Copper adsorption in diatom cultures

Melchor González-Dávila*, J. Magdalena Santana-Casiano, Luis M. Laglera

Facultad de Ciencias del Mar, Departamento de Química, Universidad de Las Palmas de Gran Canaria,
Las Palmas de Gran Canaria 35017, Spain

Received 15 May 1999; received in revised form 14 December 1999; accepted 15 December 1999

Abstract

The organic ligands naturally present in seawater, on the cell surface groups, and those released by the marine phytoplankton species, *Thalassiosira weissflogii* and *Phaeodactylum tricornutum*, and their physico-chemical interaction with copper ions were studied using differential pulse anodic stripping voltammetry DPASV as a function of pH, temperature, salinity and biomass. The acid–base properties were characterized from titration curves of diatom suspensions with proton. Three \( pK_a \) values were determined for each diatom, all of which were similar. Titration curves with copper allowed us to determine the specific adsorption of copper in a heterogeneous adsorption model. An iterative method that combines both Scatchard and Van den Berg–Ruzic approaches was used in order to determine the complexing capacity and the binding constants. High-affinity surface groups of both algae have similar affinity for copper but the concentration of these groups is 45% per cell higher for *P. tricornutum* as compared to *T. weissflogii*. The low-affinity groups in *T. weissflogii* (9.37) have higher stability constants than those in the *P. tricornutum* (9.0). After 36 h equilibrium, a ligand concentration of \( 18.6 \times 10^{-16} \) M cell\(^{-1}\) *P. tricornutum* and \( 25.0 \times 10^{-16} \) M cell\(^{-1}\) *T. weissflogii* with a conditional stability constant of log \( K' \) = 9.8 and log \( K' \) = 10.0, respectively, was exuded into the original seawater. In the presence of lead, high specificity was observed for the lowest stability constant ligands for copper, while the highest stability constant ligands were affected more by the presence of lead in solution. The ligands of *T. weissflogii* were less affected.

Keywords: adsorption; complexation; copper; diatoms; seawater

1. Introduction

Biological surfaces contain various functional groups, e.g., amino acid, carboxylic, hydroxy carboxylic and hydroxy groups, which may interact with H\(^+\) ions and metal ions. The distribution of chemical species between the solid and liquid phases is of particular interest to aquatic and environmental chemists because, to a large extent, particulate and colloidal matter regulate metal ion concentrations in natural media. Biological surfaces, especially biogenic organic particles (phytoplankton and biological debris) in transporting and settling material, play a major role in the ocean by binding heavy metals and transferring them through the water column and to the sediments, thereby regulating the concentration of dissolved metal ions (Sunda, 1990; Bruland et al., 1991; González-Dávila, 1995; Volesky and Holan, 2000).
1995). As a whole, the chemical properties of heavy metals with respect to their interaction with suspended particles, sediments and aquatic organisms are strongly affected by the chemical forms in which dissolved metals exist in seawater (Brand et al., 1983; Sunda, 1990; Knauer et al., 1997). Although we are beginning to understand more about the organic complexation of some metals, especially copper (Van den Berg, 1984; Kramer, 1986; Coale and Bruland, 1988), more studies are needed to understand the toxicity, bioavailability, geochemical reactivity and the organic interactions in natural media.

In this work, we have examined the binding of copper to the surface and to the ligands produced by living algae, specifically to understand the significance of both processes in seawater. Two diatoms were used in our studies: 

\textit{Thalassiosira weissflogii} that appears in estuarine areas, and 

\textit{Phaeodactylum tricornutum} that appears in intertidal areas. Anodic stripping voltammetry (ASV) with blank subtraction was used to measure equilibrium partitioning between surfaces and solutions to determine surface complex formation equilibrium constants (Goncálves et al., 1987; Gonzalez-Davila et al., 1995). In order to get a better understanding of the adsorption process and the effect of dissolved extracellular ligands, the effect of pH, temperature, salinity, algal biomass and the presence of lead (a highly toxic element that has been found to be complexed with organic ligands; Capodaglio et al., 1990) have been studied.

2. Experimental

Cultures of the diatoms \textit{P. tricornutum} ccm 631 and \textit{T. weissflogii} ccm 1051 were obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton, Bigelow laboratory for Oceanic Sciences, ME. They were cultured in continuous culture using a standard nutrient medium for 2 (Guillard and Ryther, 1962). Natural seawater (S = 36.78) used for the preparation of the medium and for most of the studies was collected northwest of Gran Canaria island (The Canary Islands) at the European Station for Time Series in the Ocean, Canary Islands (ESTOC) and also 1 mile off the coast from \sim 10 \text{ m depth}. Axenic cultures were maintained at 292 \pm 1 \text{ K}, under 12-h light and 27 \text{ \mu mol of photon m}^{-2} \text{ s}^{-1} (\text{ photon flux density}) in a growth chamber. All chemicals were of reagent-grade or the highest obtainable grade.

The culture medium of algae in stationary phase was harvested and centrifuged at 4000 rpm for 15 min, then washed four times with 0.45 \text{ \mu m} filtered natural seawater and resuspended in fresh seawater. The sample was then stored in the refrigerator until used, always within 24 h. The concentration was determined previous to the study by optical counting with a hemacytometer and, where necessary, algae dry weights were determined by filtering the algal suspension through 0.45 \text{ \mu m} cellulose nitrate membrane filters (Millipore acid-washed filters) and drying at 110\text{\degree}C. The final cell concentration in our studies was kept at about 1 to 3 \times 10^7 \text{ cells l}^{-1}.

2.1. Acid–base titrations

The algae from the culture media were centrifuged at 4000 rpm for 15 min in 100 ml polypropylene beakers. Dead cells (formaldehyde-treated) were washed four times with a sterile 0.7 \text{ M} NaCl solution to remove exudates and debris. Finally, the algae were resuspended in 25 ml of deaerated 0.7 \text{ M} NaCl. The pH was measured in a titration system similar to that used in our earlier studies (González-Dávila et al., 1995). Algae concentrations were 4 \text{ g dry weight l}^{-1}. The total proton exchange capacity was determined by titrating the algae with an excess of 0.1 \text{ M} HCl.

2.2. Complexation of copper

The cultured algae were harvested by centrifugation, washed, and resuspended in a 0.45 \text{ \mu m} filtered seawater. For each sample, a short reaction time (15 min) determined by kinetic measurements was used for the adsorption studies. All the samples were equilibrated at a given pH, temperature and salinity being studied. The cell solution was then filtered through 3 \text{ \mu m} (HA Millipore acid-washed) filters, and split into two fractions. The first fraction was acidified with concentrated HCl to pH 2 and microwave-treated (CEM-MDS-81D; 630 W, 30 min) to determine the total dissolved copper. The second fraction was used directly in order to determine the
labile copper (Gonçalves et al., 1987). Copper bound to the surfaces of algae was calculated from the difference between total added copper (including that initially present in the seawater) and total dissolved copper. Organically complexed dissolved copper in the adsorption studies was calculated by the difference between the total dissolved and the labile copper. The concentration of copper in the samples was determined by using differential pulse anodic stripping voltammetry DPASV. In voltammetric analysis, it must be assumed that organic ligands with a weak affinity for the metal are included in the labile fraction (Coale and Bruland, 1988; González-Dávila et al., 1995). Those ligands of very strong metal affinity that are fully titrated initially do not equilibrate with the added metal and have no contribution to the estimation of the metal affinity of the organic complexes in solution. This concept is known as the analytical window and refers to the metal affinity range of the measurement (Van den Berg and Donat, 1992; Van den Berg et al., 1990).

Measurements were performed using PAR 303 static drop mercury electrode with the PAR model 348B polarographic analyzer system connected to a computer. The reduction potential was −0.6 V, the rate was 2 mV s⁻¹, the pulse height was 50 mV, and the deposition time was 10 min. The sensitivity of the method determined in acidic media was 0.8 nA nM⁻¹ min⁻¹ (Gonçalves et al., 1987).

For the seawater complexation studies in the presence of algae, a cell solution was left in equilibrium for 36 h, then filtered using 3 μm filters, and finally, trace metals were added to the solution, whereafter, it was left to equilibrate overnight.

3. Results and discussion

Fig. 1 shows the acid–base titration curves for the diatoms, *P. tricornutum* and *T. weissflogii*, in 0.7 m NaCl solution. The shape of the titration curves indicates that the surfaces contain a large variety of weaker acid–base groups. The model used for the description of oxide surfaces (Stumm and Morgan, 1981; Keifer et al., 1997), was applied between pH = 3 and pH = 10 to evaluate the data. The intrinsic acidity constants pK⁺₁, pK⁺₂ and pK⁺₃ were obtained from the plot of pK vs. surface charge Q.

Values of pK⁺₁ = 9.12 ± 0.02, pK⁺₂ = 6.68 ± 0.03 and pK⁺₃ = 3.77 ± 0.02 were obtained as the result of different titrations of *P. tricornutum*. Values of pK⁺₁ = 9.14 ± 0.02, pK⁺₂ = 6.72 ± 0.03 and pK⁺₃ = 3.96 ± 0.02 were determined for *T. weissflogii*. The pK⁺ values under 4 are related to terminal carboxyl groups in proteins, while the pK⁺ values around 9 are related to α-amino groups of asparagine, glycine, alanine, leucine (pK between 8 and 9.5). The pK⁺ values around 6.7 can be related to the presence of primary amino groups, probably due to the presence of chitin (pK⁺₂ = 6.8; Gonzalez-Dávila and Millero, 1990).

The binding of trace metals on the cell surface groups is the first step necessary for the uptake of metals into the cell. Fig. 2 shows the uptake kinetics of 7.02 × 10⁻⁸ M Cu(II) on 2.03 × 10⁷ cells l⁻¹ *P. tricornutum*, including the study of the behavior of labile and complexed Cu(II) in solution. For *P. tricornutum* and *T. weissflogii* (data not shown), the inorganic dissolved copper sharply decreases during the first 15 min until its concentration reaches less than 2.5 and 5 nM, respectively, for both algae. After this first step, both the uptake and labile Cu(II) concentrations decrease linearly with the square root of time (inset in Fig 1), a characteristic result of a diffusion-controlled process (Crank, 1976). The uptake of Cu(II) for both algae is quite similar (50% of the initial value). Organically complexed dissolved
copper is around 45–50% of the initial value. Only 1% of the initial Cu(II) for *P. tricornutum* and 5% for *T. weissflogii* is present as inorganic dissolved copper, which corresponds to a pCu of 10.2 and 9.84, respectively. We can observe from Fig. 2 that inorganically complexed copper is taken up by algae, while the organically complexed dissolved copper did not change.

When phytoplankton cells are in equilibrium with seawater, copper binding on the cell surface groups takes place. The copper also binds with organic ligands present not only in the solution but also excreted or exuded by the cells. Metal complexing ligand concentrations and conditional stability constants were calculated by titration using the methods of Van den Berg and Kramer (1979) and Ruzic (1982). If the transformation results are linear, it is assumed that the natural chelators are within a single class of ligands, with one representative conditional stability constant. When the analytical technique is used over a wide spectral window, a linearizing plot as proposed by Scatchard or Van den Berg–Ruzic yields separate linear regions. This indicates that more than one type of ligands can be estimated. The most usual model is to consider two kinds of binding sites. This model provides a good fit of the result data in spite of the simplistic assumption on which it is based. It is characterized by a low concentration of ligands with a high conditional stability constant ([L₁] and *K*¹*cond*) and a higher concentration of ligands with a lower binding strength ([L₂] and *K*²*cond*).

Due to the well-known heterogeneity of the complexing material, the estimation of log *K*’ is an averaged value of the stability constant of natural ligands inside the analytical window. The modeled values of *K*’ and total ligand concentration obtained are not independent of each other for the same type of ligand and dependent on the detection window associated with the method used. Another aspect to be considered is the different metal ion loading conditions present in the different studies that can produce a gradual change of log *K*’ (Buffe et al., 1990). In our work, we will present and compare complexing data obtained under different metal ion loadings. These differences were always lower than one order of magnitude, and, consequently, the error associated to the estimation of log *K*’ is lower than the analytical error.

Values for the complexing parameters in a single ligand model are estimated through the use of a nonlinear fit of the Langmuir isotherm, similar to the one proposed by Gerringa et al. (1995). In order to determine the concentrations of the separate ligand classes and their respective conditional stability constants, a new iterative method has been developed. It allows one to determine independently the complexing capacity of both class of ligands when the two-site model is employed (Laglera et al., submitted). This method is characterized by using the Scatchard plot for the determination of L₁ and the Van den Berg and Ruzic plot for L₂. In our case, the total ligand

Table 1

<table>
<thead>
<tr>
<th></th>
<th><em>C</em>₀ (nM)</th>
<th>log <em>K</em>_{Cu,L₁}</th>
<th>log <em>K</em>_{Cu,L₂}</th>
<th>Alga</th>
<th><em>C</em>₀ (nM)</th>
<th>log <em>K</em>_{Cu,L₁}</th>
<th>log <em>K</em>_{Cu,L₂}</th>
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<tbody>
<tr>
<td>107 ± 2</td>
<td>7.48 ± 0.02</td>
<td>8.89 ± 0.02</td>
<td></td>
<td><em>P. tricornutum</em></td>
<td>146 ± 6</td>
<td>7.97 ± 0.04</td>
<td>9.38 ± 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>T. weissflogii</em></td>
<td>162 ± 2</td>
<td>8.16 ± 0.01</td>
<td>9.57 ± 0.01</td>
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</table>
concentration $C_1$ (nM) and conditional stability constant $K_{\text{cond}}^{\text{Cu}^{2+}}$ are shown in Table 1 for the original seawater and after both algae were left during 36 h in equilibrium. An inorganic side reaction coefficient ($\alpha$) for Cu(II) of 25.7 at pH = 8.02 (Millero and Hawke, 1992; Santana-Casiano et al., 1995) was used. It can be clearly observed that the addition of cells increases both the total ligand concentration and the conditional stability constants of seawater, revealing that these algae excrete ligands with higher stability constant than those initially present in the seawater. If the addition of algae increases the ligand concentration by 40 nM for $P$. tricornutum and the constant rises by approximately 0.5 units, the excreted ligands should have a conditional stability constant of 9.8. For the $T$. weissflogii, the constant of the new ligands should be around 10. This organically complexed dissolved copper will compete with the copper adsorbed to the surface groups. In order to characterize the speciation of copper in natural seawater, adsorption on the cell surface groups should be considered.

Fig. 3 shows the adsorption isotherms of two $T$. weissflogii cell concentrations in seawater at pH$_t$ = 8.02. The complexation of copper with the algal surfaces was interpreted using the Langmuir equation at constant pH, assuming that the adsorption of Cu occurs in two types of surface groups, within the range of Cu concentration used, considering that charge effects are negligible at constant pH. Fig. 4 presents the Scatchard linearization of the adsorption isotherm for two $P$. tricornutum concentrations, where the presence of at least two types of ligands is observed. The four parameters defining the system have been evaluated by using our iterative method that independently determines the complexing capacity of both types of ligand. The data are presented in Table 2 and the fitting lines in Fig. 4 correspond to the model output. This model removes the problems associated with both the linearization of the Van den Berg–Ruzic plot at low concentrations (negative values may appear at very low concentrations and low statistic weighting for the initial data) and with the linearization of Scatchard plot at high concentrations (concentration of points due to the experimental errors at high copper concentrations) (Apte et al., 1988; Van den Berg and Donat, 1992; Ruzic, 1996; Miller and Bruland, 1997). The data in Table 2 show that the high-affinity surface groups of both algae present similar affinity for copper, but the concentration of these groups is 45% per cell higher for $P$. tricornutum than for $T$. weissflogii.

The constants we have determined from the titration data are conditional to the pH and seawater composition considered. By using the acid–base titration curves for both diatoms discussed previously and considering the competition between proton and copper for the same binding sites, intrinsic adsorption constants, $K_s^a$ have been computed. They allow us to determine the conditional adsorption constant at other values of pH and are shown in
It also includes the intrinsic adsorption constants, $K_{ads}^s$, valid for any pH value. $pK_{P. tricornutum}$ (pH = 8.02) = 9.11. $pK_{T. weissflogii}$ (pH = 8.02) = 9.30.

Table 2 presents the conditional stability constants determined considering only one kind of site on the cell surface via a nonlinear fit of the titration data. These constants can be used to compare adsorption of copper on cell surface groups and complexation by diatom exudates (see Table 1). As shown above, the complexing capacities of the exudates from both diatoms have higher affinity for copper than the surface groups (four times higher for $P. tricornutum$ and three times for $T. weissflogii$ surface groups).

For a relatively constant algal concentration, the amount of copper that will be sorbed on the cell surface via a nonlinear fit of the titration data. These constants can be used to compare adsorption characteristics in a heterogeneous model for the surface groups of $P. tricornutum$ and $T. weissflogii$ respectively.

Table 2 (González-Dávila et al., 1995). The values for the apparent acidity constants at pH = 8.02 and NaCl 0.7 M, are log $K_{ads}^s$ = 9.11 and log $K_{ads}^p$ = 9.30 for $P. tricornutum$ and $T. weissflogii$, respectively.

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Table 3 Adsorption parameters considering a homogeneous surface, conditional to a pH$_s$ = 8.02. The last two columns show the complexing capacity of the seawater medium after 36 h equilibrium with 2.06 x 10$^7$ cell l$^{-1}$ $P. tricornutum$ and 2.21 x 10$^7$ cell l$^{-1}$ $T. weissflogii$ and the estimated complexing capacity for the exudates produced by these algae.

Table 4 Adsorption characteristics in a heterogeneous model for the surface groups of $P. tricornutum$ and $T. weissflogii$ at pH$_s$ = 8.02. It also includes the intrinsic adsorption constants, $K_{ads}^s$, valid for any pH value. $pK_{P. tricornutum}$ (pH = 8.02) = 9.11. $pK_{T. weissflogii}$ (pH = 8.02) = 9.30.
Table 4
Comparison of Cu(II) complexation parameters in seawater after 36 h equilibrium with $2.06 \times 10^7$ cell l$^{-1}$ P. tricornutum and $2.21 \times 10^7$ cell l$^{-1}$ T. weissflogii in the absence (columns 1 and 2) and in the presence (columns 4 and 5) of 100 nM Pb(II)

<table>
<thead>
<tr>
<th>$C_1$ (nM)</th>
<th>log $K_{Cu}^{2+}$</th>
<th>Alga</th>
<th>$C_1$ (nM)</th>
<th>log $K_{Cu}^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>146 ± 6</td>
<td>9.38 ± 0.04</td>
<td>P. tricornutum</td>
<td>152 ± 6</td>
<td>9.35 ± 0.06</td>
</tr>
<tr>
<td>162 ± 2</td>
<td>9.57 ± 0.01</td>
<td>T. weissflogii</td>
<td>172 ± 6</td>
<td>9.50 ± 0.06</td>
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geneous and heterogeneous models, show that P. tricornutum decreases its adsorptive capacity in the presence of lead. Using the heterogeneous model, the highest affinity ligands reduce their complexing capacity by 35% and the lowest conditional stability constant ligands reduce their concentration by 15%. The lowest stability constant ligands are more selective for copper than the highest ones in the presence of lead. For T. weissflogii, the reduction of the adsorptive capacity in the homogeneous approach is only 10%, whereas in the heterogeneous system, the decrease observed in the adsorptive capacity agrees better with the highest error in the experimental data. Table 5 depicts the high specificity of the lowest stability constant ligands for copper in the presence of lead. The highest stability constant ligands are more affected by the presence of lead |
Table 5
Comparison of Cu(II) adsorption parameters both in a heterogeneous and homogeneous model in the presence of 1.42 × 10^7 cell l^-1 P. tricornutum and 2.12 × 10^7 cell l^-1 T. weissflogii in the absence and in the presence of 100 nM of Pb(II)

<table>
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<tr>
<th>Alga</th>
<th>Heterogeneous model</th>
<th>Homogeneous model</th>
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<tbody>
<tr>
<td></td>
<td>$G_{\text{max},1} \times 10^{16}$</td>
<td>$K_{\text{H},1}$</td>
</tr>
<tr>
<td>$P. \text{tricornutum}$</td>
<td>−Pb 1.2 ± 0.6</td>
<td>10.75 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>+Pb 0.8 ± 0.2</td>
<td>10.52 ± 0.2</td>
</tr>
<tr>
<td>$T. \text{weissflogii}$</td>
<td>−Pb 2.7 ± 0.3</td>
<td>10.75 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>+Pb 1.6 ± 2.0</td>
<td>10.89 ± 0.6</td>
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</table>

of copper bound to cell surface groups as salinity decreases. The stability constants determined for the different salinity studies allow us to determine the linear regressions between both parameters for the two algae:

\[
\log K_{\text{H}}^{\text{cond}} = 10.164 - 0.165\sqrt{S};
\]

\[
r^2 = 0.993; \quad P. \text{tricornutum},
\]

\[
\log K_{\text{H}}^{\text{cond}} = 10.607 - 0.175\sqrt{S};
\]

\[
r^2 = 0.999; \quad T. \text{weissflogii}.
\]

When the experiments are carried out at different temperatures from 6°C to 45°C in seawater for $P. \text{tricornutum}$ (Fig. 7), we found increased adsorption and a decrease in inorganic complexed copper at higher temperatures. The organically complexed copper is not affected. This finding, together with the kinetic studies (Fig. 1), suggests that inorganic copper alone is adsorbed to the surface cell. It is possible that complexed copper increases with temperature. However, we do not observe this behavior in our experiments probably due to the greater amount of ligands being excreted by algae, due to the temperature stress. For $T. \text{weissflogii}$, an increase in temperature produces a decrease in both the labile and adsorbed concentration, whereas the complexed and total dissolved copper concentration increases. In this case, complexation is promoted by increasing temperature, which reduces the inorganic copper together with the copper previously adsorbed. The specific adsorption energy, $E$, determined according to González-Dávila et al. (1995), gives $6.69 \pm 0.28$ kJ mol$^{-1}$ for $P. \text{tricornutum}$ and $-4.25 \pm 0.17$ kJ mol$^{-1}$ for $T. \text{weissflogii}$. The positive specific adsorption energy of Cu$^{2+}$ may be interpreted as the heat of hydration of Cu(II), which is higher than its heat of adsorption.

Fig. 6. Effect of pH on the Cu(II) speciation in the presence of $1.19 \times 10^{7}$ cell l$^{-1}$ $T. \text{weissflogii}$ (total Cu(II) concentration, $6.27 \times 10^{-8}$ M).

Fig. 7. Effect of temperature on the Cu(II) speciation in the presence of $1.4 \times 10^{7}$ cell l$^{-1}$ $P. \text{tricornutum}$ in seawater (total Cu(II) concentration, $6.98 \times 10^{-8}$ M).
4. Conclusions

ASV has been shown to be a useful method when determining adsorption properties on biological surfaces. Heterogeneous surfaces with ligand concentrations of 2–8 nM for the highest stability constants ligands (log $K' = 10.7 \pm 0.1$) and 20–30 nM for the lowest stability constant ligands (log $K' = 9.1 \pm 0.2$) have been established for Cu on both diatoms. The values of the adsorptive capacities for T. weissflogii are the highest. However, the determination of the seawater complexing capacity by ASV only allows us to use a homogeneous model, even if more than one type of ligand is present. Accordingly, after 36 h equilibrium, a ligand concentration of $18.6 \times 10^{-16}$ M cell$^{-1}$ P. tricornutum and $25.0 \times 10^{-16}$ M cell$^{-1}$ T. weissflogii (conditional stability constant of log $K' = 9.8$ and log $K' = 10.0$, respectively) is exuded into the original seawater. The exuded compounds have more affinity for copper than the cell surface groups, when the inorganic concentrations are kept at levels below toxic values (Brand et al., 1983). The excretion of organic ligands with different properties from the surface groups can also be affected by the pH. The maximum complexation is reached around 1 pH unit lower than in adsorption.

When the lead concentration of twice the maximum adsorptive capacity of the surface groups is added, high-affinity ligands on cell surface groups for copper in both algae are found to be less specific than low-affinity ligands. The specificity of the T. weissflogii surface ligands that reveal affinity for copper is higher than that shown for P. tricornutum. We are unable to define the exact nature of this type of complexing ligands. However, the results underline the importance of complexation with organic ligands for elements such as copper, which are involved in naturally occurring biological processes.

Acknowledgements

We gratefully acknowledge the critical and constructive criticisms offered to us by the anonymous reviewers. We would like to thank Prof. Frank J. Millero for the review of the manuscript and valuable suggestions.

References


