

## Interaction of Pb, Ni and Cd with Aquatic Humic Substances of Amazonian Blackwater Rivers

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In this study, the extraction and characterization of aquatic humic substances (AHS) from two blackwater rivers in the Amazon basin was carried out in order to assess the seasonal effects in the AHS structure. In addition, an investigation of the complexing capacities (CC) of the structures containing the metal ions Cd<sup>II</sup>, Ni<sup>II</sup> and Pb<sup>II</sup> was also done. The characterization (of the AHS) was done using UV-Vis, molecular fluorescence, and infrared spectroscopy. AHS from the Negro River showed more aromatic characteristics in its structures and molecular weight, than the AHS from the Carú River. The AHS studied presented a seasonal pattern characterized by higher humification levels during the rainy season. For all the metals, the AHS complexing capacity was higher for the Negro River. The highest complexing capacities were observed for the Ni<sup>II</sup> assays, which were 0.56 and 0.12  $\mu\text{mol Ni}^{\text{II}} \text{mg}^{-1}$  dissolved organic carbon (DOC), for AHS RN and AHS RC-II, respectively. We concluded that the transport, bioavailability, and toxicity, of these metal species should be higher for Carú River.

**Keywords:** Amazon, humic substances, blackwater, complexation, characterization, Negro River

### Introduction

In the aquatic environments, the organic matter (OM) can stem from autochthonous and allochthonous sources, being formed by simple and defined, or complex structures, with different reactivity within the functions of its chemical characteristics, microbiological and photochemical processes by which the precursor material had been altered.<sup>1-3</sup> An important fraction of OM is present in a humified form (humic substances, HS). It is a result of the degradation process of animal and vegetal residue, and the association of molecules by weak interactions.<sup>4,5</sup> The substances are known by their reactivity, and capacity, to interact with metals due to many functional groups present in their structures, such as, aromatic moieties,

aliphatic, phenolics, carboxylic and, alcohols. For example, the carboxylic groups are responsible for the metal complexation ability of HS. This characteristic plays an important role in the transport, bioavailability, toxicity, and accumulation, of these elements in the environment.<sup>6-8</sup> Depending on the concentration or speciation, the metal species can be considered as beneficial, or toxic. Ni, Cd, and Pb are elements derived from natural and anthropic processes. With the latter, being the main sources coming from the industrial activities of mining, smelting, batteries, and fossil fuels.<sup>9</sup>

Many studies<sup>6,8,10-14</sup> had been done to evaluate the origin and seasonal effects of HS in diverse aquatic systems. The Amazon basin represents worldwide relevance, it is the largest basin, and ranks first in terms of hydrologic discharge.<sup>15</sup> Its rivers are classified by physical-chemical characteristics such as whitewater, clearwater, and

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blackwater.<sup>16</sup> Whitewater rivers have a low dissolved OM content, and a high content of suspended solids, like the Amazon, and Madeira Rivers.<sup>17</sup> On the other hand, blackwater rivers show a dark-brown water color, with pH variables between 3.8 to 4.9, due to the high amounts of dissolved OM, represented mainly by the HS, in the waters of Negro, Urubu and Carú Rivers.<sup>18,19</sup>

Studies<sup>12,13,20-22</sup> were conducted to characterize, and to assess, the complexing capacity (CC) of aquatic humic substances (AHS) with metal species, in the rivers of the Amazon basin. Most of these studies were conducted in the Negro River. The studies were done using either an ultrafiltration system, or potentiometers, using different metals, such as Cu, Ni, Cd, Pb, Zn and Hg. These studies showed AHS CC varies according to the molecular weight, pH, or sampling season, among other parameters.<sup>12,13,20,22</sup>

However, studies of interaction of the AHS from other blackwater rivers in the Amazon basin are still non-existent. In this study, even though the area is not highly impacted by the anthropogenic actions, it comprises a region in which industrial development is increasing. In this study, we made the seasonal characterization of AHS extracted from the Carú and Negro Rivers. Then, investigated their CC with Pb, Cd and Ni in order to generate information on the manner in which these metals transport, and availability.

## Experimental

### Study area

The Carú River is located around the municipality of Itacoatiara in the central region of the Amazon Basin, on the banks of the Amazon River. Its location provides port activities, commerce and tourism. In addition, it is the third most populated city in the state of Amazonas and is a region in development. The power generation is thermoelectric. The region is also considered to be an important agricultural pole of the Brazilian North region.

In turn, the city of Manaus, the most populous of the state of Amazonas, is located at the confluence of the Negro and the Solimões Rivers. The municipality is characterized as the main urban center of the North region, having several industrial activities.

### Samples collection and AHS extraction

Two different blackwater rivers in the Amazon basin were sampled for AHS extraction (Figure 1). The physicochemical parameters are detailed in a previous study.<sup>23</sup> The Negro River located in Manaus city (03°04'13.74"S 60°08'26.92"W) is the major blackwater river in the Amazon basin, while the Carú River is located around Itacoatiara city, in Amazonas's state eastward (03°02'21.7"S 058°37'27.9"W); for this river, the AHS were extracted in the dry season, (November/2015) (AHS RC-I) and the rainy season, (July/2016) (AHS RC-II). Water samples were collected, and transported, to the laboratory. AHS from the Negro River (AHS RN) was extracted at the Laboratory of Environmental Analytical Chemistry (INPA, Manaus-AM) and AHS RC at the laboratory of the Federal University of Amazonas (UFAM, Itacoatiara-AM). Surface water employed in each AHS extraction was filtered to remove leaves and branches and acidified to pH 2.0 (6.0 mol L<sup>-1</sup> HCl). AHS extraction was carried out following the procedure prescribed by International Humic Substances Society (IHSS) using a polymer resin for the retention of AHS.<sup>24</sup> The humic extracts were purified using a cation exchange resin (DOWEX 50WX8, hydrogen form, 200-400 mesh, Sigma-Aldrich) and freeze dried.

### Aquatic humic substances characterization

The AHS solutions were prepared in NaHCO<sub>3</sub> (0.05 mol L<sup>-1</sup>, pH 8.0) with 5.0 mg L<sup>-1</sup> of dissolved organic



**Figure 1.** Sampling sites of water to extraction of humic substances at Negro River in Manaus and in the Carú River in Itacoatiara (adapted from Google Maps).

carbon (DOC). UV-Vis absorbance of the AHS from the Negro (AHS RN), and the Carú River (AHS RC-I: dry season; AHS RC-II: rainy season) were measured to evaluate chromophoric characteristics, by  $E_2:E_3$  ratio ( $a_{275}/a_{365}$  nm),  $S_R$  (spectral slope:  $S_{275-295}/S_{350-400}$ ) and aromaticity degree ( $52.509-6.780(E_2:E_3)$ ).<sup>25,26</sup> The spectra were carried out by using a Spectrophotometer 2600 (Shimadzu, Japan), in 200 to 800 nm using a 1 cm quartz cuvette. Ultrapure water (MilliQ) was used for blank determination. Fluorescence spectra scans were obtained by an emission-excitation matrix (EEM) using a Spectrofluorimeter Lumina (ThermoScientific). Emission wavelengths ranged from 270 to 600 nm and excitation wavelengths ranged from 230 to 500 nm at 600 nm  $s^{-1}$ . HIX (humification index) and BIX (freshness index) were determined using fluorescence data.<sup>27-29</sup> Fourier-transform infrared (FTIR) spectra were obtained by using the attenuated total reflectance (ATR) mode in a spectrometer (Spectrum Two UATR, PerkinElmer, USA) of 4,000-400  $cm^{-1}$  with a resolution of 4  $cm^{-1}$ . Elemental analysis (C, H, N and S) was conducted in a CE Instruments Elemental Analyzer (Fision, EA 1108, USA).

#### Interaction of AHS and metals

The complexation capacities (CC) of AHS were determined by employing an ultrafiltration system, using a tangential flow (TF-UF) equipped with a 1 kDa membrane, that allowed the AHS and the metal solutions to flow through the system, and when the flow is stopped the solution is forced to pass through the membrane, in this way, the non-complexed metal is able to percolate through it.<sup>7,8</sup> For this, 10.0 mg  $L^{-1}$  of AHS RN were prepared (0.1 mol  $L^{-1}$   $NaNO_3$ , pH 5.0) and 50.0 mg  $L^{-1}$  of AHS RC-II (0.1 mol  $L^{-1}$   $NaNO_3$ , pH 5.0) with a final volume of 100.0 mL. The metal concentrations ranged from 0.04 to 24.9  $\mu mol L^{-1}$ , 0.08 to 47.8  $\mu mol L^{-1}$  and 0.02 to 13.5  $\mu mol L^{-1}$  for cadmium(II) ( $Cd^{II}$ ), nickel(II) ( $Ni^{II}$ ) and lead(II) ( $Pb^{II}$ ), respectively. After the equilibrium time (ASH RC-II:  $Pb^{II}$  60 min;  $Ni^{II}$  35 min;  $Cd^{II}$  35 min; ASH RN:  $Pb^{II}$  30 min;  $Ni^{II}$  30 min;  $Cd^{II}$  20 min) of AHS-metal complex formation, the TF-UF flow was stopped for the collection of 2.0 mL, in order to quantify the  $[metal]_{free}$ . All experiments were conducted at the ambient temperature of the laboratory (20 °C). CC was determined using the intersection of two linear sections of the titration curve (plots of  $[metal]_{free}$  vs.  $[metal]_{added}$ ). The CC values were normalized by using the dissolved organic carbon (DOC) concentration in each experiment. In addition, the stability constant ( $K_c$ ) was determined according to the Scatchard model.<sup>20,30</sup>

#### Metal quantification

To assess the CC of the AHS in this study, the total concentrations of Cd, Ni, and Pb were determined by using an atomic absorption spectrophotometer with graphite furnace atomization, and a Zeeman background corrector (GFAAS) (AA280Z, Varian, Australia). The chemical modifier, Pd/ $Mg(NO_3)_2$ , (1500/1000 mg  $L^{-1}$ ) solution, was employed in the atomization process to determine the metal species. The following instrumental parameters were used<sup>8,23,31</sup> (lamp, monochromator crack, detection wavelength): Cd (5.0 mA, 0.5 nm and 228.8 nm), Pb (10.0 mA, 0.5 nm and 283.3 nm) and Ni (10.0 mA, 0.2 nm and 232.0 nm). The certified reference material, 1640a NIST® SRM®-trace elements in natural water, was employed as an analytical quality control for the quantification of metals by GFAAS. The laboratory also participated in the use of a proficiency test, applied by EMBRAPA, and the results (z score) have been considered satisfactory ( $z < 2$ ).

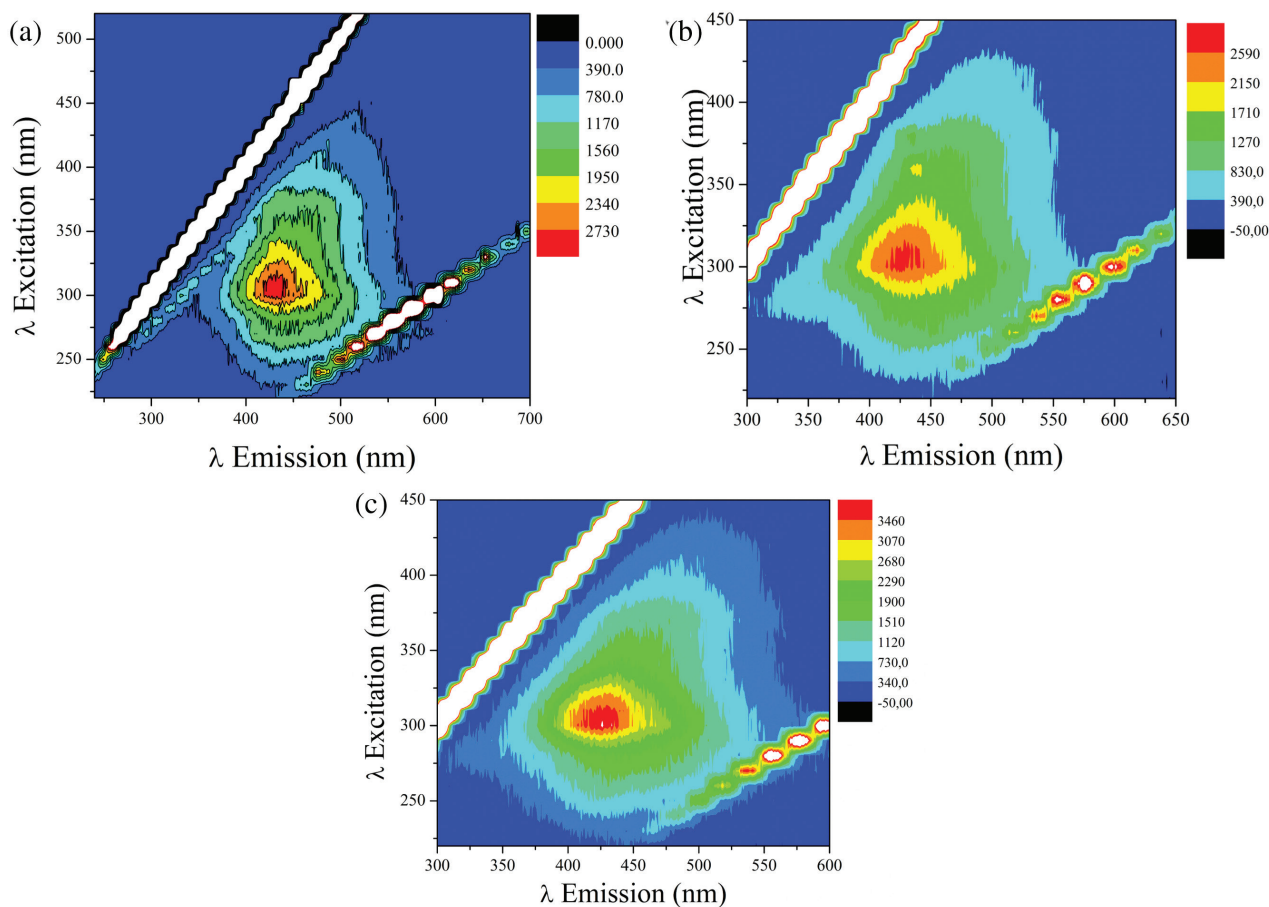
## Results and Discussion

#### Characterization of AHS

Figure 2 shows the fluorescence spectra obtained from the EEM of the AHS RN, RC-I, and RC-II, also the AHS RN had a maximum fluorescence intensity ( $F_{max}$ ) at  $\lambda_{exc}/\lambda_{em} = 300/431$  nm. For AHSs from the Carú River, the sample extracted during the dry season (RC-I) had  $F_{max}$  at  $\lambda_{exc}/\lambda_{em} = 300/426$  nm and AHS RC-II, the sample extracted during the rainy season, had  $F_{max}$  at  $\lambda_{exc}/\lambda_{em} = 310/420$  nm. Main peaks can be classified as peak C, humic-like ( $\lambda_{exc}/\lambda_{em} = 320-365/420-470$ ), which was assigned to the presence of the allochthonous humic materials, identified in soil, in freshwater and ocean samples.<sup>32,33</sup>

In the emission wavelengths, there was a slight blue shift, (shorter) observed in the AHS from the Carú River, when compared to the RN sample. This can be related to less extension of the electron system or conjugated bounds. In other words, this behavior was attributed to the lower presence of fluorophores that contain aromatic rings in their structures. This characteristic is associated with the arising, and transformation, of the HS, such as photodegradation reactions.<sup>33</sup>

The ratios, and spectral indexes, determined by UV-Vis and fluorescence measurements, are presented in Table 1. A higher HIX (12.36) was obtained for AHS RN, indicating this AHS as the most humified. The increasing of HIX was related to Zsolnay *et al.*,<sup>29</sup> with the rise of emission wavelengths due to the presence of polycondensated molecules. For RC samples, the HIX was slightly higher for AHS RC-II, indicating the influence of seasonality, in



**Figure 2.** Total fluorescence spectra obtained in EEM mode for AHS (5 mg L<sup>-1</sup> DOC, pH 8.0): (a) RN, (b) RC-II and (c) RC-I.

which there was a greater input of most degraded organic matter in the rainy season. BIX index is indicative of the autochthonous dissolved organic matter (DOM) presence (likely microbial). The AHS RC-II sample showed the greatest value (0.42), which corroborated with the HIX results. It is worth mentioning that an aquatic system with expressive autochthonous production usually shows  $BIX > 1.0$ .<sup>29,34</sup> Therefore, all the AHS showed little autochthonous influence.

The aromaticity degree was determined using UV-Vis measurements, the results were 31.5, 25.0 and 27.9 for AHS RN, RC-I, and RC-II, respectively (Table 1). The higher aromaticity degree assigned to AHS RN agreed with the other indexes and indicated a greater presence of aromatic structures in this sample. The spectral ratio  $E_2:E_3$  was inversely related to the aromaticity, and molecular weight.<sup>25</sup> Thus, in the humic material from the Negro River, we observed lower  $E_2:E_3$  ratios (3.09), which were 4.05 and 3.63 in the Carú samples. Another index related to the molecular weight is the spectral slope ( $S_R$ ). The lower values have been correlated to greater molecular weight.<sup>25</sup> Therefore,  $S_R$  also corroborated with the other index, the AHR RN, which was assigned to the sample with the greater

**Table 1.** Spectral indexes obtained by UV-Vis and fluorescence measurements of aquatic humic substances from Carú (RC-I and RC-II) and Negro (RN) Rivers

Sample	HIX <sup>a</sup>	BIX <sup>b</sup>	$E_2:E_3$ <sup>c</sup>	$S_R$ <sup>d</sup>	Aromaticity degree <sup>e</sup>
RC-I	6.96	0.40	4.05	2.68	25.0
RC-II	7.45	0.42	3.63	2.64	27.9
RN	12.36	0.34	3.09	2.36	31.5

<sup>a</sup>HIX: humification index; <sup>b</sup>BIX: freshness index; <sup>c</sup>ratio of a250 nm and a365 nm; <sup>d</sup>spectral slope ratio,  $S_{275-295}/S_{350-400}$ ; <sup>e</sup>aromaticity degree =  $52.509 - 6.780 (E_2:E_3)$ .

molecular weight. The AHSs from the Carú River had  $S_R$  of 2.68 and 2.64.

Elemental composition and atomic ratios for the AHSs are shown in Table 2. The percentage of C is in accordance with values obtained from previous samples extracted from the rivers.<sup>35</sup> The percentage of C ranged from 35.4 to 43.2%, which is the lowest percentage for AHS RC-I. The percentage of C and N also agree with the results found for the AHS RN in the literature.<sup>12,36</sup> There was a higher variation of percentage of N in these samples, which can be related to the presence of less degraded N in the material

of AHS RC-I and RC-II, 1.6 and 1.1%, respectively. The percentage of S for AHS RC were slightly higher than reported by Cory *et al.*,<sup>37</sup> and varied to 3.0 and 3.6%, for AHS RC-I and RC-II, respectively (Table 2).

**Table 2.** Elemental composition (C, H, N and S) and atomic ratios of AHS from Carú River (RC-I and RC-II) and Negro River (RN)

AHS	C / %	H / %	N / %	S / %	H/C	C/N
RN	41.6	4.1	0.6	–	1.17	74.52
RC-I	35.4	3.0	1.6	3.0	1.01	25.75
RC-II	43.2	3.7	1.1	3.6	1.02	46.79

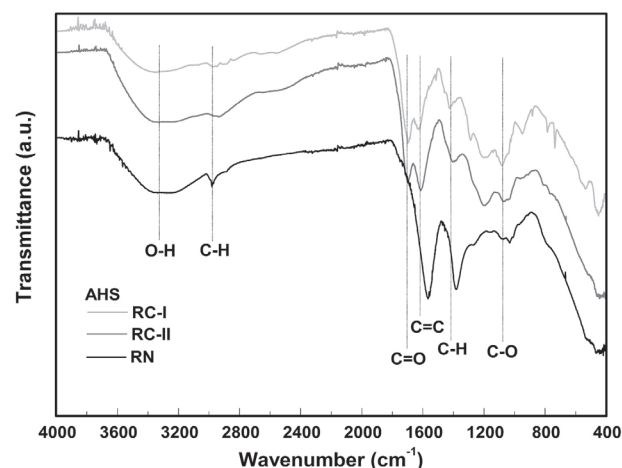
–: not determined. AHS: aquatic humic substances.

The lower values H/C atomic ratio indicate the presence of more humified material and they were correlated to higher HIX indexes, for the AHS the results were 1.17, 1.01 and 1.02, for RN, RC-I and RC-II, respectively, similar results as observed for fulvic fraction extracted from other blackwaters.<sup>29,33,37</sup>

The carbon and nitrogen relation, expressed by the C/N ratio, is related to the degree of humification in a sample.<sup>38</sup> Low values are associated with higher N amounts in the sample, which indicates the preservation of incorporated N in the structures that contain less humified material. Thus, AHS RN showed a higher C/N ratio (74.52), this is in agreement with the HIX index (Table 1). For the AHS RC-I, and RC-II, the C/N ratios were 25.75 and 46.79, respectively. These results showed seasonality effects in the AHS from the Carú River. AHS, and RC-II, isolated during the flooded period, showed more humification than the AHS RC-I sample extracted during the dry season. This can be associated to more intense contribution of humified organic material leached from soils. The enrichment of DOC during the rainy season is noted in the literature.<sup>39,40</sup> The DOC concentration has been associated to the presence of markers, such as lignin, which, is leached in the litter, from the runoff of the surface soils. Thus, the AHS RC-I structures, extracted during the dry period, had likely been formed by more labile compounds, mostly due to the influence of autochthonous, which was also revealed in the fluorescence spectrum (Figure 2).

Figure 3 shows the FTIR spectra of AHS from the Negro and Carú Rivers. Bands around 3300 cm<sup>-1</sup> were attributed to stretching of the O–H of hydroxyl groups, such as, phenols, alcohols, and carboxylic acids.<sup>41,42</sup> A band at 2980 cm<sup>-1</sup> is related to C–H binding stretching of aliphatic structures (alkanes), which was most evident in the RC-I. For AHS RC-I and RC-II, bands observed in the region of 1700 cm<sup>-1</sup> were also attributed to the stretching of the carbonyl (C=O), present in the structures as carboxylic

acids, amides, esters, ketones, and aldehydes. These bands were not present in the AHS RN FTIR spectra. However, the bands could have been overlapped by the intense bands observed at 1562 cm<sup>-1</sup>, which were attributed to the stretching of C=C, suggesting that the carbonyl groups may be linked to aromatic moieties, thus, only showing one intense peak in the spectra. These data corroborated with the high aromaticity highlighted in the spectral indexes (Table 1).



**Figure 3.** FTIR spectra of samples of aquatic humic substances (RC-I, RC-II and RN).

At 1380 cm<sup>-1</sup> a defined band, associated to the bending of (C–H) in the –CH<sub>3</sub> groups was observed, also, a slight shifting was observed in the AHS RN around 1415 cm<sup>-1</sup>. In the spectra of AHS from the Carú River, the main difference observed was the presence of a band at 1200 cm<sup>-1</sup>, which was also attributed to the bending of C–O, in the carboxylic, phenolic, and/or aromatic, compounds. The stretching of bands around 1300 to 1000 cm<sup>-1</sup> are related to the C–O of the aryl-alkyl-esters, or, polyalcohol, that are characteristics of polysaccharides or simple carbohydrates.<sup>43</sup> Both AHS samples from the Carú River presented bands in this region, specifically at 1075 and 1069 cm<sup>-1</sup> for RC-I, and RC-II, respectively. The decrease in RC-II indicates the low stage of degradation of the organic material in this sample.

#### AHS complexation capacity with Cd<sup>II</sup>, Ni<sup>II</sup> and Pb<sup>II</sup>

The CC was determined as value of the intersection of both linear regressions in the titration curves and, normalized, by the DOC concentrations in each sample. Figure 4 illustrates the typical titration curves ([metal]<sub>added</sub> vs. [metal]<sub>free</sub>) for the complexation assays between, AHS RC-II and RN, with the Cd<sup>II</sup>, Ni<sup>II</sup>, and Pb<sup>II</sup>, the complexation capacities of both AHSs are shown in Table 3.

For determination of K<sub>c</sub>, the empiric data of the complexation assays between AHS RN, and RC-II with

metal ions ( $\text{Ni}^{\text{II}}$ ,  $\text{Cd}^{\text{II}}$  and  $\text{Pb}^{\text{II}}$ ) were treated, using the Scatchard method. Scatchard plots for the interaction of AHS from RN and RC-II are reported in the Supplementary Information section (Figure S1). In this approach, the complexation is considered as 1:1 with a finite number of binding sites. For this purpose, a linearization of  $[\text{metal-ligand}]$  vs.  $([\text{metal-ligand}]/[\text{metal}]\text{free})$  is made and Kc is the slope of its linear regression. For experiments with  $\text{Ni}^{\text{II}}$ , both AHSs showed a similar behavior. The complexation is defined by two different sites, in which Kc1 and Kc2 can be determined by the two different linear sections (Figure S1).<sup>20</sup> For  $\text{Cd}^{\text{II}}$  assays, just one Kc was determined for both AHS (RN and RC-II), meaning that, only one binding site is able to complex  $\text{Cd}^{\text{II}}$  in these samples (Table 3). For  $\text{Pb}^{\text{II}}$  distinct results were observed for both AHS samples using two different sites in the complexation reaction of AHS RN ( $\log \text{Kc1} = 1.23$  and  $\log \text{Kc2} = 0.26$ ) and only one for AHS RC-II ( $\log \text{Kc1} = 0.81$ ).

Among all the metal ions tested in this study ( $\text{Cd}^{\text{II}}$ ,  $\text{Ni}^{\text{II}}$  and  $\text{Pb}^{\text{II}}$ ), AHS from the Negro River had the highest CC. This AHS interaction, observed for  $\text{Ni}^{\text{II}}$ , was six times higher than the CC of the AHS RC-II, while the CC with  $\text{Pb}^{\text{II}}$  and  $\text{Cd}^{\text{II}}$  was three and sixteen times higher than that observed in the AHS samples from the Carú River. These results are in agreement with the observation of the AHS RN FTIR spectra, that showed more aromatic and carboxylic moieties in its structure.

These differences observed in the CC of AHS samples from the blackwater rivers can be attributed to the distinct humic structures. Although these AHS stem from common precursor materials, other environmental conditions, such as, the degradation processes, and the physical-chemical parameters (pH, ionic strength) could influence the chemical structure diversity. Furthermore, many of the chemical groups presented in the AHS molecules could arrange in a supramolecular structure, resulting in distinguished interactions between the binding sites.<sup>44</sup>

The metal ions  $\text{Ni}^{\text{II}}$ ,  $\text{Cd}^{\text{II}}$  and  $\text{Pb}^{\text{II}}$  have an ionic radius of 0.69, 0.97 and 1.19 Å, respectively, which can contribute to the differences observed in the CC of the active binding

sites of AHS.<sup>45</sup> We concluded that the complexation order observed for AHS RN was inverse to the ionic radius of the metal ions studied (CC:  $\text{Ni}^{\text{II}} > \text{Cd}^{\text{II}} > \text{Pb}^{\text{II}}$ ), meaning, the smaller the ionic radius, the larger the capacity of AHS interactions with binding sites present in the humic material. For AHS RC-II, we observed that the CC values were higher for  $\text{Ni}^{\text{II}}$  than for  $\text{Cd}^{\text{II}}$  and  $\text{Pb}^{\text{II}}$ , also justifying that the interaction between the  $\text{Ni}^{\text{II}}$  and the SHA binding sites of RC-II is greater when compared to the other two metal ions.

Sargentini Jr. *et al.*<sup>12</sup> carried out the CC studies of the fractionated AHS (1.0 mg L<sup>-1</sup> of AHS, pH 5.0) from the Negro River, using  $\text{Ni}^{\text{II}}$ ,  $\text{Cu}^{\text{II}}$ ,  $\text{Zn}^{\text{II}}$ ,  $\text{Cd}^{\text{II}}$  and  $\text{Pb}^{\text{II}}$  solutions. It was verified that the fraction 50-100 kDa showed the major CC of the metal ions,  $\text{Pb}^{\text{II}} > \text{Cd}^{\text{II}} > \text{Zn}^{\text{II}} > \text{Cu}^{\text{II}} > \text{Ni}^{\text{II}}$ , with the order of the interactions being unlike the one observed in the present study. These results show a relationship between variations in the AHS composition and, its structural arrangements. In this study, the AHS RN was less saturated (low H/C ratio), with greater C/N (more humified) (Table 2).

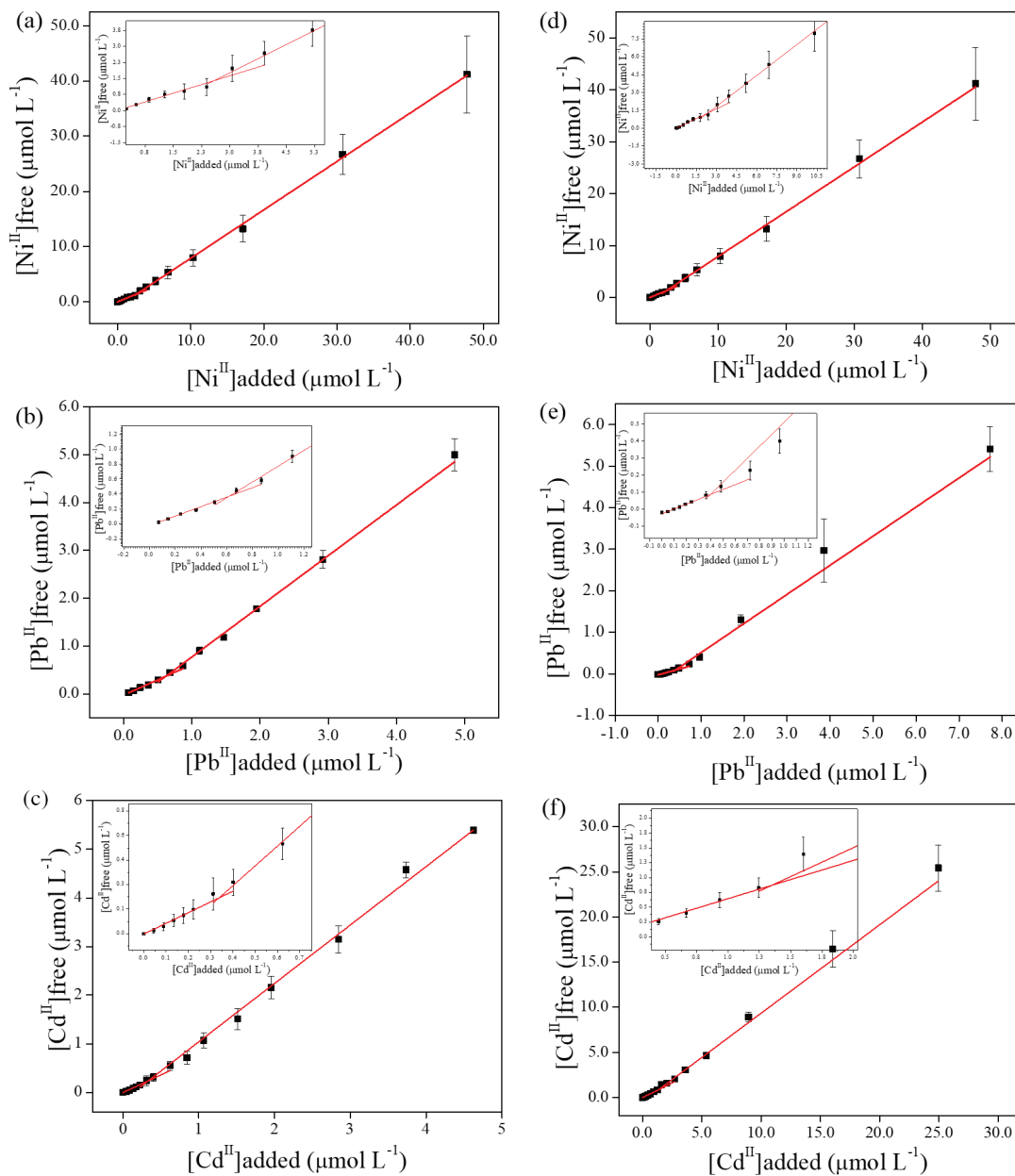
In another study<sup>46</sup> it was determined that the CC of the dissolved organic matter from three Amazonian rivers, the Itapecuru, Bacanga and Pericumã, had 8.84, 24.1 and 1.01 mg of DOC L<sup>-1</sup>, respectively. The complexation assays were conducted using the metal ions  $\text{Cd}^{\text{II}}$ ,  $\text{Pb}^{\text{II}}$  and  $\text{Cu}^{\text{II}}$  with the pH at 6.0. The CC results with  $\text{Cd}^{\text{II}}$ , similar to results found in AHS RN and RC-II ranged from 0.04 to 0.10  $\mu\text{mol Cd mg}^{-1}$  DOC. However, for  $\text{Pb}^{\text{II}}$  assays, the dissolved organic matter present in these rivers showed a higher interaction, (ranged from 0.11 to 0.81  $\mu\text{mol Pb}^{\text{II}} \text{mg}^{-1}$  DOC) than the AHS studied in the present work (ranged from 0.03 to 0.10  $\mu\text{mol Pb}^{\text{II}} \text{mg}^{-1}$  DOC).<sup>46</sup>

Besides the ion metal properties or the pH, the CC of the AHS depends on the chemodiversity of the structures and, the degree of humification (microbiological and chemical degradation and the molecular association). Comparing the characteristics of the AHS RN and RC-II, with the CC determined here, both samples have features of the organic material of blackwater rivers, the AHS from the Negro

**Table 3.** Complexation capacities (CC) and stability constant ( $\log \text{Kc}$ ) for AHS from Negro (RN) and Carú Rivers (RC) with  $\text{Cd}^{\text{II}}$ ,  $\text{Ni}^{\text{II}}$  and  $\text{Pb}^{\text{II}}$

	Aquatic humic substances					
	RN			RC-II		
	CC / ( $\mu\text{mol metal mg}^{-1}$ DOC)	$\log \text{Kc1}$	$\log \text{Kc2}$	CC / ( $\mu\text{mol metal mg}^{-1}$ DOC)	$\log \text{Kc1}$	$\log \text{Kc2}$
$\text{Cd}^{\text{II}}$	0.33	0.13	–	0.02	1.09	–
$\text{Ni}^{\text{II}}$	0.56	0.15	1.52	0.12	0.04	1.82
$\text{Pb}^{\text{II}}$	0.10	1.23	0.26	0.03	0.81	–

–: not determined. DOC: dissolved organic carbon.



**Figure 4.** Typical plots for complexation capacity of AHS RC-II with (a) Ni<sup>II</sup>, (b) Pb<sup>II</sup>, (c) Cd<sup>II</sup>; and of AHS RN with (d) Ni<sup>II</sup> (e) Pb<sup>II</sup>, (f) Cd<sup>II</sup>.

River showed a high degree of humification, and more likely diverse chemical groups, in its structure, which can be related to the larger CC observed for Ni<sup>II</sup>, Cd<sup>II</sup> and Pb<sup>II</sup>.

## Conclusions

The AHS RN presented higher aromatic fractions, and larger size molecular structures, when compared to the AHS from RC. The bands in the infrared spectrum were observed at 3300 cm<sup>-1</sup> for the AHS RN, which is indicative of the presence of hydroxylated groups, such as those present in phenols, alcohols and carboxylic acids, favoring the complexation with metals. Also, the band observed at

1562 cm<sup>-1</sup> attributed to the stretching of the aromatic C bond. The effect of seasonality on AHS characteristics was also observed, indicating a higher degree of humification for HS extracted from samples representative of the flooded period. In the dry period the AHS RC received more autochthonous influence. Therefore, the characteristics of AHS are strongly dependent on origin and seasonality.

The complexing capacity values obtained from the AHS RN with the metal ions Cd, Ni and Pb, were higher than the values obtained from the study of CC with AHS RC, the results were corroborated by the characterization of AHS. The decreasing order of CC for the AHS RN was Ni<sup>II</sup> > Cd<sup>II</sup> > Pb<sup>II</sup>, while in AHS RC it

was  $Ni^{II} > Pb^{II} > Cd^{II}$ . Thus, the values enable us to know the amount of metal complexed in these blackwater aquatic environments, allowing to infer to their possible behavior in each aquatic environment and to contribute to the studies of the availability, transport, and toxicity. In this sense, if these metals are present and available to be transported, we conclude that the lower complexing capacity of AHS from the Carú River could mean higher bioavailability and toxicity of these metals in this aquatic environment.

## Supplementary Information

Supplementary data (Scatchard plots) are available free of charge at <http://jbcbs.sbq.org.br> as PDF file.

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## Author Contributions

Isabela C. Constantino was responsible for methodology (equal); Juliana G. Viana for methodology (equal); Priscila A. Teixeira for methodology (equal); Altair B. Moreira for conceptualization (equal), formal analysis (equal); Giovanna P. Gama for methodology (equal); Fabiana M. M. Paschoal for formal analysis (equal); Ézio Sargentini Jr. for formal analysis (equal); Márcia C. Bisinoti for funding acquisition (equal), investigation (equal) and project administration (equal).

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