Copper and cadmium complexation by *Cylindrospermopsis raciborskii* exudates

Alessandra Emanuele Tonietto, Natalia L. Oliveira, Ana Teresa Lombardi and Adriano Polpo

ABSTRACT

*Cylindrospermopsis raciborskii* is a potentially toxic cyanobacterium that excretes organic materials which act as ligands for metals. Metal ligands may be characterized for their strength of association, e.g., stability constants, which can be either thermodynamic (K) or conditional (K'). In this research we examined K and K' for Cu and Cd complexes with three molecular weight fractions (>30 kDa; 30–10 kDa; 10–3 kDa) of the cyanobacteria EOM. Complexation capacities of the excreted organic materials (EOM) for metals were determined at several ionic strengths ($1.0 \times 10^{-2}$, $5.0 \times 10^{-2}$, $1.0 \times 10^{-1}$, and $5.0 \times 10^{-1}$ mol L$^{-1}$) at pH 6.6 ± 0.1, with ligands for which no data for their acidity constants are available; these constants are thus conditional for this specific pH. Bayesian statistics showed that with a probability of 95–100% the EOM have two different ligands for Cu but only one for Cd, that ligands for Cu were stronger than for Cd (94–100% probability), and that the smallest EOM fraction had the highest strength of association for Cu ($\log K_{CuL}$ 13.5). The lowest affinity was obtained for Cd ($\log K_{CdL}$ 8.6) complexed to any molecular weight fraction. The present findings have important ecological implications, since the metal–ligand association is dynamic, and together with a diversity of ligands it can act as an environmental metal buffer. As a result, higher metal loads may be necessary for the detection of toxicity.

**Key words** | cyanobacteria, exudates, potentiometry, stability constant, trace metals

INTRODUCTION

Phytoplankton cells and the organic products that they excrete contribute to the formation of dissolved organic materials (DOM) in aquatic environments, and because of their ability to associate with metals, they affect the speciation, bioavailability and toxicity of metals (Buffle 1988). Cadmium (Cd) and copper (Cu) are considered major contaminants in aquatic ecosystems. As with the majority of metals, they have a tendency to accumulate in food webs, and can inhibit growth or cause the death of organisms (Buffle & Tercier-Waerber 2003). The strength of association between metals and ligands in the organic materials can be represented by stability constants. These are values that, in addition to environmental applications, industry can use for the preparation of structured nanofilms (Freire-Nordi et al. 2005), for definition of parameters for the sequestration of metals in industrial effluents (Nemeth et al. 2011), and for the support of metal specificity in chelation therapy (Riha et al. 2013).

Natural DOM and excreted organic materials (EOM) produced by phytoplankton cells act as metal ion buffers in aquatic environments, a property that is related to their capacity to bind metal ions at both high and low strengths. These ligands are normally referred to as high affinity and low affinity ligands and are ecologically important because they are related to the availability and the toxicity of metals to aquatic organisms (Batley et al. 2004).

The strengths of association of natural ligands, e.g., their affinity for trace metals can be qualitatively characterized via the determination of conditional stability constants (K') and thermodynamic stability constants (K). They provide information on how tightly a metal combines with a ligand, but K' is dependent on environmental conditions, such as pH and ionic strength, whereas K is an absolute value and is not dependent on such variables (Muller & Batchelli 2015). So, in comparison with conditional stability...
constants, the thermodynamic equivalent gives more meaningful information regarding freshwater ecosystems, in contrast to the high salinity in marine environments. This occurs because the ions necessary for ionic strength adjustment in K’ determinations competes with the metal of interest for the binding sites in the DOM, so disturbing the metal/ligand associations and sub-estimating the complexation capacity; this effect is absent in the thermodynamic constants.

The conditional stability constants of associations between metals and natural organic materials, such as algae-excreted compounds, aquatic organic matter, humic acid and fulvic acids, are widely documented in the literature (Buffler 1988; Muller & Batchelli 2013; Tonietto et al. 2014), but few studies have reported on the thermodynamic constants (Croot et al. 1999; Town & Filella 2000; Wiramanaden et al. 2008). This is possibly due to the difficulties in determining the absolute value.

We report on the conditional and thermodynamic stabilities constants of EOM produced by the toxic cyanobacterium Cylindrospermopsis raciborskii. The EOM was fractionated into three molecular weight fractions and differences in metal affinity within fractions and the metals Cu and Cd were obtained. This study is a contribution to the knowledge of the biogeochemical cycles of Cu and Cd and to the fate of EOM in aquatic environments.

**METHODS**

*Cylindrospermopsis raciborskii* was isolated from Barra Bonita reservoir (São Paulo, Brazil), where it dominates, and it is maintained in the culture collection of the Botany Department at Universidade Federal de São Carlos (SP, Brazil). *Cylindrospermopsis raciborskii* is a cyanobacterium whose blooms are increasing in frequency in water reservoirs worldwide. This organism is extremely aggressive and can tolerate climate change, it is competitive in tropical eutrophic environments and can produce and release lethal toxins (hepatotoxins, and neurotoxins) according to Berger et al. (2006). In addition, it excretes copious amounts of organic materials (Tonietto et al. 2014).

*Cylindrospermopsis raciborskii* was grown in 7 L glass carboys at pH 7.8 at controlled temperature (23 ± 1 °C) and light intensity. An irradiance of 42 μmol m⁻² s⁻¹ was provided by 40 W fluorescent tubes with a light–dark cycle of 12/12 hours. The cyanobacterium was cultured in ASM-1 medium (Gorham et al. 1964), and experimental cultures were inoculated with exponentially growing cells and kept under continuous aeration by gentle bubbling with filtered and moist air. Chlorophyll a concentration was monitored throughout algal growth. It was extracted with 90% acetone and determined in a spectrophotometer at 665 nm (Pharmacia Biotech Novaspec II) according to Parsons et al. (1984). At the end of the exponential growth phase (ca. 37 days after inoculating), the EOM was obtained by tangential filtration through 0.65 μm hollow-fiber polysulfone cartridges (GE, QuixStand Systems; UFP-6-E-4A – A/G Technology Corporation, Piscataway, NJ, USA). The particulate material was discarded, and the filtrate that contained the EOM was further fractionated into three molecular weight fractions. These were obtained by further tangential filtration through hollow-fiber polysulfone cartridges (UF-P30-E-4A of 30 kDa, UFP-10-E-4A of 10 kDa and UFP-3-E-4A of 3 kDa – A/G Technology Corporation). Cartridges were washed according to the procedure described in the operating guide (A/G Technology Corporation). The different molecular weight fractions were freeze-dried (Hetodywinner) and stored in a freezer (−20 °C) until analysis.

The EOM produced by *C. raciborskii* was characterized for its dissolved organic carbon (DOC) content using a Shimadzu TOC-VCPH carbon analyzer (Japan). Potassium hydrogen phthalate (Nacalai Tesque) and sodium hydrogen carbonate (Nacalai Tesque) were used as the standards.

Complexation capacities of the EOM for Cu and Cd were determined in previously filtered (0.22 μm acetate membrane – Sartorius) samples containing 100–150 mg L⁻¹ that was quantified as DOC. This concentration of EOM gave DOC concentration similar to that in Barra Bonita reservoir (Tonietto et al. 2014). Copper and Cd were determined through ion-selective electrodes (ISE – Thermo Scientific, USA).

The titrations (n = 3) with Cd or Cu were performed on the EOM molecular weight fractions and monitored using ISE at controlled temperature (25 ± 1 °C) and pH (6.6 ± 0.1), and buffered with 7.5 × 10⁻³ mol L⁻¹ of PIPES (piperazine-N,N’-bis[2-ethanesulfonic acid], Sigma-Aldrich).

The technique used for determination of the K is based on Fatibello-Filho (2012). This involves previous determination of conditional stability constants (K’) at several ionic strengths (I) that are then plotted as K’ vs. I⁻¹/₂, and a linear regression calculated. The thermodynamic stability constant is represented by the linear coefficient of the straight line, thus at zero ionic strength. So, each EOM fraction was titrated in four different ionic strengths (1.0 × 10⁻², 5.0 × 10⁻², 1.0 × 10⁻¹, and 5.0 × 10⁻¹ mol L⁻¹) that were adjusted with ultrapure NaNO₃ (Sigma-Aldrich). The conditional stability constants (logKₘₖ) were calculated using

\[
\text{log} K_{\text{ML}} = \text{log} K’ - \frac{1}{2} \hat{m}_c \text{I}^{\frac{1}{2}}
\]
Scatchard plot analysis (Scatchard et al. 1957). This analysis revealed two main classes of binding ligands in the EOM that we classified as class L₁ (binding ligands with logK_{ML} ~ 12–14) and class L₂ (binding ligands with logK_{ML} ~ 7–10). This classification has also been used in Town & Filella (2000). By ligand class it should be understood a composition of ligands and not only one ligand.

For the titrations, standard solutions of Cu and Cd (Titrisol, Merck) were diluted to 1.0 × 10⁻³ mol L⁻¹ and added to the samples through standard additions from 4.0 × 10⁻⁷ to 3.5 × 10⁻⁵ mol L⁻¹. Potential readings were obtained using a pH/ISE meter (Thermo Scientific Orion Star A214 Benchtop pH/ISE meters). Cu (Thermo Scientific, 9429BN) and Cd (Thermo Scientific, 9448BN) electrodes were used with an Ag/AgCl double-junction reference electrode (Thermo Scientific, model 900200). The ISE linear range for Cu was extended, going from 2.8 × 10⁻¹³ to 3.5 × 10⁻⁵ mol L⁻¹ and for Cd the range was 5.4 × 10⁻¹¹ to 1.0 × 10⁻⁵ mol L⁻¹, using metal ion buffers according to Lombardi & Vieira (2000). Blank titrations were performed to evaluate if the pH/ISE meter (Thermo Scientific Orion Star A214 Benchtop pH/ISE meters). Cu (Thermo Scientific, 9429BN) and Cd (Thermo Scientific, 9448BN) electrodes were used with an Ag/AgCl double-junction reference electrode (Thermo Scientific, model 900200). The ISE linear range for Cu was extended, going from 2.8 × 10⁻¹³ to 3.5 × 10⁻⁵ mol L⁻¹ and for Cd the range was 5.4 × 10⁻¹¹ to 1.0 × 10⁻⁵ mol L⁻¹, using metal ion buffers according to Lombardi & Vieira (2000). Blank titrations were performed to evaluate if the pH buffer, PIPES (Sigma-Aldrich), and the ionic strength adjuster, NaNO₃ (Sigma-Aldrich), influenced the metal complexing properties of the samples. We did not use a fixed equilibration time; this was based on the stabilization of the potential. It was only after the potential (mv) stabilization that the next titration point was added. The lower the metal concentration to be added in the solution, the longer the equilibrium time. For the first additions in the titration curve, the equilibrium times were longer (~2 h); however, with the decrease of available ligands, equilibrium time also decreased and, by the end of the titration, approximately 5 min was enough for the equilibrium to be reached. Always, before each EOM titration, the potential for the metal ion buffer was obtained (so linearity was extended to lower values) and a complete calibration curve was performed to verify the Nernstian behavior of the electrode response and to convert the titration data from nV to pH or pCd using the Nernst equation (Lombardi & Vieira 2000). This means that regression equations for the calibrations were obtained daily and for each titration, since only one titration was performed per day. All labware was cleaned by washing with neutral detergent, immersed for 7 days in 10% HNO₃ (J.T. Baker), and rinsed with distilled and deionized water.

The statistical analysis was performed under the Bayesian paradigm. In this case, the parameters are considered random variables, which allow us to compare them via probabilities. Different from analysis of variance (ANOVA), which is based on the frequentist paradigm, the statistical analysis we performed tells us about the probability of one K being greater than the other. This means that for a variable A that is obtained for different experimental conditions, such as A₁ and A₂, we can determine the probability of A₁ > A₂ and also A₂ > A₁. In our case, A represents the strengths of association between ligands and metals.

For the calculation of the thermodynamic stability (K_{ML}), a linear regression model was used that considered all of the ionic strengths and treatments used to obtain the K_{ML} for the Cu and Cd ligands (Gelman et al. 1995; Koop et al. 2007). Using the posterior distribution of the linear regression parameters, we evaluated the posterior distribution of the K_{ML} for each treatment and compared them. As an uncertainty measure, we used the posterior probability of ordering K_{ML} so providing the probability of the K_{ML} of one treatment to be greater than the other. Additionally, we plotted the posterior densities for graphical comparison. Thus, one can analyze and compare the different K_{ML} values obtained for all treatments, even if one of the ionic strengths is not available. The method used to compare the different K_{ML} values is an alternative to ANOVA.

For all experiments, previous information was not used to evaluate the posterior distribution. Thus, all results are based only on the observed data.

RESULTS

The average slope for both Cu (28.0 ± 1.6 mV decade⁻¹) and Cd (27.8 ± 1.9 mV decade⁻¹) electrode calibrations (n = 36 for each metal) are in agreement with the expected Nernstian slope of 29.6 mV decade⁻¹ for a divalent ion electrode (Rijstenbil & Gerrings 2002).

Figure 1 exemplifies a Cu (Figure 1(a)) and Cd (Figure 1(b)) titration for the EOM lowest molecular weight fraction (10–5 kDa). It revealed more ligands for Cu than for Cd, since Cd reached a complexation capacity (10 μmol L⁻¹) before Cu (15 μmol L⁻¹). For Cu, a two-segmented curve was obtained after Scatchard plot analysis, but only one for Cd. This confirmed that two ligands with different conditional stability constants (Figure 1(c)) were present for Cu (L₁ and L₂), but only one for Cd (Figure 1(d)).

The conditional stability constants for the fractionated EOM and the metals Cu and Cd that were used for the thermodynamic constant calculations are presented in Table 1. Plotting these results for calculation of the thermodynamic stability constants resulted in a negative slope after adjusting the linear regression model, since the lower values of conditional stability constants were obtained.
with the increase in ionic strength. Considering Cu, the EOM lowest molecular weight fraction had the highest conditional stability constants, which was followed by the largest molecular weight fraction. The intermediate molecular weight EOM (30–10 kDa) exhibited the lowest conditional stability constants. However, no clear trend was observed for Cd.

To compare the logKML for the different metals and molecular weight fractions, we obtained their posterior densities, which are plotted in Figure 2.

Figure 2(a) shows the KML posterior densities for Cu ligands (L₁) considering the different EOM molecular weight fractions and their ordering. It shows that the KML obtained for the larger molecular weight fraction (logKML’s mean = 12.6) represents weaker associations in relation to that for the KML of the smaller fraction (logKML’s mean = 13.5) with 99% probability. The KML for the intermediate molecular weight fraction (30–10 kDa) is smaller than the two others, with >99.9% probability (logKML’s mean = 10.9). These results agree with our hypothesis that was based on the conditional stability constant analysis.

Figure 2(b) shows the probability posterior densities for KML for the Cu L₂ ligands. The ordering is similar to that observed for Cu L₁ ligands. The logKML’s mean = 9.1 for the intermediate (30–10 kDa) molecular weight fraction is lower than that associated with the higher molecular weight fraction (logKML’s mean = 9.8) with 95% probability. The smaller molecular weight fraction (10–3 kDa) presented the highest logKML’s mean (11.6) among all, with a probability of >99.9%.

For Cd, the KML posterior densities for the different molecular weight fractions are presented in Figure 2(c). The probability is 48% that the KML for the Cu L₂ ligands. The ordering is similar to that observed for Cu L₁ ligands. The logKML’s mean = 9.1 for the intermediate (30–10 kDa) molecular weight fraction is lower than that associated with the higher molecular weight fraction (logKML’s mean = 9.8) with 95% probability. The smaller molecular weight fraction (10–3 kDa) presented the highest logKML’s mean (11.6) among all, with a probability of >99.9%.

For Cd, the KML posterior densities for the different molecular weight fractions are presented in Figure 2(c). The probability is 48% that the KML for the intermediate molecular weight fraction will be higher than that for the smallest fraction. Because this value is close to 0.5, there is no defined ordering between these two KML, meaning they are similar and their mean values are ca. 8.7. The statistical analysis also revealed that for the KML for Cd associated with the higher molecular weight fraction (logKML’s mean = 8.5), the probabilities are lower (both ca. 89%) than for the KML associated with either the 30–10 or 10–3 kDa molecular weight fractions. Thus, for the metal Cd, the higher
molecular weight fractions resulted in the lowest KML; however, the intermediate and lower molecular weight fractions of the EOM presented similar KML values. We emphasize that these KML values apply for pH 6.6 ± 0.1.

The class 1 and class 2 ligands concentrations (CL) for Cu (mol L⁻¹) and class 1 for Cd (mol L⁻¹) considering the three molecular weight fractions (>30 kDa; 30–10 kDa; 10–3 kDa) of the cyanobacteria EOM with the metals are presented in Table 2.

These results indicate that concentrations of class 1 ligands available for copper are higher than those obtained for the class 2 ligands for this metal.

**DISCUSSION**

The logK'ML values obtained in the present research varied within the metals Cu and Cd and the molecular weight fractions of *C. raciborskii* EOM, suggesting the presence of ligands with different chemical composition (Table 1) in the different fractions.

Considering the Cu logK'ML for the 30–10 kDa fraction at ionic strength of 0.1 mol L⁻¹, the present results are in agreement with those of Gouvêa *et al.* (2005), who studied Cu and Cd complexation with cyanobacteria EOM at 0.1 mol L⁻¹ ionic strength and detected two ligands for the metals. For Cu, Gouvêa *et al.* (2005) detected a strong ligand (logK'CuL 9.2–9.5) and a weak ligand (logK'CuL 6.7–8.3). Considering the 0.01 mol L⁻¹ ionic strength, the present results are in agreement with those of Tonietto *et al.* (2014). Using voltammetry, they determined K'ML for molecular weight fractionated *C. raciborskii* EOM and, for the intermediate fraction, obtained values of the logK'CuL similar to those in the present research. However, for the larger (>30 kDa) and smaller (10–3 kDa) fractions, Tonietto *et al.* (2014) obtained lower logK'CuL values. These differences may be due to the different techniques used, since voltammetry detects labile metals while ISE (used in the present research) detects free metal ions.

The logK' decrease as the ionic strength increased (Table 1) is in agreement with the results of Bezerra *et al.* (2009) that evaluated Cu complexing properties of humic acid using ISE and at three ionic strengths. Similar to our results, the authors obtained an inverse relation between logK'CuL and ionic strength. This is due to the electrolyte

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**Table 1** | Strengths of association reported as the conditional stability constant (logK'ML) for the ligands at the molecular weight fractions of the cyanobacteria excreted organic materials (>30 kDa, 30–10 kDa, and 10–3 kDa) with Cu and Cd using different ionic strengths

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Ionic strength mol L⁻¹</th>
<th>&gt; 30 kDa</th>
<th>30–10 kDa</th>
<th>10–3 kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cu class 1</strong></td>
<td>0.01</td>
<td>12.87 ± 0.38</td>
<td>10.92 ± 0.17</td>
<td>15.58 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>11.66 ± 0.32</td>
<td>10.28 ± 0.14</td>
<td>12.53 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>11.07 ± 0.29</td>
<td>9.71 ± 0.21</td>
<td>11.88 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>9.71 ± 0.18</td>
<td>9.04 ± 0.28</td>
<td>9.99 ± 0.34</td>
</tr>
<tr>
<td><strong>Cu class 2</strong></td>
<td>0.01</td>
<td>10.54 ± 0.40</td>
<td>8.74 ± 0.10</td>
<td>11.31 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>8.84 ± 0.31</td>
<td>8.45 ± 0.10</td>
<td>11.22 ± 0.39</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>7.62 ± 0.16</td>
<td>8.00 ± 0.22</td>
<td>9.80 ± 0.37</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>7.81 ± 0.32</td>
<td>NC</td>
<td>8.51 ± 0.15</td>
</tr>
<tr>
<td><strong>Cd</strong></td>
<td>0.01</td>
<td>8.14 ± 0.10</td>
<td>8.48 ± 0.07</td>
<td>8.58 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>8.08 ± 0.06</td>
<td>8.10 ± 0.04</td>
<td>8.05 ± 0.10</td>
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<tr>
<td></td>
<td>0.1</td>
<td>7.99 ± 0.07</td>
<td>7.90 ± 0.07</td>
<td>7.91 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>7.75 ± 0.06</td>
<td>7.36 ± 0.11</td>
<td>7.34 ± 0.05</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation (*n* = 3).
NC (not calculated): no complexation capacity.

**Figure 2** | Distributions a posteriori of the thermodynamic stability constants (logKML) between the EOM fractions and (a) Cu L₁, (b) Cu L₂, and (c) Cd ligands. Points represent > 30 kDa, dashed line 30–10 kDa and solid line 10–3 kDa.
The thermodynamic stability constants for Cu and the C. raciborskii EOM (8.5–13.5) are lower than that for Cu and EDTA according to Croot et al. (1999). These authors showed that logK_{Cu_{EDTA}} is ∼17.9, meaning the cyanobacteria EOM form weaker complexes with the metal, so being more susceptible to Cu release into the environment than Cu-EDTA. The Cu-EDTA constant was obtained in pH 7.7–8.2, a condition of totally deprotonated synthetic ligand. The acid–base properties of C. raciborskii natural ligands were not considered in the present investigation; however, we can reason that at pH 6.6, where H^+ ions can compete with Cu and Cd for the ligands, the thermodynamic constant for Cu:C. raciborskii EOM would be lower than in a situation of totally deprotonated EOM. Moreover, we call attention to the fact that EDTA is a synthetic ligand whereas C. raciborskii EOM is a natural and complex ligand. This cyanobacteria EOM is composed of carbohydrates, organic acids, amino acids and lipids, so carrying carboxylic, amines, phosphates and sulfhydryl groups, and in smaller proportion lipids. These groups can act as ligands for metal complexation, presenting affinity and specificity according to their composition and spatial conformation, as well as with culture growth phase (Tonietto et al. 2014). So, the lower logK for Cu:C. raciborskii EOM in comparison with the Cu-EDTA can be due to both the probable partial deprotonated natural ligand that was analyzed and the, in fact, lower complexation capacities. In a different study, Tsang et al. (2006) investigated the thermodynamic stability constant of Potomac River (USA) DOM for Cu and observed its value to be within 21.5 and 32.0. These are considered high values for a natural DOM and means there is little tendency for the metal to be released in environmental conditions, unless degradation of the organic material takes place. Nogueira et al. (2014) showed that the heterotrophic degradation of natural DOM can release previously complexed metals.

The present results showed that the cyanobacterium C. raciborskii produced ligands for Cu and Cd and that those for Cu are more tightly bound to the EOM than those for Cd, as shown by K_{ML}. For Cu, the strongest ligands were present in the lowest molecular weight fraction (10–3 kDa), while the intermediate fraction presented lower metal complexing properties. However, for Cd, the intermediate and 10–3 kDa fractions had similar K_{ML}, which were both higher than that for the larger molecular weight fraction.

It is evident that comparison of complexation constants with those reported in literature for different classes of organic ligands is difficult because of the variety of properties.

**Table 2** | Complexation capacity reported as ligand concentrations (C_{L}) for class 1 and class 2 for Cu (mol L^{-1}) and class 1 for Cd (mol L^{-1}) for the molecular weight fractions of the cyanobacteria excreted organic materials (>30 kDa, 30–10 kDa, and 10–3 kDa) with the metals Cu and Cd, using different concentrations of ionic strength

<table>
<thead>
<tr>
<th>Ligands concentration (C_{L}) / DOC (10^{-4} mol g^{-1})</th>
<th>Fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionic strength (I) mol L^{-1}</td>
<td>&gt; 30 kDa</td>
</tr>
<tr>
<td>0.01</td>
<td>9.98 ± 0.56</td>
</tr>
<tr>
<td>0.05</td>
<td>14.3 ± 1.13</td>
</tr>
<tr>
<td>0.1</td>
<td>6.02 ± 0.75</td>
</tr>
<tr>
<td>0.5</td>
<td>8.66 ± 0.94</td>
</tr>
<tr>
<td></td>
<td>class 2 for Cu</td>
</tr>
<tr>
<td>0.01</td>
<td>3.01 ± 1.51</td>
</tr>
<tr>
<td>0.05</td>
<td>0.75 ± 0.19</td>
</tr>
<tr>
<td>0.1</td>
<td>7.34 ± 4.14</td>
</tr>
<tr>
<td>0.5</td>
<td>1.51 ± 0.38</td>
</tr>
<tr>
<td></td>
<td>class 1 for Cd</td>
</tr>
<tr>
<td>0.01</td>
<td>7.91 ± 1.69</td>
</tr>
<tr>
<td>0.05</td>
<td>8.66 ± 3.58</td>
</tr>
<tr>
<td>0.1</td>
<td>7.34 ± 1.69</td>
</tr>
<tr>
<td>0.5</td>
<td>8.66 ± 0.38</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation (n = 3). NC (not calculated): no complexation capacity.

effect, e.g., in high ionic strength solutions, an increase in attractive and repulsive forces between the electrolyte and the metal ions involved in the complexation equilibrium. This shields the metal ions and lower stability constants are observed (Skoog et al. 2007).

Tonietto et al. (2014) showed that C. raciborskii EOM with molecular weight >30 and 30–10 kDa contain higher proportion of carbohydrates in relation to proteins, whereas the 10–3 kDa carry similar content of carbohydrates, proteins and lipids. The authors showed that while Cu ligands correlated with carbohydrates, Cd ligands correlated with the proteinaceous materials. Thus, carbohydrates and proteins in similar amounts in the lowest molecular weight fraction may be responsible for the higher logK_{Cu_{L}} obtained in the present research. This confirms other results in which relationships between amino acids/proteins and metal complexing properties have been demonstrated. Gonzalez-Davila et al. (1995) showed that the amino acids glycine and tyrosine favor metal complexation; the Gonzalez-Davila et al. (1995) study investigated exudates from the marine phytoplankton Dunaliella tertiolecta.
CONCLUSIONS

We demonstrated the presence of at least two ligand classes for Cu and one class for Cd in the EOM produced by the cyanobacterium C. raciborskii. The posterior means of the thermodynamic stability constants for the Cu-EOM L1 ligands in the larger (>30 kDa; logKCuL’s mean = 12.6) and smaller (10–3 kDa; logKCuL’s mean = 13.5) molecular weight fractions were higher than those obtained for the intermediate molecular weight fraction (30–10 kDa; logKCuL’s mean = 10.9). For the Cu L2 ligands, both intermediate (logKCuL’s mean = 9.1) and larger (logKCuL’s mean = 9.8) molecular weight fractions had lower logKML than the smallest molecular weight fraction (logKCuL’s mean = 11.6). For the Cd-EOM, all of the thermodynamic stability constants were similar, but the larger fraction presented a slightly lower logKCuL.

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