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Original paper

THE INITIAL ADSORPTION OF Pb^{2+} TO *Dunaliella salina*

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ABSTRACT

The adsorption process of metal ion to algal surface groups and complexes to become organic material at all growth phases could be affecting their behaviour in seawater. The differential pulse anodic stripping voltammetry (DPASV) method was used to determine the adsorption and interaction between the Dunaliella salina and Pb^{2+}*. The rate of adsorption was found in two steps; first a relative fast adsorption step (10 min) and second, slower or diffusion-controlled uptake into the cells. The parabolic equation could be performed* ($[Pb^{2+}]_{sorbed} = 1.31 \times 10^{-7} + 0.062 \times 10^{-7} \sqrt{t}$) *to describe the relationship between metal ion adsorption and uptake by the algae as a function of time. The high affinity constant is similar to the complexing capacity by the alga and no significant effect of cell density on binding capacity. This shows that ligands produced by the algae play an important part in buffering concentration of free metal concentration.*

Keywords: Adsorption, Pb^{2+}, *Dunaliella salina*

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INTRODUCTION

Interactions between metal ions, particulate matter and dissolved organic matter can be used to determine the distribution and chemical speciation of trace metals in the marine environment. Particulate matter and organic ligands produced by biological activities have important implications for geochemical cycling, activity, and environmental toxic effects of trace metals (Xue and Sigg, 1993; Gonzales-Davila, 1995). The interaction between metal ions (Cu^{2+}, Pb^{2+}, Cd^{2+}, Zn^{2+}, etc.) and natural particles (micro-algae, bacteria, yeast, etc.) is important for predicting the behaviour of metal ions in aqueous systems (Huang *et al.*, 1991; Crist *et al.*, 1992).

The cells of phytoplankton can control the dissolved metals concentration in the water column, by means of surface reactions. Cell surface area can affect the adsorption rate of metal ions (Muhaemin, 2004). These cells contain various functional groups such as N-terminal NH_2-groups, C-terminal COO^- groups, S-terminal SH groups, and side chain amino acid residues (Huang *et al.*, 1991; Muhaemin, 2005). Adsorption of metal ions to cell surface groups is followed by slow transport step across the cell membrane and into the cytoplasm (Garnham *et al.*, 1992) the presence of organic ligands regulates the bioavailability, bioaccumulation, toxicity, and transport of metal ions through biological membranes (Anderson and Morel, 1982; Muhaemin, 2005). Metal organic complexation could influence their transport and cycling by decreasing or increasing its adsorption onto the suspended inorganic and organic particles (Florence, 1982; Donat and Bruland, 1990); and the studies on it varied (Donat and Bruland, 1990, Bruland, 1992, Donat and van der Berg, 1992; Donat *et al.*, 1994). Metal ions adsorption and complexation by dissolved organic ligands in sea water system have been studied for few metals such as copper, zinc, and cadmium (Xue and Sigg, 1990; Gonzales-Davila, 1990; Gonzales-Davila *et al*, 1994; Muller and Kester, 1991). All of them showed that the metal ions binding to micro-alga exudates have significant effect on metal speciation higher than the binding to the micro-alga surfaces.

Although lead has no known physiological requirement, it has an effect to micro-algae growth and even the micro-algae activities. Lead may displace nutrient metals from sites of biological molecules (ligands) and even alter normal metabolic function/activities (Santana *et al.*, 1995). Here the initial binding of lead to micro-alga and to the ligands produced/excreted by the micro-algae has been studied.

MATERIALS AND METHODS

The algae used were unicellular marine green algae *D. salina*. It was collected from Balai Budidaya Laut (BBL, Mariculture Centre) Hanura pure isolate. The research was conducted on October-December 2005.

The number of cells (2×10^7 cell.l^{-1}) in suspension of 38 mg.l^{-1} (dry weight) was determined. When the concentration was high (10^8 cell.l^{-1}), 250 ml of cultures were centrifuged at 4000 rpm for 15 min, then washed 5 times with 0.45 µm filtered seawater (Millipore acid washed filter). This cell content was diluted to get 1 l of sample with a cell number in the range 1-3 $\times 10^7$ cell.l^{-1}. The sample was stored in refrigerator for 24 h before use. Lead stock solution of 100 µg.l^{-1} was prepared using sterilized distilled water.

Differential Pulse Anodic Stripping Voltammetry (DPASV) was used for metal determination. 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. An Ag/AgCl electrode was used as the reference electrode and coiled platinum wire as the auxiliary electrode. The reduction potential was -0.8 V for Pb^{2+}. The scan rate was 2 mV/s, the pulse height was 50 mV, and the deposition time was 2 min. The research was conducted in triplicates with anticipated relative standard deviations of less than 10%.

Each experimental point records a measurement of an individual batch after equilibration time of 10 min at required temperature (25 ^{0}C). After filtration, the electrochemically labile metal concentration was tested in untreated sample. The total dissolved metal concentration was tested after acidification (pH = 2) with 2 M HCl and microwave treatment. The amount of metal adsorbed by cell was determined by Langmuir mass law (Santana *et al.,* 1995). The sensitivity of the determination was measured in each experimental condition to analyze the metal concentration.

RESULTS AND DISCUSSION

Uptake kinetics of Pb^{2+} by the algae

The uptake kinetics is shown in **Fig. 1**. The process follows two steps sorption kinetics. Initial Pb^{2+} adsorbed by the cells rapidly for the first 10 minutes as they took up 59.7% (1.50 M Pb^{2+}) of the total amount of

Pb^{2+} assimilated by the algae. Pb^{2+} uptake continues even until the end of the experiment. It indicates that the surface binding occurs first and takes place rather fast than the first 10 minutes.

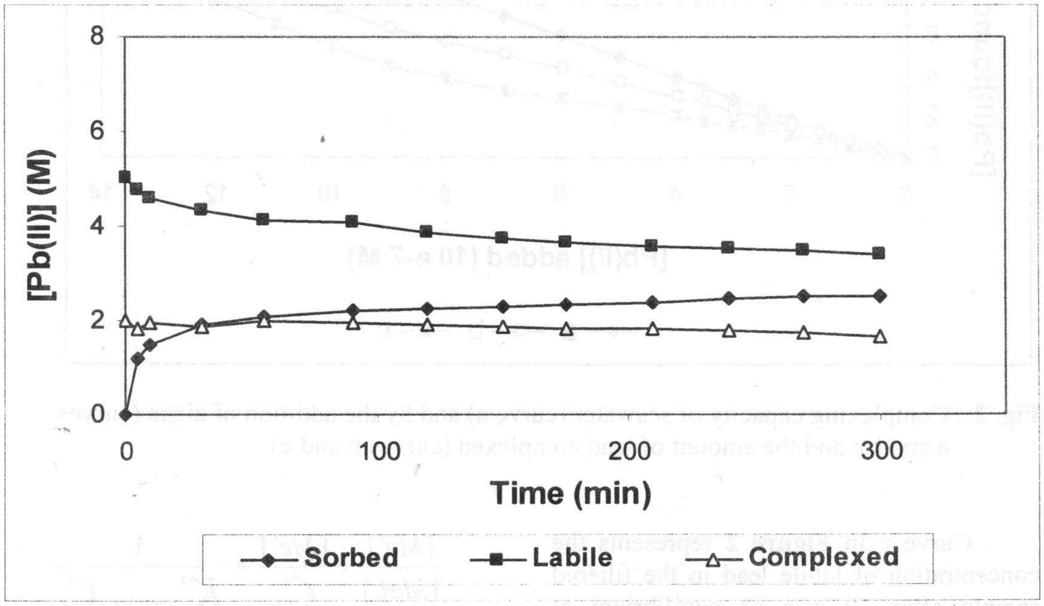
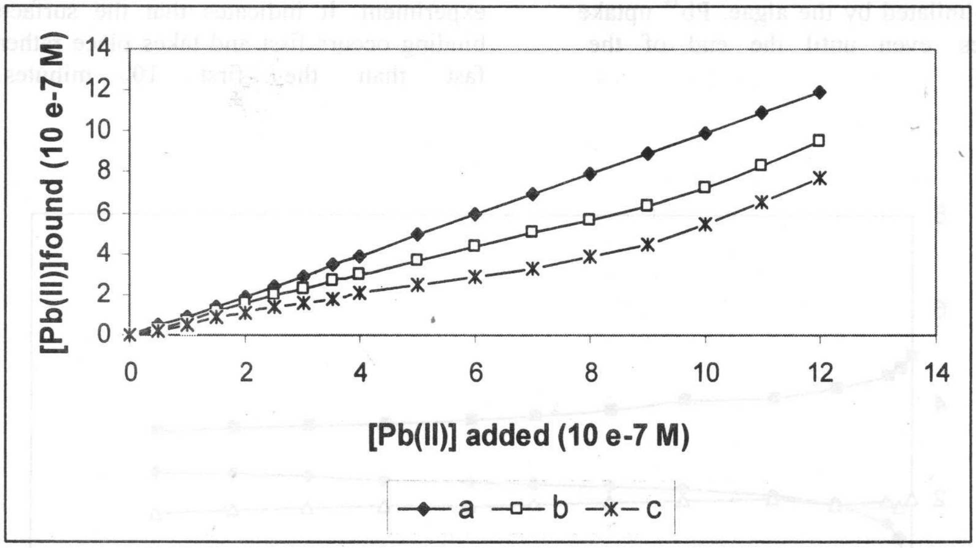


Fig. 1. The kinetic uptake of Pb^{2+} by the *Dunaliella salina* in seawater (pH=7.9; 25 ^{0}C). The changes in the complexed and labile lead concentration as a function of time are also included. Initial lead concentration is 8×10^{-7} M.

Further, Pb^{2+} uptake by the live cells slows down as it may be controlled by the diffusion process through the cell wall, or regulated by intracellular metabolic processes (Muhaemin, 2004). After 15 minutes, Pb^{2+} uptake will conform the parabolic diffusion model $\mathbf{[Pb^{2+}]_{sorbed} = 1.31 \times 10^{-7} + 0.062 \times 10^{-7} \sqrt{t}}$. It indicates that intracellular diffusion may be rate limiting step. After 5 hours equilibration time, 31.4 % of initial Pb^{2+} was assimilated by the cells. In this study, 10 min equilibration time was used in order to obtain pseudo-equilibrium with the algal surface.

3.2 Pb^{2+} sorption by Dunaliella salina

Fig. 2. shows a typical determination of the complexing capacity of seawater for Pb^{2+}. It shows the changes of the complexing capacity of seawater (curve b and c) as a consequence of both the adsorption of lead onto the cell walls and the subsequent complexation by the exudates produced and excreted by the algal cells.



algae

Fig. 2. Complexing capacity of seawater (curve a) and by the addition of algae (curves a and b); and the amount of lead complexed (curves b and c)

Curve c in **Figure 2** represents the concentration of labile lead in the filtered sample after 10 min of equilibrium at natural pH. For the determination of the total dissolved lead in the presence of algae (curve b), the samples were acidified to pH=2 after filtration and equilibrated for 3 h.

The amount of metal complexed with the non-labile ligands present in seawater is determined taking into account curve b and c (labile metal). The shift of peak potential when Pb^{2+} is added to the algal suspension or to the solution of the algae after centrifugation at different pH values well correspond to the hydrolysis of the metal (Santana *et al.*, 1995). It shows that the labile organic lead complexes are not formed in addition to the non-labile lead complexes.

Figure 2 shows a typical determination of the complexing capacity of seawater for Pb^{2+}. Based on van den Berg-Ruzic plot (van den Berg, 1982), the natural complexing capacity of seawater can be evaluated:

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

where [Me'] is the concentration of the metal ion in all inorganic forms; [MeL] is the concentration of organically complexed metal; C_L is the total ligand concentration in seawater; and $K'_{cond(Me')}$ is the conditional stability constant calculated with respect to [Me']. The conditional stability constant of the form $K'_{cond(Me^{2+})}$ could be calculated with respect to the free Me^{2+}; where α is the inorganic side reaction coefficient of metal in seawater

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

The value 0.028 was chosen to normalize the conditional stability constant with the respect to free metal Pb^{2+}, in natural seawater for Pb^{2+}, C_L is 120 ± 3 (nM), ***log***

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$\boldsymbol{K_{Me(II)}}$ is 6.79 ± 0.07, and $\boldsymbol{\log K_{Me}^{2+}}$ is 8.34 ± 0.03, in Seawater and exudates for Pb^{2+}, $\boldsymbol{C_L}$ is 157 ± 13 (nM), and $\boldsymbol{\log K_{Me}^{2+}}$ is 8.40 ± 0.13.

The addition of algal cells into the seawater could change the complexing capacity of the seawater due to the release of exudates from the algal cells. Only a slight increase in the ligand concentration was observed in the filtrates of the algae. It showed that at the beginning of the culture, the presence of algal cells does not affect the value of complexation capacity of Pb^{2+} ion. The same phenomenon has been observed by Santana *et al* (1995) for *Dunaliella tertiolecta*, and Stolzberg and Rosin (1977) for *Skeletonema*.

The mass law equation could describe the step of metal binding ion onto biological surfaces. The following type of surface complex suggests:

$$\equiv SOH + Pb^{2+} \leftrightarrow \equiv SOPb^{2+} + H^{+};\ K_1^{s} \qquad (3)$$

$$2 \equiv SOH + Pb^{2+} \leftrightarrow (\equiv SO)_2 Pb^{2+} + 2H^{+};\ \beta_2^{s} \qquad (4)$$

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

$$\equiv S_iH + Pb^{2+} \leftrightarrow S_iPb^{2+} + H^{+};\ K_{Pb,i}^{s} \qquad (5)$$

Where $\equiv S_i$ describes a (deprotonated) bidentate surface chelating site. The algal surface acid base properties may be characterized by:

$$\equiv SH_2^{+} \leftrightarrow SH + H^{+};\ K_a,1 \qquad (6)$$

$$\equiv SH \leftrightarrow S^{-} + H^{+};\ K_a,2 \qquad (7)$$

Because the functional groups on the surface are not identical; it is obtained by applying the law of mass action to a mixture of surface chelating sites:

$$\equiv SH + Pb^{2+} \leftrightarrow \equiv SPb^{2+} + H^{+};\ K_{\beta}^{s} \qquad (8)$$

The equilibrium state above is a function of both the hydrogen ion and free metal concentration. The free metal concentration of lead as a function of total lead in the sea water suspension is affected by the production of extracellular organic matter with metal complexing properties coming from the algae (Santana *et al.*, 1995). For quantitative study of metal adsorption to algal surface, binding to exudates must be considered onto the equation. In the presence of algae, the total Pb(II) added is

$$[Pb^{2+}]_T = [Pb^{2+}]_{inorg} + \Sigma_i \{\equiv S_i - Pb\} + \Sigma_n [PbL_n]$$
$$= \alpha^{-1} [Pb^{2+}]_{free} + \Sigma_i \{\equiv S_i - Pb\} + \Sigma_n [PbL_n] \qquad (9)$$

Where $[Pb^{2+}]_{inorg}$ describes the concentration of Pb^{2+} in all the inorganic forms (labile lead), α is the fraction of free metal, and $\Sigma_i \{\equiv S_i - Pb\} + \Sigma_n [PbL_n]$ describe the concentration of lead adsorbed in the algal surface and binding with the soluble exudates. The binding of lead to the exudates was calculated in order to determine the true free concentration of lead in equilibrium with the metal adsorbed.

Based on the equation (6), equilibrium constant may be defined for a given pH. In terms of conditional constant as (considering the charges):

$$K_H^{S} = \frac{\{\equiv SPb\}}{\{\equiv SH\}[Pb^{2+}]} \qquad (10)$$

The equilibrium constant above 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; Santana casiano *et al.*, 1995).

In natural seawater, a single toxic metabolic species rarely exist and the presence of a multiplicity of metal ions often gives rise to interactive effects. The expression of the effect on the algae may vary, and depend on species of algae, metal combination, and levels of metal concentration (Bruland *et al.* 1991; Muhaemin, 2004; Santana casiano *et al*, 1995). The addition of other metal ions, such as Cu^{2+}, has been found to increase the labile and total dissolved lead in the solution. It could decrease in the adsorbed and complexed amount of Pb^{2+} (Santana casiano *et al*, 1995). Gonzales-Davila *et al* (1994) showed that the uptake of lead and copper in two-metal system describes antagonistic behaviour in the adsorption of lead due to a competition for adsorption sites on the cell walls.

The research may only the linear relationship be analyzed due to the limited measurements made at low concentrations. The treatment used assumes that metal complexation can be adequately represented by a single ligand and complexes having 1:1 stoichiometry ratio. The presence of two ligands or sites, with sufficient affinities for the metal ions, has been shown to cause the non linear behaviour of the plots at lower concentrations (van den Berg, 1984; Santana casiano *et al.*, 1995). These simple treatments of metal-organic interaction only describe an average stability constant. 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 (Donat *et al.*, 1994).

Equation (10) can also be transformed in terms of a Langmuir isotherm where $\{\equiv SPb\}$ and $\{S_T\}$ correspond to Γ_{Pb} and Γ_{Max}, and are the amount of metal ion adsorbed in $mol.cell^{-1}$, and maximum value of metal ion adsorption complexing capacity.

$$\Gamma_{Pb} = \frac{\Gamma_{\max}\left[Pb^{2+}\right]}{\left(K_H^S\right)^{-1} + \left[Pb^{2+}\right]} \qquad (11)$$

$$\{\equiv Pb\} = \frac{\{S_T\}\left[Pb^{2+}\right]}{\left({}^{*}K_H^S\right)^{-1} + \left[Pb^{2+}\right]} \qquad (12)$$

Figure 3 shows the forms of Langmuir isotherms (Equation 12) for the adsorption of lead on two different amounts of *Dunaliella salina* cells. 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. It demonstrates that a two site Langmuir isotherm can reproduce the experimental data very well (see determination coefficient, R^2). No significant differences were observed for studies carried out at two different concentrations of cells. It shows that the functional groups of algal surfaces co-ordinately 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 is understandable because the metal ions bind first to the surface functional groups having the highest affinity and subsequently to those groups having a lower affinity. The complexation capacities for the Pb^{2+} binding with algal surfaces are only valid for the specified conditions. The adsorption and binding of Pb^{2+} shows that algae influences 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 Pb^{2+} ions.

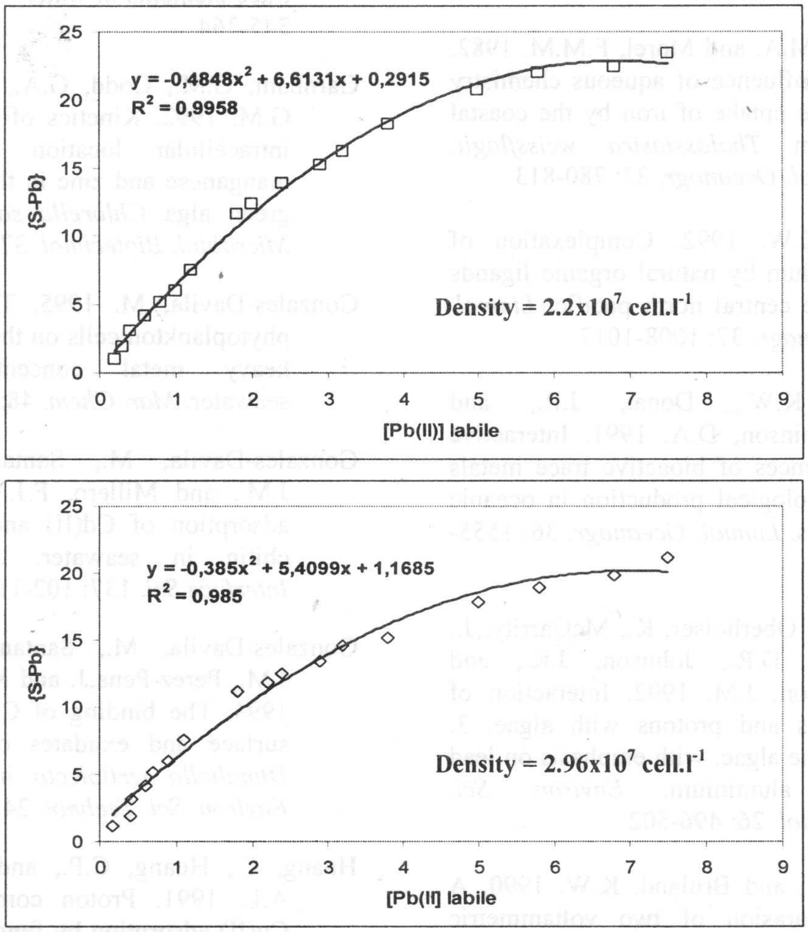

Fig. 3. Adsorption isotherms of Pb^{2+} and algae according to the Langmuir equation (Eq. 12) at two different algae concentration. X-ordinate ($x\ 10^{-7}$ M); Y-ordinate ($x\ 10^{-15}$ $mol.cell^{-1}$)

CONCLUSIONS

The research shows that lead can be adsorbed by *Dunaliella salina* and complexed by the algae. The ligands produced by the algae compete with the surface groups of lead ions. There was no significant effect of cell density on binding capacity. At least, it shows that ligand produced by the algae plays an important role in buffering concentration of free metal (Pb^{2+}) concentration.

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