Toxicological and Environmental Chemistry, Vol. 73, pp. 191–206
Reprints available directly from the publisher
Photocopying permitted by license only

BINDING OF LEAD TO *P. TRICORNUTUM* AND *T. WEISSFLOGII* CULTURES IN SEAWATER

J. MAGDALENA SANTANA-CASIANO*,
MELCHOR GONZALEZ-DAVILA, LUIS M. LAGLERA
and JESUS PEREZ-PEÑA

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

Titrations using differential pulse anodic stripping voltammetry (DPASV) to detect electroactive lead were carried out in *Phaeodactylum tricornutum* and *Thalassiosira weissflogii* seawater cultures to determine the extent of lead complexation with both dissolved ligands and cell surface groups. Adsorption and complexation parameters in a heterogeneous model were determined as a function of pH, temperature, and salinity and cell concentration by using a new iterative method. Lead forms high stable complexes in seawater and organically complexed lead fraction constitutes a significant portion of the total lead. Higher affinity for lead is observed by the surface groups of *T. weissflogii* in respect to *P. tricornutum* showing that the functional groups of *T. weissflogii* are more specific in the presence of 100 nM Cu(II) in solution.

Keywords: Adsorption; complexation; lead; diatoms; cultures; seawater

INTRODUCTION

Trace metals tend to be complexed by organic and inorganic ligands in natural waters and seawater. The inorganic speciation of heavy metals such as lead has been studied extensively, although the results are at times conflicting [1,2]. Organic complexation of some metals, like copper and zinc, has been

* Corresponding author.

extensively studied in seawater [3–6] while, relatively little is known about lead. Lead is a toxic element with a residence time of ~ 2 years in surface waters where the largest biological activity is concentrated. Capodaglio *et al.* [7] found that the organically complexed lead fraction constitutes a significant portion of the total (50%) in surface waters of the Eastern North Pacific. A high specificity was observed for the natural ligands presented in the seawater for lead with no apparent explanation due to the fact that lead is not involved in naturally occurring biological processes. Wells *et al.* [8] also found that organic metal complexing ligands significantly dominated the dissolved speciation of lead (67–94%) in Narragansett Bay, influenced by multiple classes of metal binding ligands as well as in south San Francisco Bay [9]. A marine chlorophyte, *Dunaliella tertiolecta* also has been shown to release extracellular ligands that bind Pb, Cu and Cd [10,11]. The conditional stability constants of these chlorophyte exudates determined by DPASV are 1–3 order of magnitude lower than those measured in Narragansett Bay. These findings are consistent with suggestions that microorganisms actively condition seawater to facilitate optimal metal conditions for growth [12]. It has been also pointed out that some soluble metal ligand complexes in coastal seawaters are a by-product of intracellular metal detoxification mechanisms [13,14]. Cell surface groups also act as important metal binding ligands fraction at the cell concentration generally used in culture [15,16]. Phytoplankton cells present in these systems can provide relatively large surface areas with high affinity for metal binding.

In this study we investigated the role of the two diatoms *P. tricornutum* and *T. weissflogii* in the lead chemistry in seawater cultures, not only by surface reactions, but also by metal uptake and by production of extracellular organic matter with metal complexing properties. For a more complete understanding of the binding process, the studies were also carried out in the presence of copper (100 nM) and at varying temperatures, pH and salinity of the seawater solution.

CONCEPT AND TERMS

The complexation equilibrium between lead ion and a class of natural organic ligands, both in the surface or in solution, $[S^{n-}]$ or $[L^{n-}]$, respectively, with a stoichiometric ratio 1 : 1, can be defined by using a thermodynamic equilibrium constant $K = [PbL^{2-n}]/([Pb^{2+}][L^{n-}])$. Thermodynamic constants can be transformed to estequiometric constants by the use of activity coefficients ($K' = K/(\gamma_{PbL}/(\gamma_{Pb}\gamma_{L}))$) and by considering side-reactions of lead and

organic ligands with inorganic species. In a seawater media with concentration of Ca^{2+} and Mg^{2+} as high as 0.064 M in both ions [17], complex formation with these ions takes place and competition for the trace metal binding sites are expected. However, as other authors have found [15] and as we will show in this study, complex formation with Ca^{2+} and Mg^{2+} is not so extensive as that with trace metals. High concentrations of those metals representative of those encountered in natural seawater, competitively represses surface complex formation with traces of metals. According to Gamble *et al.* [18] the alkaline metal ions are not adsorbed by the biomass. This group of metal ions does not form complexes with most ligands [19]. The free lead concentration can be expressed as a function of the concentration of dissolved lead present in all the inorganic forms [Pb′] through $[Pb^{2+}] = [Pb']/\alpha_{Pb}$. The total ligand concentration present not bound with lead is, thus, $[L'^{n-}] = [L']/\alpha_{L'}$, where α_{Pb} and $\alpha_{L'}$ are the corresponding side-reaction coefficients [20]. For the experimental condition used in our study, the complexation equilibrium can be expressed as a conditional stability constant, K'_{cond} related to K' by side-reaction coefficients: $K'_{cond} = [PbL]/([Pb'][L']) = K'/\alpha_{Pb'}\alpha_{L'}$. With differential pulse anodic stripping voltammetry (DPASV), [Pb′] is the inorganic lead fraction detected, [PbL] is calculated by the difference between the total Pb and [Pb′], and K'_{cond} is calculated. However, as α_L, for natural ligand is not known, the K'_{cond} cannot be converted to a thermodynamic stability constant; however K'_{cond} and [L′] for the Pb–ligand interaction in seawater allow an estimation of the extent of lead complexation with organic ligands.

Metal complexing ligand concentrations ($[L_T]$ for dissolved and Γ_{max} for surface ligands) and conditional stability constants were calculated from the titration data after transformation using both Langmuir and Scatchard linearization methods [21]. If the Langmuir transformation ([Pb′]/[PbL] against [Pb′]) results are linear it is assumed that the natural chelators are within a class of ligands having one representative conditional stability constant. The y-intercept of these linearized data gives the reciprocal product of K'_{cond} and $[L_T]$, while the reciprocal slope of the line gives $[L_T]$. These values are verified with Scatchard linearizations, where the ratio of [MPb]/[Pb′] is plotted against [MPb]; the y-intercept is K'_{cond} $[L_T]$ and the x-intercept is $[L_T]$.

Distinctively separate linear regions within a transformation curve indicate that more than one class of ligand exists. Generally, two kinds of ligands can explain the observed experimental data: a low concentration of ligands $[L_1]$ having a high conditional stability constant $K'_{cond,1}$ and a higher concentration of ligands $[L_2]$ with a lower binding strength, $K'_{cond,2}$. These four parameters can be evaluated through the Scatchard linearization [22] or through the van den Berg–Ruzic linearization, which is equivalent to the

Langmuir transformation. However, assessing values for $[L_1]$ and $K'_{cond,1}$ through van den Berg–Ruzic plot may be not accurate due to the high curvature in the corresponding region of the plot (iterative method may result in negative y-intercept). The same can be described for the assessing of $[L_2]$ and $K'_{cond,2}$ values through the Scatchard linearization due to error in the determination on the metal complexed at high metal additions. We have developed an iterative method, which determines the four parameters in two kinds of ligands model, by using the Scatchard method for the high affinity ligands and the van den Berg–Ruzic method for the low affinity ligands. The method also computes the complexation parameters (both on the surface and on the dissolved ligands) taking into account the contribution of each ligand on the determination of the other [23].

EXPERIMENTAL

Natural seawater ($S = 36.78$) used for preparation of the medium and for most of the studies was collected northwest of Gran Canaria (The Canary Islands) at the European Station for Time Series in the Ocean, Canary Islands (ESTOC) and also 1 mile off the coast from ~ 10 m depth. Cultures of the diatoms *Phaeodactylum tricornutum* ccmp 631 and *Thalassiosira weissflogii* ccmp 1051 were obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton, Bigelow laboratory for Oceanic Sciences, ME. They were cultured in a continuous culture using a standard nutrient medium f/2 [24]. Axenic cultures were maintained at 292 ± 1 K, under 12-h light and 27 μmol of photon $m^2 s^{-1}$ (photon flux density) in a growth chamber. All chemicals were reagent grade or the highest obtainable grade.

The alga in stationary phase were harvested and centrifuged at 4000 rpm for 15 min, then washed four times with 0.45 μm filtered natural seawater and resuspended in fresh seawater. Cell concentration was determined previously to the study by being optically counted with a hemocytometer. Final cell concentration in our studies was kept in $1-3 \times 10^7$ cells L^{-1} range.

Complexation of Lead

The cultured alga were harvested by centrifugation, washed, and resuspended in a 0.45 μm filtered seawater. For each data point, a short reaction time (10 min) determined by kinetic study was used for the adsorption studies. All the samples were equilibrated at a given pH, temperature and salinity studied. The cell solution was then filtered by 3 μm (HA Millipore acid-washed) filters, and split into two fractions. The first fraction was acidified with concentrated

HCl until pH 2 and microwave treated (CEM-MDS-81D; 630 W, 30 min) in order to determine the total dissolved lead. The second fraction was used directly in order to determine the labile lead [15]. Lead bound to the surfaces of algae was calculated from the difference between total added lead (including that initially present in the seawater) and total dissolved lead. Organically complexed dissolved lead in the adsorption studies, was calculated by the difference between total dissolved and labile lead. The concentration of lead in the samples was determined by using DPASV. Measurements were performed with the PAR 303 static drop mercury electrode using the PAR model 348B polarographic analyser system connected to a computer. The reduction potential was −0.8 V, the rate was 2 mV/s, the pulse height was 50 mV and the deposition time was 10 min. The sensitivity of the method was 4.9 nA nM^{-1} min^{-1}.

For the seawater complexation studies in the presence of algae, a cell solution was allowed to be equilibrated during 36 h, filtered by 3-μm filters and then, trace metals were added to the solution and allowed to equilibrate overnight.

RESULTS AND DISCUSSION

Figure 1 shows a plot of concentration changes for labile, complexed and sorbed lead (initial concentration 5×10^{-8} M) as a function of reaction time in the presence of 1.64×10^7 cell L^{-1} *P. tricornutum*. The labile Pb(II) initially decreases rapidly for a few minutes; pseudoequilibrium with the algal

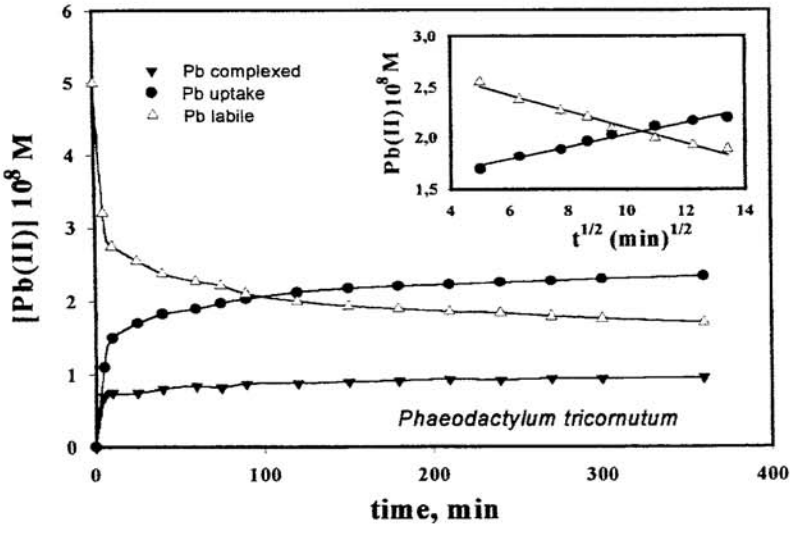

FIGURE 1 Uptake kinetics of Pb(II) (50 nM) on 1.64×10^7 cell L^{-1} *P. tricornutum*. Inset: Pb(II) labile and uptake during the second step as a function of the square root of reaction time.

surface seems to be attained. Subsequently, its concentration decreases more slowly. Before penetrating the cell membrane, the metal cations as well as any nutrient are first transferred to and through an inert structure, the cell wall. After the first rapid step, where the metal becomes surface co-ordinated to the algal surface groups, both, the labile and sorbed Pb(II) concentration varies with the square root of time (Figure 1, inset). This secondary slower uptake is a diffusion controlled process, most likely the diffusion into the inside of the cell. After 10 min, the uptake conforms the parabolic diffusional model [25] $[Pb(II)]_{sorbed} = 1.43(0.04)10^{-8} + 0.061(0.003)10^{-8}\sqrt{t}$ and $[Pb(II)]_{sorbed} = 1.55(0.02)10^{-8} + 0.052(0.003)10^{-8}\sqrt{t}$ for *P. tricornutum* and *T. weissfloggi*, respectively. After 6 h equilibrium time, 20% more lead is assimilated by the *T. weissflogii* as compared to *P. tricornutum*. Figure 1 (inset) also shows for *P. tricornutum* (and applicable to *T. weissflogii*) that the uptake of lead comes from inorganic labile lead, which also follows a parabolic dependence with square root of time, which is consistent with the observation that metal toxicity is related to changes in the free metal ion activities [26]. The slight increase in the amount of organically complexed lead with time is in accordance with the results of Capodaglio *et al.* [7] who showed that the association of lead with ligands is not as fast as observed for copper.

The time required for the adsorption equilibrium of lead between the solution and the surface has been determined as the intercept of both lines, and kept in all our studies at 10 min. In order to assess quantitatively the binding of heavy metal ions to cell surfaces, suspensions of microorganisms were titrated either with increments of lead at the natural pH of seawater ($pH_t = 8.02$), or at given metal concentration with acid. However, the presence of lead in a biological system has shown [7] high affinity for complexing ligands that competes with lead sorbed on the surface cell. In such a way, prior to the study of binding capacities to the cell surface, studies were carried out in order to define the lead complexing capacity of natural seawater samples and seawater with algal exudates. Figure 2 clearly shows that the addition of algae changes the complexing capacity of natural seawater, plotted according to the van den Berg–Ruzic plot. By using a value of $\alpha = 0.028$ to normalize the conditional stability constants with respect to free metal concentration [27], Table I shows the complexing characteristics of the Canary Islands seawater. A ligand concentration of 23 nM is found with a stability constant of 8.89, slightly lower than those found in the North Pacific Ocean [7], but similar to others found in previous studies [11]. When phytoplankton cells in exponential phase are added and kept in equilibrium for 36 h, an increase in the organic ligands initially present in the seawater is

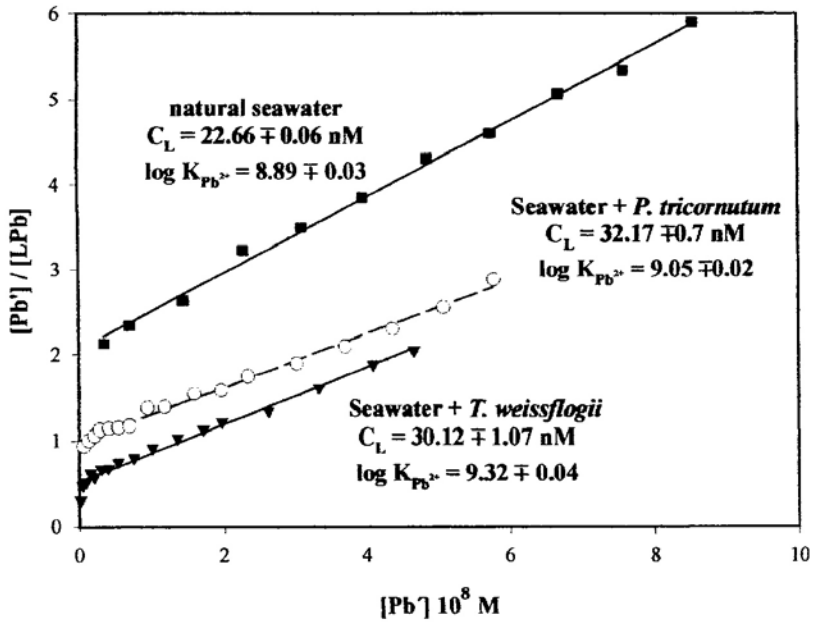

FIGURE 2 Seawater complexation characteristics after 36 h equilibrium with 1.34×10^7 cell L^{-1} *P. tricornutum* and 1.32×10^7 cell L^{-1} *T. weissflogii.*

TABLE I Complexation parameters for natural seawater and after 36-h equilibrium period with 1.34×10^7 cell L^{-1} *P. Tricornutum* and 1.32×10^7 cell L^{-1} *T. Weissflogii* ($\alpha_{Pb} = 35$)

Alga	*Natural seawater*			*Seawater after 36-h equilibrium*		
	C_L (nM)	log $K_{Pb(II)}$	log K_{Pb}^{2+}	C_L (nM)	log $K_{Pb(II)}$	log K_{Pb}^{2+}
P. tricornutum	22.7 ± 0.06	7.34 ± 0.03	8.89 ± 0.03	32.7 ± 0.7	7.50 ± 0.02	9.05 ± 0.02
T. weissflogii				30.1 ± 0.7	7.77 ± 0.04	9.32 ± 0.04

observed which produces an increase in the conditional stability constant. Table I shows that organic ligands in solution increase until 32 nM while the stability constant achieves 9.32 for *T. weissflogii.* If we consider the increase in both parameters, an estimation of the stability constant for the exudates of both alga can be carried out resulting in a log $K'_{PbL} = 9.3$ and 9.8 for *P. tricornutum* and *T. weissflogii*, respectively. It must be kept in mind that this simple treatment of metal–organic interaction only provides an average constant because a variety of ligands exist and because the different methods have different detection windows [28]. The experimental data allows us to apply the two kinds of ligands model to the *T. weissflogii* exudate suspension (36-h pre-equilibrium), resulting in $[L_1] = 0.9 \pm 0.9$ nM ($K_1 = 10.90 \pm 0.3$); $[L_2] = 33.6 \pm 1.3$ nM ($K'_2 = 9.17 \pm 0.03$). These results clearly show that lead forms high stable complexes in seawater and that organically complexed lead fraction contributes to a significant portion of the total and must be considered in speciation models for lead in surface seawater.

Figure 3 shows the titration curves of suspensions of *T. weissflogii* (two concentrations) with increments of lead and is plotted according to the usual form of Scatchard plot at $pH_t = 8.02$. Two kinds of ligands are clearly found for both alga. The adsorption parameters determined by using the iterative method are shown in Table II. The lines in Figure 3 correspond with the adsorbed lead determined by using the computed parameters, showing the accuracy of the proposed method. Higher affinity for lead is observed by the *T. weissflogii* in respect to *P. tricornutum* showing that the functional groups of *T. weissflogii* are more specific. When we compare the value of the conditional stability constant of the organic dissolved ligands $K'_{cond(Pb^{2+})}$ in the presence of *T. weissflogii* and *P. tricornutum* determined in an

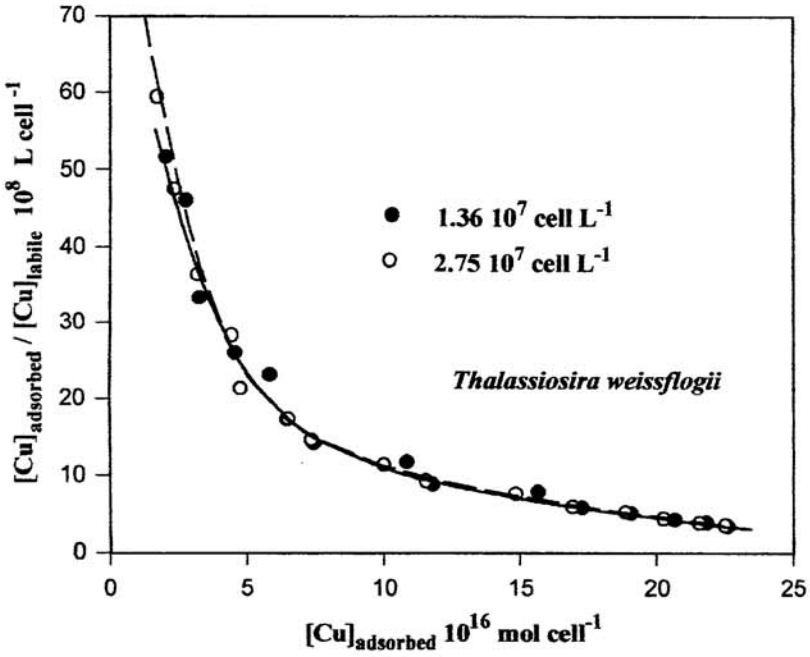

FIGURE 3 Schatchard linearization of the adsorption isotherms for two *T. weissflogii* cell concentrations. The curves correspond with model output.

TABLE II Adsorption characteristics in a heterogeneous model for the surface groups o *P. tricornutum* and *T. weissflogii* at $pH_t = 8.02$. Intrinsic adsorption constants, $K^S_{\beta,i}$, valid for an pH are also included

Alga	*cell* L^{-1} (10^7)	$\Gamma_{max,1}$ (10^{16})	$\log K_{H,1}$	$\log K^S_{\beta,1}$	$\Gamma_{max,2}$ (10^{16})	$\log K_{H,2}$	$\log K^S_{\beta,2}$
P. tricornutum	1.55	3.1 ± 0.2	10.52 ± 0.05	11.64 ± 0.15	22.9 ± 0.5	9.19 ± 0.01	10.31 ± 0.1
	2.67	4.8 ± 0.3	10.37 ± 0.05	11.49 ± 0.17	24.7 ± 0.4	8.94 ± 0.01	10.06 ± 0.1
T. weissflogii	1.36	4.3 ± 0.8	10.76 ± 0.11	12.06 ± 0.25	27.4 ± 3.0	9.04 ± 0.02	10.34 ± 0.1
	2.75	3.8 ± 1.0	10.88 ± 0.07	12.18 ± 0.19	27.4 ± 2.7	9.08 ± 0.03	10.38 ± 0.1

homogeneous model consideration (Table III) (9.32 and 9.05, respectively), with the value of the conditional constants for the algal surfaces (9.53 and 9.42, respectively), it is clearly found that the complexation constants of the algal exudates are lower than the adsorption constants. However, if we compare the constants for the surface with the constants estimated for the exudate (9.8 and 9.3 for *T. weissflogii* and *P. tricornutum*, respectively) we observe that the ligands produced by *T. weissflogii* have more affinity for lead than the surface groups. In Figure 4 we have exemplified the lead speciation in the presence of *T. weissflogii* (2.75 cell L^{-1}) at $pH_t = 8.02$ in Gran Canaria coastal seawater ($S = 36.82$); complexed lead by oceanic seawater is also included for comparison. Due to the high affinity ligands on the surface of the algae, higher concentrations of lead are adsorbed at low concentrations

TABLE III Adsorption parameters considering an homogeneous model. The last two columns show the complexing affinity of seawater after 36-h equilibrium with 1.34×10^7 cell L^{-1} *P. Tricornutum* and 1.32×10^7 cell L^{-1} *T. Weissflogii*

Alga	*cell* L^{-1} (10^7)	Γ_{max} (10^{16}) (mol cell^{-1})	$\log K_H^{'S}$	$\log K_\beta^S$	$\log K'_{PbL}$ (medium)	$\log K'_{PbL}$ (exudates)
P. tricornutum	1.55	24.1 ± 0.5	9.40 ± 0.02	10.52 ± 0.11	9.05 ± 0.04	~9.3
	2.67	25.0 ± 0.8	9.32 ± 0.03	10.44 ± 0.12		
T. weissflogii	1.36	25.7 ± 0.4	9.47 ± 0.03	10.77 ± 0.12	9.32 ± 0.01	~9.8
	2.75	25.9 ± 0.7	9.43 ± 0.02	10.74 ± 0.11		

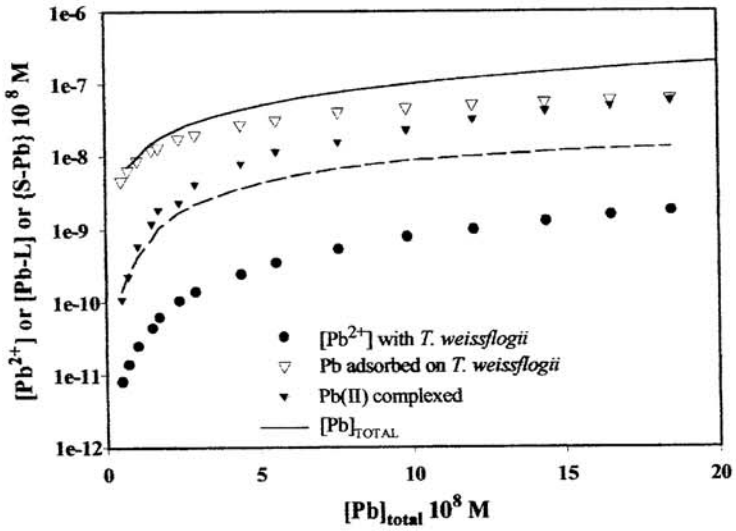

FIGURE 4 Speciation of Pb(II) in coastal Canary Islands seawater in the presence and absence of 2.75×10^7 cell L^{-1} *T. weissflogii* at $pH_t = 8.02$. Dotted curve represents the organically complexed lead in an oceanic seawater ($[L_2] = 22.7$ nM, $\log K' = 8.89$).

compared with the amount of lead complexed. At lead concentrations higher than the binding capacity of the high affinity sites, lead distributes between surface groups on the algae and organic dissolved ligands. Thus, when a lead concentration of 10 nM is added to a solution containing 2.75×10^7 cell L^{-1} *T. weissflogii* in seawater $pH_t = 8.02$ and after 10 min equilibrium period, the ASV labile fraction, that is, the inorganic lead fraction, represents only an average of 9% of the total.

The lead complexed by the dissolved ligands is only 6% while the lead adsorbed is 85% due to the high binding constant of the high affinity sites. If the concentration of total lead was increased to 100 nM, 29% would be labile, 23% organically complexed dissolved lead and 47% adsorbed on the surface groups. The free lead fraction calculated by application of side-reaction coefficients, is 25 pM at 10 nM of total lead, which represents 0.3% of the total lead. If only inorganic ligands were considered, concentration about half the value expected will be observed. In the absence of algae, the inorganic fraction represents an average of 39% of the total while the free lead fraction represents 1.9% of the total lead concentration.

The conditional constant is a function of both proton concentration and the apparent proton adsorption constant. Pb(II) intrinsic adsorption constant valid for any pH can be computed from the conditional state, considering the proton competition with Pb(II) ions for the same sites. By using a value for the apparent acidity constants [29] of $\log K_a^a = 9.11$ and $\log K_a^a = 9.30$ for *P. tricornutum* and *T. weissflogii*, respectively ($pH_t = 8.02$, NaCl 0.7 M), Tables II and III list the intrinsic adsorption constants estimated from the two models. Based on the two-site model, the intrinsic high-affinity constant and the intrinsic low affinity constant of *P. tricornutum* are 11.55 ± 0.33 and $10.20 \pm 0.36\ (\mu M)^{-1}$, respectively, while the intrinsic high affinity constant and intrinsic low affinity constant of *T. weissflogii* are 12.15 ± 0.23 and $10.37 \pm 0.23\ (\mu M)^{-1}$, respectively. These intrinsic values are independent of pH and can be used at any pH values.

To investigate the specificity of the surface ligands which show affinity for lead, copper was added to the seawater sample in large excess (100 nM) with respect to the lead complexing ligand. 1.34×10^7 cell L^{-1} *P. tricornutum* and 1.32×10^7 cell L^{-1} *T. weissflogii* were kept in equilibrium during 36 h, removed and the complexing capacity of the solution determined as in Figure 1. According to Table IV the ligands produced by both alga are highly specific for lead with a slight decrease in the stability constant for *T. weissflogii*. For this algae, it was possible to apply a two sites model showing that while the high affinity ligands were not affected (the $\log K'_{L,1}$ was 10.90 in both studies), the low affinity ligands changed from 9.17 ± 0.03

TABLE IV Pb(II) complexation parameters in seawater after 36-h equilibrium with 1.34×10^7 cell L^{-1} *P. tricornutum* and 1.32×10^7 cell L^{-1} *T. weissflogii* without and with 100 nM Cu(II)

Alga	*Without Cu(II)*		*With* 100 nM *Cu(II)*	
	C_L (nM)	$\log K_{pb}^{2+}$	C_L (nM)	$\log K_{pb}^{2+}$
P. tricornutum	32.7 ± 0.7	9.03 ± 0.02	33.1 ± 1	$8.98 \pm .02$
T. weissflogii	32.1 ± 1.1	9.32 ± 0.04	32.8 ± 0.7	9.17 ± 0.05

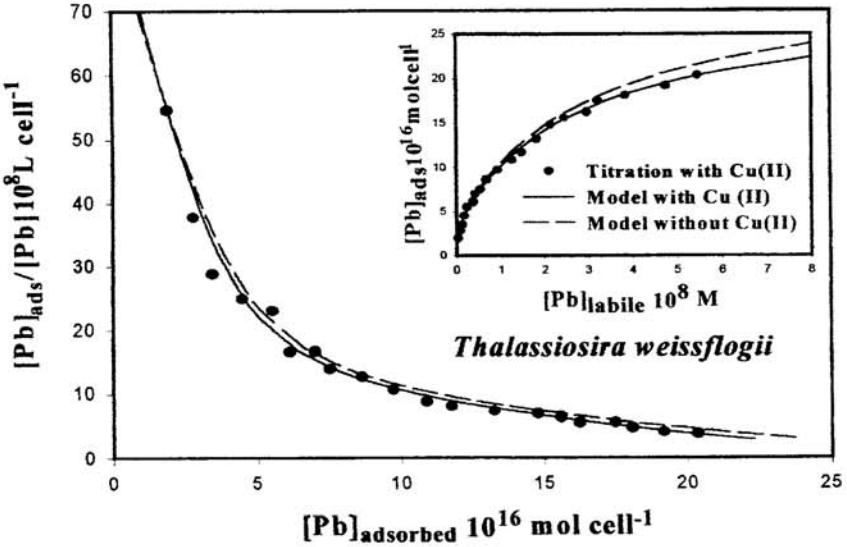

FIGURE 5 Scatchard plot for the adsorption of Pb(II) on 2.75×10^7 cell L^{-1} *T. weissflogii* in the presence of 100 nM Cu(II). Dotted lines represent model output in the absence of Cu(II). Inset: Langmuir isotherm for the same data.

to 9.07 ± 0.05. In any case, the high specificity of both kinds of dissolved ligands for lead is clearly observed. Studies were also carried out in order to study the effect of copper in the adsorption of lead. One hundred nM Cu(II) is around three times the adsorption capacity of both, 1.33×10^7 cell L^{-1} *P. tricornutum* (40 nM) and 1.36×10^7 cell L^{-1} *T. weissflogii* (35 nM). Figure 5 shows the lead adsorption characteristics for *T. weissflogii* in the presence of 100 nM Cu(II), after 10-min equilibration period. For *P. tricornutum* (data not shown), the high affinity ligand concentration is reduced from 4.0 ± 1.1 to 1.5 ± 0.6 nM, while the conditional adsorption constants increased from 10.45 ± 0.12 to 10.86 ± 0.3. These values show the ligands displaced by copper correspond with those with lower affinity inside the high affinity ligand class. In the *T. weissflogii* experiments (Figure 5), only a slight reduction in the adsorption capacity of the low affinity ligands is found (from 27.4 to 24.9 nM, with and without Cu(II), respectively). These results show the high specificity of the surface and dissolved ligands of both alga (in special *T. weissflogii* ligands) for lead, even in the presence of 100 nM Cu(II).

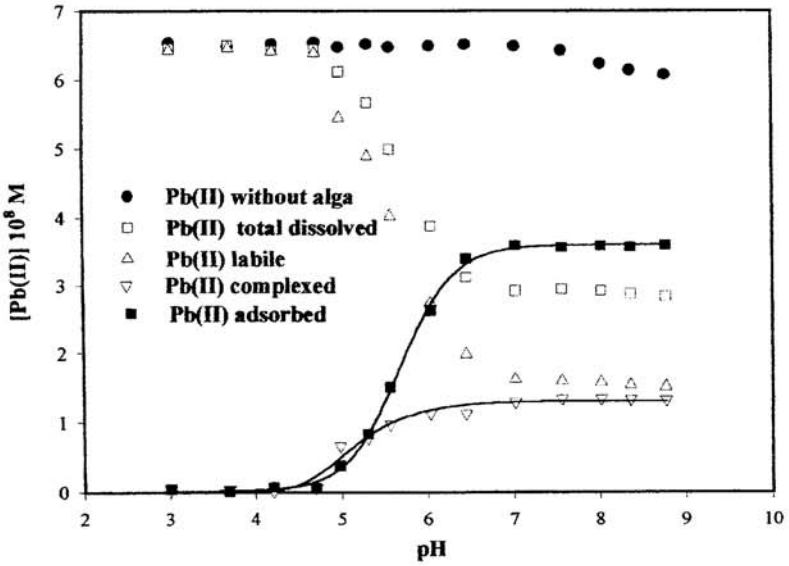

FIGURE 6 Effect of pH on the Cu(II) (65 nM) speciation in the presence of 2.7×10^7 cell L^{-1} *T. weissflogii.*

An acidimetric titration curve of a biomass suspension at a constant total concentration of the heavy metal shows directly the dependence of the metal binding on pH. Figure 6 exemplifies this pH-dependence for the binding of Pb(II) (65 nM) to the surface sites of the cell *T. weissflogii* (2.70×10^7 cell L^{-1}). Similar results as those given in Figure 6 were obtained for *P. tricornutum.* Since the functional groups in marine algae are acidic, the availability of free sites depends on pH. This corresponds to increased metal cations binding with the increasing pH of the sorption system. In addition to the role of protons in changing the state of active metal ion binding sites, the speciation of the metal ion in solution is dependent upon the decreasing solubility of the metal complexes with increasing pH. Since adsorption depends not only on the attraction of the sorbate to the solid surface but also on its lypophobic behaviour, it means that for most metals adsorption increases with increasing pH. In this sense, we have studied (Figure 6) the effect of pH in the speciation of lead both with and without the presence of algae. In the absence of algae, a decrease in the lead concentration at pH higher than 7 is due to complexation with the organic ligand naturally present in the seawater and to adsorption of lead on the plastic wall. The hydroxo complexes of Pb(II) such as $PbOH^+$ are considered fully labile [30]. At low pH values, protons and metal ions compete for the same binding sites, with more sites being available for metal ion sorption at higher pH values [31]. Figure 6 shows that for pH higher than 7, the speciation of lead in a seawater solution containing algae is quite constant. The nature of the functional groups of the organic compounds

excreted by the algae may not be the same as the surface groups, because the pH of maximum adsorption and complexation are not coincidental.

As it has been pointed out, ion exchange and complex formation can be considered as processes occurring in the biological surface groups [32–35]. Major cations present in seawater such as alkaline earth metals may compete for sites where trace metals are being bound. In order to study the effect of changes in the seawater composition on the sorption of lead on the algal surface groups, experiments were designed by varying the salinity of the seawater solution keeping the pH constant by addition of acid or base. Figure 7 shows for *P. tricornutum* (2.67×10^7 cell L^{-1}) the binding capacity for Pb(II) (62 nM) at a pH = 8.02 increases (16%) as seawater is diluted from $S = 36$ to $S = 12$ together with an increase in the Pb(II) complexed (45%) and a decrease in the labile fraction (69%). For the alga *T. weissflogii*, the Pb(II) complex increases only by 18% while the adsorption increases by 11% when a seawater solution of 36 salinity is diluted to 12. Similar behaviour for the adsorption of Pb(II) by *Rhizoclonium* [36] and by *D. Tertiolecta* [11] has been shown. When salinity changes at a constant pH, the surface charge and double-layer capacitance of the algal surface groups is modified. Obviously, this charge must lead to electrostatic attraction of cations. Moreover, the salinity changes the speciation of metals [27], both major and trace metals, changing their biochemical availability. In our studies, the increase in metal adsorbed is accomplished by an increase in the complexed fraction, presumably due to an increase in the amount of extracellular exudation products favoured by the osmotic changes in the interior of the cells. This increase in the complexed

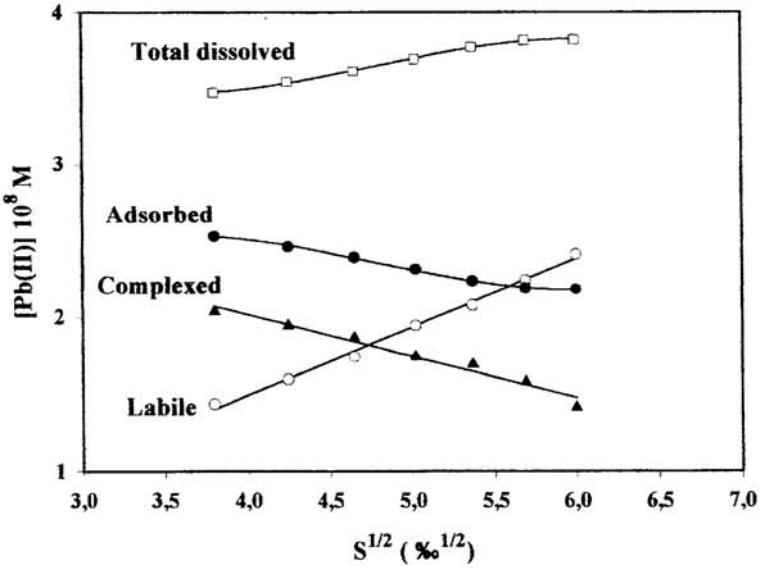

FIGURE 7 Effect of salinity in the speciation of Pb(II) (62 nM) in the presence of 2.67×10^7 cell L^{-1} *P. tricornutum*.

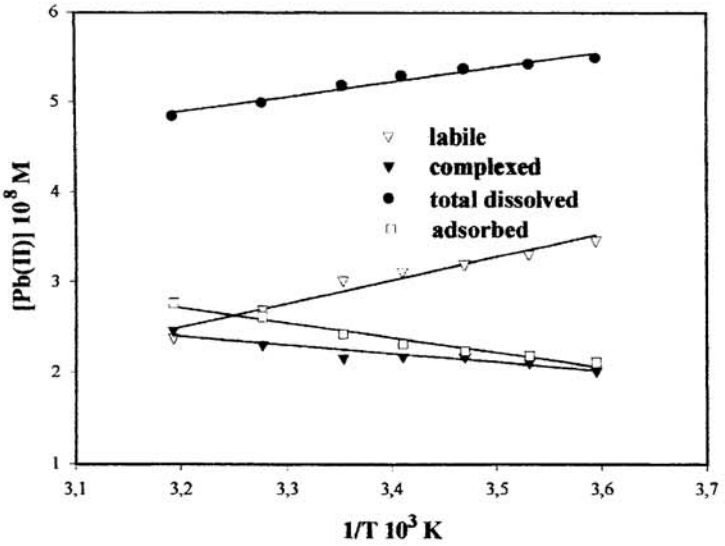

FIGURE 8 Effect of temperature in the Pb(II) (76 nM) speciation in the presence of 2.74×10^7 cell L^{-1} *P. tricornutum.*

fraction should be higher than we found because as seawater is diluted in the experimental design, the amount of natural organic ligands in seawater is also diluted.

Adsorption studies at different temperatures were carried out in order to determine the effect of temperature on the total process and to determine the specific adsorption energy. Figure 8 presents the effect of temperature on the speciation of lead (76 nM) in *P. tricornutum* seawater culture (2.7×10^7 cell L^{-1}). When the temperature increased from 6°C to 45°C, the speciation of lead in the presence of both alga is strongly affected. In the *P. tricornutum* culture, the complexed lead concentration increases by 10%, the labile fraction is highly decreased due to the increase in both the complexed lead and the adsorbed lead, which increases by 25%. In the *T. weissflogii* culture, the same behaviour is found. The complexed and adsorbed fractions increase with temperature by 35%, decreasing the inorganic lead fraction. The specific adsorption energy, E, determined accordingly [10] gives 8.35 ± 0.60 kJ mol^{-1} for *P. tricornutum* and 9.17 ± 0.53 kJ mol^{-1} for *T. weissflogii.* The positive specific adsorption energy of Pb^{2+} may be interpreted as the heat of hydration of Pb(II) being higher than its heat of adsorption.

References

[1] R.H. Byrne, Inorganic lead complexation in natural seawater determined by UV spectroscopy, *Nature* **290**, 487–489 (1981).

[2] D.R. Turner, M. Whitfield and A.G. Dickson, The equilibrium speciation of dissolved components in freshwater and seawater at 25°C and 1 atm pressure, *Geochim. Cosmochim. Acta* **45**, 855–881 (1981).
[3] C.M.G. van den Berg, Determination of complexing capacity and conditional stability constants of complexes of copper (II) with natural organic ligands in seawater by cathodic stripping voltammetry of copper–catechol complex ions, *Mar. Chem.* **15**, 1–18 (1984).
[4] K.H. Coale and K.W. Bruland, Copper complexation in the Northeast Pacific, *Limnol. Oceanogr.* **33**, 1084–1101(1988).
[5] W.G. Sunda and S.A. Huntsman, Feedback interactions between zinc and phytoplankton in seawater. *Limnol. Oceanogr.* **37**, 25–40 (1992).
[6] F.L.L. Muller, Interactions of copper, lead and cadmium with the dissolved, colloidal and particulate components of estuarine and coastal waters, *Mar. Chem.* **52**, 245–268 (1996).
[7] G. Capodaglio, K.H. Coale and K.W. Bruland, Lead speciation in surface waters of the Eastern North Pacific, *Mar. Chem.* **29**, 221–233 (1990).
[8] M.L. Wells, P.B. Kozelka and K.W. Bruland, The complexation of dissolved Cu, Zn, Cd and Pb by soluble and colloidal organic matter in Narragansett Bay, RI, *Mar. Chem.* **62**, 203–217 (1998).
[9] P.B. Kozelka, S. Sanudo-Wihelmy, A.R. Flegal and K.W. Bruland, Physico-chemical speciation of lead in South San Francisco Bay, *Estuar. Coast Shelf Sci.* **44**, 649–658 (1997).
[10] M. González-Dávila, J.M. Santana-Casiano, J. Pérez-Peña and F.J. Millero, Binding of Cu(II) to the surface and exudates of the alga *Dunaliella tertiolecta* in seawater, *Environ. Sci. Technol.* **29**, 289–301 (1995).
[11] J.M. Santana-Casiano, M. González-Dávila, J. Pérez-Peña and F.J. Millero, Pb^{2+} interactions with the marine phytoplankton *Dunaliella tertiolecta*, *Mar. Chem.* **48**, 115–129 (1995).
[12] K.W. Bruland, J.R. Donat and D.A. Hutchins, Interactive influences of bioactive trace metals on biological production in oceanic waters, *Limnol. Oceanogr.* **36**, 1555–1577 (1991).
[13] J.G. Lee, B.A. Ahner and F.M.M. Morel, Export of cadmium and phytochelatin by the marine diatom *Thalassosira weissflogii*, *Environ. Sci. Technol.* **30**, 1814–1821 (1996).
[14] M.N. Hughes and R.K. Poole, *Metals and Microorganism* (1st edn.), p. 412. Chapman & Hall. London (1989).
[15] M.L.S. Gonçalves, L. Sigg, M. Reutlinger and W. Stumm, Metal ions binding by biological surfaces: voltammetry assessment in the presence of bacteria, *Sc. Total Envrion.* **60**, 105–119 (1987).
[16] H. Xue and L. Sigg, Binding of Cu(II) to algae in a metal buffer, *Wat. Res.* **24**, 1129–1136 (1990).
[17] F.J. Millero, *Chemical Oceanography* (2nd edn.), p. 469. CRC Press, Inc, Boca Raton (1996).
[18] D.S. Gamble, M. Schitzer, H. Kerndorff and C.H. Langford, Multiple metal ion exchange equilibria with humic acid, *Geochim. Cosmochim. Acta* **47**, 1311–1323 (1983).
[19] J.M. Tobin, D.G. Cooper and R.J. Neufeld, Uptake of metal ions by *Ryzopus arrhizus* biomass, *Appl. Envir. Microbiol.* **31**, 821–828 (1988).
[20] A. Ringbom and L. Harju, Determination of stability constants of chelate complexes. Part I. Theory, *Anal. Chim. Acta* **59**, 33–47 (1972).
[21] M. González-Dávila, The role of phytoplankton cells on the control of heavy metal concentration in seawater, *Mar. Chem.* **48**, 215–236 (1995).
[22] G. Sposito, On the use of the Langmuir equation in the interpretation of adsorption phenomena: II. The two-surface Langmuir equation, *Soil. Sci. Soc. Am. J.* **46**, 1147–1152 (1982).
[23] C.M.G. van den Berg, Determination of copper in sea water by cathodic stripping voltammetry of complexes with cathechol, Anal. Chim. Acta **16**, 195–207 (1984).
[24] R.R.L. Guillard and J.H. Ryther, Studies of marine planktonic diatoms. I. *Cyclotella nana Hustedt* and *Detonula confervacea* (Cleve) gran, *Canad. J. Microbiol.* **8**, 229–238 (1962).
[25] J. Crank, *The Mathematics of Diffusion* (1st edn.), p. 361, Oxford University Press, New York (1976).
[26] W. Sunda and R.L. Guillard, The relationship between cupric ion activity and the toxicity of copper to phytoplankton, *J. Mar. Res.* **34**, 511–529 (1976).
[27] F.J. Millero and D.J. Hawke, Ionic interactions of divalent metals in natural water, *Mar. Chem.* **40**, 19–48 (1992).

[28] J.R. Donat, K. Lao and K.W. Bruland, Speciation of copper and nickel in South San Francisco Bay: a multimethod approach, *Anal. Chim. Acta*, **284**, 547–572 (1994).
[29] M. González-Dávila, J.M. Santana-Casiano and L.M. Laglera, Copper adsorption in diatoms cultures, *Mar. Chem.* (in press).
[30] M.L.S. Gonçalves, L. Sigg and W. Stumm, Voltammetric methods for distinguishing between dissolved and particulated metal ion concentrations in the presence of hydrous oxides: a case study of lead (II), *Environ. Sci. Technol.* **19**, 141–146 (1985).
[31] S. Schiever and B. Volesky, Modelling of the proton–metal ion exchange in biosorption, *Environ. Sci Technol.* **29**, 3049–3058 (1995).
[32] R.H. Crist, J.R. Martin, D. Carr, J.R. Watson, H.J. Clarke and D.R. Crist, Interaction of metals and protons with algae. 4. Ion exchange vs adsorption models and a reássessment of Scatchard plots; ion-exchange rates and equilibria compared with calcium alginate, *Environ. Sci. Technol.* **28**, 1859–1866 (1994).
[33] S. Schiever and B. Volesky, Modelling multi-metal ion exchange in biosorption, *Environ. Sci. Technol.* **30**, 2921–2927 (1996).
[34] S. Schiever and B. Volesky, Ionic strength and electrostatic effects in biosorption of protons, *Environ. Sci. Technol.* **31**, 1863–1871 (1997).
[35] C. Huang, C.P. Huang and A.L. Morehart, Proton competition in Cu(II) adsorption by fungal mycelia, *Water Res.* **25**, 1365–1375 (1991).
[36] R.H. Crist, K. Oberholser, J. McGarrity, D.R. Crist, J.K. Johnson and J.M. Brittsan, Interaction of metals and protons with algae. 3. Marine algae, with emphasis on lead and aluminum, *Environ. Sci. Technol.* **26**, 496–502 (1992).