^{-1}$ for the adsorption capacity, $\Gamma_{\text{max}}$, and a value of $7.99 \pm 0.25$ for $\log K^S$ are obtained. A quantity of $15 \pm 2 \times 10^{-15}$ mol cell$^{-1}$ less Pb$^{2+}$ is adsorbed in this case, being replaced by Cu$^{2+}$. The lack of changes in the conditional constant suggests that the sites occupied by Pb(II) are the same.

The uptake of lead and copper in a two-metal system shows antagonistic behaviour in the adsorption of lead due to a competition for adsorption sites on the cells walls. However, we have found (Gonzalez-Davila et al., 1994) that lead ions appeared to have no effect on the uptake of copper. A similar behaviour has been found by Ting et al. (1991) for cadmium and zinc on Chlorella vulgaris. Zinc and copper are essential elements which play an important role in many enzyme systems and they are universally required by all algae (Sunda, 1988/1989). Cadmium has recently been found to substitute for Zn in Zn-starved phytoplankton (Price and Morel, 1990). In contrast, lead is toxic non-essential element that serves no known biological function. Thus, the uptake of lead is considerably lower than the uptake of copper under the same environmental conditions.

4. Conclusions

It has been demonstrated that lead can be adsorbed by the alga Dunaliella tertiolecta and complexed by the exudates excreted by the algae.
The adsorption of Pb\(^{2+}\) on the surface groups of the algae in seawater can be correlated with a surface complex formation equilibria which considers the existence of two kinds of sites, high affinity and low affinity sites. The ligands produced and excreted by the algae compete with the surface groups for lead ions. The complexing constant for Pb\(^{2+}\) with the exudates is always higher than the adsorption constants. Variation in factors such as pH, temperature and salinity have additional (positive or negative) effects on the adsorption and complexation of lead. When Cu\(^{2+}\), which forms more stable complexes with the algal surface groups, is present, it is preferentially adsorbed, leaving most of the Pb\(^{2+}\) in the solution phase.

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