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Article

Influence of Seasonality on the Interaction of Mercury with Aquatic Humic Substances Extracted from the Middle Negro River Basin (Amazon)

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As elevadas concentrações de mercúrio em diferentes matrizes ambientais na Amazônia têm sido atribuídas a atividades mineradoras. Entretanto, altas concentrações de mercúrio também estão presentes no solo e na água em locais como a Bacia do médio Rio Negro, distantes de fontes emissoras antropogênicas. A região Amazônica é caracterizada por dois diferentes regimes sazonais, bem definidos em períodos de cheias e vazantes. O objetivo deste trabalho foi investigar as influências sazonais na interação entre mercúrio e substâncias húmicas aquáticas (AHS), que são os principais complexantes orgânicos naturais. Os resultados obtidos por análise estatística multivariada dos dados mostraram que as substâncias húmicas possuem diferentes características estruturais, dependendo do período sazonal. A capacidade das substâncias húmicas em formar complexos com Hg(II) não está diretamente relacionada ao seu teor de carbono, mas sim à natureza e disponibilidade dos grupos funcionais presentes em sua estrutura. Os grupos funcionais carboxílicos e aromáticos estão diretamente relacionados à maior capacidade de complexação de AHS por íons mercúrio.

High mercury concentrations in different environmental matrices in the Amazon have been attributed to mining activities. However, high concentrations of mercury are also present in the soil and water in places like in the middle of the Negro River Basin, which is far away from any anthropogenic emission sources. The Amazon region is characterized by two different regional seasons, with well-defined flood and low water periods. The objective of this work was to investigate the seasonal influences of the interaction between mercury and aquatic humic substances (AHS), which are the main agents of the natural organic complexation capacity. The results of the multivariate statistical analysis of the data showed that the humic substances had different structural characteristics, depending on each season. The ability of humic substances to form complexes with Hg(II) is not directly related to their carbon content, but to the nature and availability of the functional groups present in its structure. The functional groups are carboxylic and aromatic directly related to the higher complexation capacity of AHS by mercury ions.

Keywords: aquatic humic substances, seasonality, Negro River Basin, Amazonia, mercury

Introduction

Although the Negro River Basin is distant from anthropogenic emission sources, elevated mercury concentrations have been found in a variety of environmental matrices in this region of Amazonia. There are indications that this mercury derives from the weathering of rocks, with leaching through soils being the main means of its transport to aquatic systems.[1]

The Negro River, one of the principal tributaries of the Amazon, contains high levels of organic matter (OM), and since the biogeochemical cycling of mercury

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includes the involvement of OM, the river can contain high concentrations of the metal.[2] The nature of OM is influenced by the alternating flood and low water regime,[3] and its interaction with mercury influences the biogeochemical cycle of the metal in terms of its transport, speciation, solubility and bioavailability.[1,2,4,5]

An important characteristic of the biogeochemical cycle of mercury is the variety of routes that it can take in the environment, including its release at the soil/atmosphere and water/atmosphere interfaces, atmospheric transport, and deposition to soil and water surfaces. In contact with soils and sediments, sorption of mercury in insoluble form may be followed by methylation/demethylation. The cycle is completed by the incorporation of mercury species in precipitation, bioconversion into volatile and soluble forms, release to the atmosphere or bioaccumulation in aquatic organisms.[6-8]

The toxicity of mercury is one of the main reasons for scientific investigation of its behavior. In the Amazon region, Branches *et al.*[9] identified symptoms such as vertigo, palpitations, headaches and trembling in patients exposed to mercury vapor. From an epidemiological perspective, the main concern is exposure of riverside communities to organomercury compounds such as methylmercury. The clinical consequences of human exposure to methylmercury and other organomercury compounds are potentially serious, and include neurological effects that can be irreversible. Due to its affinity for adipose tissues, methylmercury tends to bioaccumulate faster than other mercury species.

Complexation with organic matter limits the availability of mercury to the microorganisms responsible for the methylation, and therefore also reduces the bioaccumulation of methylmercury. The main components of the organic matter present in soil, sediments and waters are humic substances (HS), which are refractory materials that have an undefined composition. The apparently large molecular size of humic substances is actually a supramolecular structure of small bio-organic molecules of a molecular mass smaller than 1000 Da, held together by weak forces, such the Van der Waals force.[10,11] Most of the environmental reactivity of HS is controlled by the content of acidic functional groups and their position in the conformational structures of those substances.[12] Although the characteristics and properties of these substances have been studied for 200 years, the interest in aquatic humic substances (AHS) has intensified over the last 30-40 years due to the greater importance in the awareness of the chemical quality of water destined for human consumption. Hence, studies of the HS properties related to the transportation, liability and complexation of metal species in aquatic systems are essential in order to better understand the behavior of these species in the environment.[13]

The strength and nature of bonding between AHS and metals are directly influenced by the molecular size of AHS, whose molar masses can vary between < 5 and > 100 kDa. Molecular size fractionation procedures can be used to reduce the polydispersion of AHS to obtain distinct fractions possessing similar properties and to enable the characterization of their interactions between metal species.[14] In order to elucidate the dynamics of mercury in the middle Negro River Basin (Amazon), a project was developed and supported by FAPESP (São Paulo Research Foundation, Brazil), with the objective to propose the biogeochemical cycle of mercury in the region. Since there are many factors that can contribute to the mobility of mercury in the environment, this study aimed to achieve broader conclusions and to investigate the interaction between AHS, major organic complexation in the environment, and mercury considering the seasonal influence in this process.

Experimental

Reagents

Analytical grade reagents (suprapur, Merck AG) were used, and the solutions were prepared using deionized water (Milli-Q, Millipore).

Water sample collection

Collection of water samples was undertaken on a monthly basis over a period of one year in order to explore the influence of seasonality on the structural characteristics and behavior of AHS in the Amazonian environment. The water sample collection points were situated on the left hand bank of the Negro River, between the Tarumã Mirim River and the Tarumã Açu River tributaries, approximately 20 km west of the Manaus City (GPS: 3 02' 22S 60 08' 21W).

Extraction of the aquatic humic substances using XAD-8 resin

XAD-8 macro-porous resin used to extract AHS was previously purified by successive processing with hydrochloric acid (0.50 mol L^{-1}), sodium hydroxide (0.50 mol L^{-1}) and methanol (24 h for each process). The procedure most used by researchers of the area in accordance with the International Humic Substances Society (IHSS)[15] was used for extraction. The samples were filtered under vacuum using Whatman No. 42 filter papers, acidified with

6.0 mol L^{-1} hydrochloric acid solution until a pH value of 3.0 was obtained, and percolated under gravity at a flow rate of 4 mL min^{-1} through glass columns (2 cm internal diameter and 25 cm tall) packed with XAD-8 resin. After saturation indicated by darkening of the resin, a volume of 10 mL of 0.01 mol L^{-1} hydrochloric acid was used to remove impurities, and the columns were then eluted with 0.10 mol L^{-1} sodium hydroxide at a flow rate of 1.2 mL min^{-1}. After the first elution, the resin was purified and the process repeated. The pH value of the final humic extract was adjusted to 5.0 (a pH similar to that of the Negro River water samples) using 0.10 mol L^{-1} hydrochloric acid.

Purification of the humic extract

Seamless dialysis membranes were prepared according to the procedure described by Town and Powell.[16] After treatment for 10 min each in a 2% solution of sodium bicarbonate and a 0.01 mol L^{-1} solution of EDTA (ethylenediaminetetraacetic acid) dissodium salt dihydrate, the membranes were washed with deionized water at 65 ºC and refrigerated at ca. 4 °C. The humic extract was concentrated using a rotary evaporator, and purified against deionized water using the dialysis membrane until it tested negative for chlorides (using 0.10 mol L^{-1} silver nitrate solution). After removing the excess of dissolved salts by dialysis, the humic extract was lyophilized according to the procedure described by Vigneault *et al.*[17] and the material was then stored in petri dishes in a desiccator.

Nuclear magnetic resonance (^{13}C NMR)

^{13}C NMR experiments with cross polarization (CP), magic angle rotation (MAS) and variable amplitude (VA) were done in a Varian Spectrometer (Unity Inova 400 model). The samples were conditioned in a cylindrical zirconium rotor, which was 5 mm in diameter (Doty Supersonic) and, rotated at 6 KHz in a Doty Supersonic probe to make the solid samples. The VACP/MAS ^{13}C NMR spectra were obtained under the following experimental conditions: a resonance frequency of 100 MHz for ^{13}C, a spectral band of 20 KHz, a proton preparation pulse of 3.8 μs, a contact time of 1 ms, an acquisition time of 12.8 ms and an interval of 500 ms. Hexamethilbenzene (HMB) was used as a reference of the chemical shift in the spectra.

Digestion of the AHS samples and the determination of mercury

A volume of 5.0 mL of 30% hydrogen peroxide solution was slowly added to 0.20 g of AHS, under agitation. When the sample was completely dissolved, 15.0 mL of sulfonitric acid solution were slowly added, using an ice bath. The solution was heated for 1 h at a temperature of 70 ºC, cooled to room temperature, and 10.0 mL of potassium permanganate (7.5%, m/v) were added. After 15 min, a volume of 5.0 mL of potassium persulfate (8%, m/v) was added. The solutions containing the AHS samples were heated for 2 h at 70 ºC, and then left to rest for 12 h. Sufficient hydroxylamine chloride solution (12%, m/v) was added to reduce the remaining potassium permanganate, and mercury determinations were performed shortly thereafter, using atomic absorption spectrometry, Analyst 300-Perkin Elmer, with cold vapor generation.[2,18]

Fractionation of AHS extracted from the water samples

AHS fractionation was performed as described by Sargentini Jr. *et al.*,[14] 250 mL of AHS (1.0 mg mL^{-1}, pH 5.0) were pumped through the sequential system for fractionation by ultrafiltration (SSFU), with collection of the following molecular size fractions: > 100 kD (F_1), 50-100 kD (F_2), 30-50 kD (F_3), 10-30 kD (F_4), 5-10 kD (F_5) and < 5 kD (F_6).

Determination of total organic carbon (TOC)

Measurements were made using the catalytic combustion technique with infrared detection (Shimadzu TOC-5000 Analyzer), in an oxygen atmosphere. The white limit detection (3σ) of the technique was 0.1 mg L^{-1} TOC.

Determination of the complexation capacities of dissolved organic matter and AHS for Hg(II) ions

The analytical procedure proposed by Burba *et al.*[19] and adaptated by Pantano *et al.*[20] and Serudo *et al.*[13] was used to determine the complexation capacities of the various AHS fractions for Hg(II) ions (Figure 1). This technique is based on a tangential ultrafiltration system (Sartorius Ultrasart X), fitted with a 1 kDa cellulose membrane to prevent passage of humic substances and HS-metal complexes having molecular sizes greater than 1 kDa. The membrane allows passage of free metal ions that are not either complexed with HS, or that have been exchanged. Prior to addition of the mercury solution, the membrane was conditioned by pumping AHS solution (250 mL) through it for around 5 min. The first aliquot (2 mL) was then filtered, corresponding to time zero (i.e., prior to addition of the mercury solution). 2 mL volumes of a solution of 1 mg L^{-1} Hg(II) in 1% (v/v) nitric acid were added to the AHS solutions. 2 mL aliquots of the solution fractions containing Hg(II) ions isolated by the filtration procedure were collected following establishment of equilibrium, which was reached after 15 min under constant agitation. Bromine chloride solutions were added to the filtrates to fix Hg^{2+} and prevent any losses. Hydroxylamine chlorohydrate (12%, m/v) was added about 5 min before analysis using cold vapor atomic absorption spectroscopy. A flask containing potassium permanganate was connected to the system in order to capture and to subsequently quantify any reduced mercury present (Figure 1).

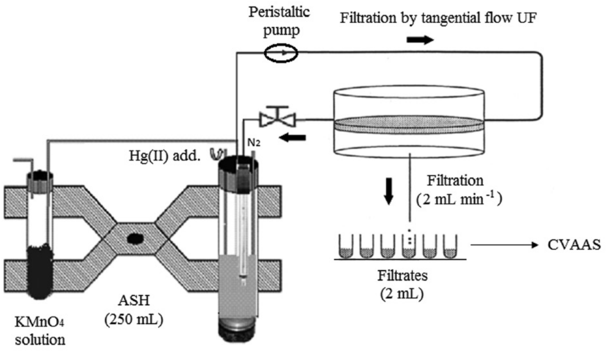

Figure 1. Scheme of the system used to determine the complexation capacity for Hg(II) ions of aquatic humic substances extracted from samples collected at the Negro River. Conditions: Sartorius Ultrasart X ultrafiltration system fitted with 47 mm diameter, 1 kDa porosity, cellulose membrane (Gelman Pall-Filtron OMEGA), AHS solution pH 6.0, and solution of 0.024% (m/v) potassium permanganate in 4% (v/v) sulfuric acid solution.

Results and Discussion

Seasonal flooding in the middle Negro River Basin increases the river level by 8-10 m, and for the study period the peak and lowest water levels occurred in July and November 2002, respectively (Figure 2).

Characterization of AHS extracted from the water samples, which were monthly collected revealed seasonal differences in their molecular structures.[2] Statistical analysis was used to classify AHS into three groups according to the degree of humification, which corresponded to the periods in which they were collected: March-May 2002, June-October 2002 and November 2002-February 2003. One sample of AHS from each group was selected for molecular size fractionation and analysis of the carbon content distribution and complexation capacity for mercury ions. The samples selected for fractionation were those collected in March, July and December 2002. According to Thurman,[21] around 50% of the dissolved organic carbon comprises fulvic and humic acids, with aquatic humic substances generally consisting of 90% fulvic acids and 10% humic acids.

Significant differences between the samples collected during both the high and low water periods were observed for both carbon distributions and the complexation capacities of the different AHS molecular size fractions (Figure 3). In the case of AHS from the March 2002 sample, approximately 65% of the carbon content was equally distributed between the fractions F_1 and F_6. For the July 2002 sample, F_1 (> 100 kDa) showed the highest carbon content, with reduced amounts in F_2 and F_5. In this situation, the total amount of carbon in fractions F_1, F_2 and F_3 was approximately 78%, indicating a predominance of carbon in the first fractions.

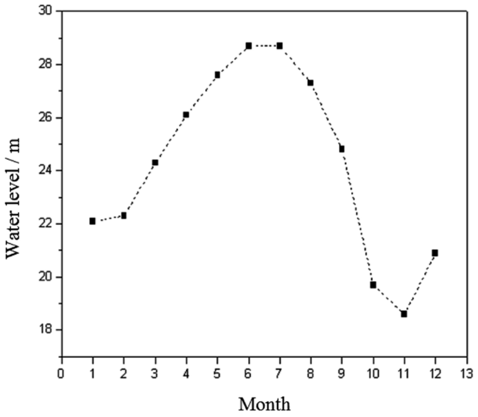

Figure 2. Monthly mean water levels of the Negro River (source: National Institute for Amazonian Research (INPA)).

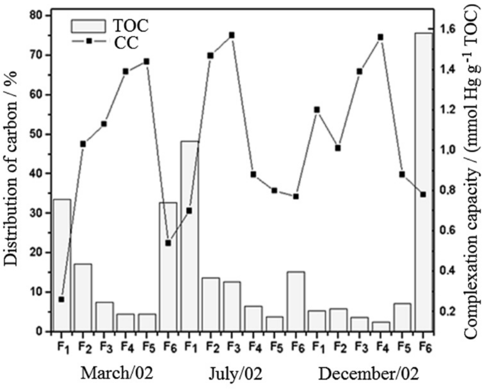

Figure 3. Carbon content distribution and complexation capacity for Hg(II) ions of the different molecular size humic fractions extracted from water samples, collected from the Negro River in March, July and December 2002 and fractionated using multiple stage tangential flow sequential ultrafiltration.

According to Guetzloff and Rice,[22] humic acid and fulvic acid isolated from the same environment are composed of molecules having distinct sizes, which are larger for humic acid and smaller for fulvic acid. This is also reflected in the molecular size interval of aquatic particles. It can therefore be inferred that the July 2002 sample contained more larger molecules (humic acid), and since AHS are typically composed of 90% fulvic acids and 10% humic acids, during this period there was a large influence of organic soil matter, in agreement with the higher river level during this period and the typically alloctone origin of humic substances found in rivers and creeks, deriving from soil and organic plant matter.[21] These results are in agreement with the ones obtained by Bisinoti *et al.*,[6] indicating that the increase of fresh OM associated with the increase in the water level causes a major change in the water chemistry of Amazonian black waters.

From Figure 3, it can be seen that for HS extracted from water collected in December 2002, fraction F_6 contained about 75% of the total carbon content, showing that this sample preferentially contained smaller molecules.

A seasonal influence on AHS is evident from the distribution of carbon in the different fractions obtained by sequential fractionation. Table 1 lists, in decreasing order, the total organic carbon contents and the complexation capacities of the different AHS size fractions extracted from samples collected in March, July and December 2002. From the carbon distributions, it can be seen that the highest carbon contents were in fractions F_1 and F_6 from the March sample. For the July and December samples, fractions F_1 and F_6 contained most carbon, respectively. For the March sample, the same fractions (F_1 and F_6) showed the lowest Hg(II) complexation capacity. Although the F_1 and F_6 fractions probably contained larger and smaller molecules, respectively, their complexation capacities were similar, which could be related to the conformation adopted by the molecules, leading to a molecular arrangement that influenced their complex similarly in both cases. For July, the lowest complexation capacity was observed in F_1, while for December F_6 and F_4 showed the lowest and highest complexation capacities, respectively. For this group of samples, it can be seen that those showing lower complexation capacities generally had higher carbon contents. Therefore, it can be inferred that the quantity of carbon present in a sample of HS is not proportional to the number of functional groups present.

Environmental studies can generate large quantities of experimental data, and exploratory statistical techniques are useful to correlate variables and assist in the interpretation of results. Hierarchical grouping analysis was used here to examine the seasonal influence on the AHS samples fractionated according to the molecular size. In Figure 4A, the formation of two groups can be seen: (a) pluviometric index and total carbon content and (b) AHS complexation capacity and fluviometric index. In Figure 4B, three main sample groupings can be seen: (a) F_1 and F_6 for March and F_6 for December, (b) F_2, F_3, F_4 and F_5 for March and F_1, F_2, F_3, F_4 and F_5 for December and (c) F_1, F_2, F_3, F_4, F_5 and F_6 for July. Group (a) is essentially composed of fractions showing the lowest complexation capacity and the highest carbon content. In groups (b) and (c), it can be seen that fractions having most similarity were those from the same

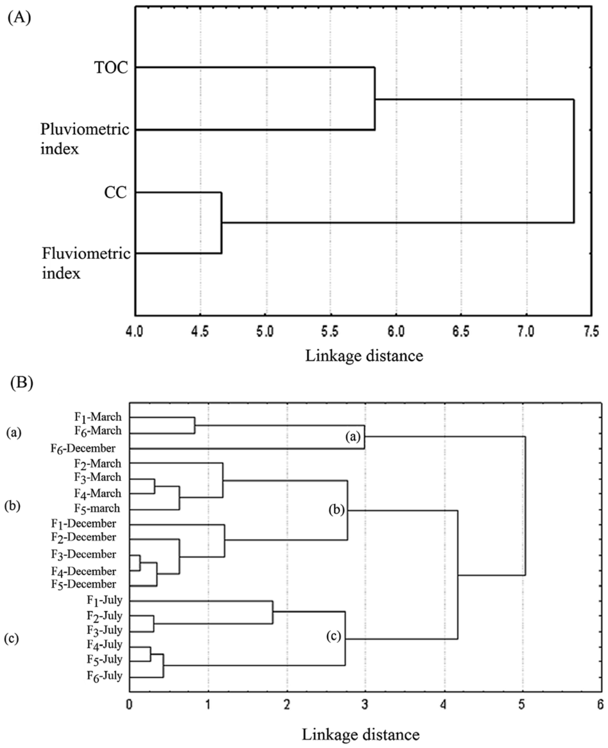

Figure 4. (A) Dendrogram obtained from determinations of the complexation capacities of aquatic humic substances for Hg(II) ions, total organic carbon contents, and pluviometric and fluviometric indices, and (B) dendrogram for aquatic humic substances extracted from the Negro River water, fractionated according to molecular size: > 100 kDa (F_1), 100-50 kDa (F_2), 50-30 kDa (F_3), 30-10 kDa (F_4), 10-5 kDa (F_5) and < 5 kDa (F_6).

Table 1. Decreasing orders of organic carbon contents and complexation capacities for different molecular size fractions of aquatic humic substances extracted from the Negro River water samples

Sample	Parameter	Decreasing order
July 2002	total organic carbon	$F_1 > F_6 > F_2 > F_3 > F_4 > F_5$
	complexation capacity of AHS for Hg(II) ions	$F_3 > F_2 > F_4 > F_5 > F_6 > F_1$
December 2002	total organic carbon	$F_6 > F_5 > F_2 > F_1 > F_3 > F_4$
	complexation capacity of AHS for Hg(II) ions	$F_4 > F_3 > F_1 > F_2 > F_5 > F_6$
March 2002	total organic carbon	$F_1 > F_6 > F_2 > F_3 > F_4 \approx F_5$
	complexation capacity of AHS for Hg(II) ions	$F_5 > F_4 > F_3 > F_2 > F_6 > F_1$

Table 2. Peak assignments for the ^{13}C NMR spectra of the aquatic humic substances extracted from the Negro River and their integrated areas (%)

Sample	Integration / %					HI	HB	HI/HB	Hg(II) / (μg g^{-1})
	0-45 ppm aliphatic	45-65 ppm C–O, C–N	65-90 ppm O–aliphatic	90-155 ppm aromatic	155-190 pm carboxil				
March 2002	50.1	3.0	17.1	9.3	9.6	59.4	29.7	2.0	8.24
December 2002	34.3	2.6	28.7	19.8	10.1	54.1	41.4	1.3	35.32
July 2002	45.1	2.8	24.3	9.2	9.3	54.3	36.4	1.5	6.70

month. Therefore, there was distinction according to the seasons, but not according to molecular size. Due to the flood/low water regime of the Amazon basin, the seasonal factor appears to be predominant in influencing the nature of AHS characterized according to the molecular size.

The complexation capacities of AHS for Hg(II) ions were determined in the original samples, before making any fractionation. The values obtained were: 1.74 mmol of Hg g^{-1} of TOC in December, 0.85 mmol of Hg g^{-1} of TOC in July and 1.04 mmol of Hg g^{-1} of TOC in March. Therefore, it is apparent that during the flood period (in July) the AHS complexation capacity was lower, while during the low water period it was higher.

Studies proposed by Serudo *et al.*[13] and Bisinoti *et al.*[23] mentioning that in soils from this region, the highest complexing capacity of soil humic substances (SHS) occurred in flooded soils. These results suggest that there may be a competition between AHS and SHS during the flood periods. Thus, the full period of the SHS complex with a higher concentration of mercury, being a lower mercury concentration in the environment available to complex with the AHS.

To further clarify the interaction between mercury and AHS, it was made of the correlation results of complexation with ^{13}C NMR data of AHS to verify the functional group which will be the primarily responsible for the complexation of mercury ions.

The calculation of the percentages of carbon groups in the structures of HS was made based on the following chemical shifts: 0-45 ppm: aliphatic carbons, 45-65 ppm: C–O and C–N groups; 65-90 ppm: O–aliphatic carbons, 90-155 ppm: aromatic carbons and 155-190 ppm: carboxylic carbon.[24] The percentages obtained for the aliphatic carbons and aromatic carbons were assigned to hydrophobic, while the groups C–O, C–N, O–alkyl and carboxylic assigned the hydrophilic carbon. Thus, the percentages of carbon-carbon non-polar and polar were used to calculate the hydrophobic index (HB) and hydrophilic index (HI), respectively (Table 2).[24]

The HI/HB ratio provides an estimate of the capacity of HS compounds interacting with different polarities. High HI/HB ratio represents a higher affinity interaction of humic compounds with higher polarity.[24] Thus, the AHS sample extracted from water samples collected in March is classified as more hydrophilic. The December sample can be classified as more hydrophobic. According to the mercury contents quantified in three samples, it is noted that the December sample quantity of mercury has a much higher than the other two, it can be inferred a directly proportional relation between hydrophobicity and mercury ions present in the AHS samples.

According to the linear relationship obtained from the Pearson correlation between data and ^{13}C RMN complexation of HS by mercury ions (Table 3), it can be seen that the aromatic carboxylic groups are strongly correlated with the ability of complex mercury, corroborating data literature.[23-25] The ratio of carboxylic groups is possibly due to the fact that these groups are the most oxidated and therefore related to the most refractory OM. In literature,[13,18] some authors have examined the relation between the complexing ability of metal species and degree of humification of OM, that is, the greater the degree of humification, the greater complexing ability of certain metal species. In general, the study of the relationship between AHS functional groups determined by ^{13}C NMR may indicate which of the samples is more efficient for the complexation of metal species influencing its availability in the environment.

The results of these studies led to a widespread tendency in changing the structural characteristics of AHS

Table 3. Parameters of correlation between the ^{13}C NMR data and the complexation capacities of the humic substances by mercury ions

Group	Linear parameter		
	ax	b	R^2
Aliphatic (0-45 ppm)	–0.0504	3.386	0.754
C–O; C–N (45-65 ppm)	–1.75	6.11	0.558
O–Aliphatic (65-90 ppm)	0.0518	–0.0015	0.4196
Aromatic (90-155 ppm)	0.0755	0.2464	0.9621
Carboxil (155-190 ppm)	1.1418	–9.8278	0.9693

R^2: correlation coefficient.

Table 4. Tendencies for changes in the characteristics of the Negro River aquatic humic substances, as a function of season

Parameter	Flood	Low water
Organic matter	recent	refractory
HS molecular size	larger molecules	smaller molecules
Total organic carbon	greater in larger fractions	greater in smaller fractions
HS complexation capacity	smaller	greater

as a seasonal function, including what time of years the largest complexation of mercury because of the structural characteristics of AHS, minimizing the availability of mercury in the environment, directly influencing the cycle mercury in the region studied (Table 4).

Conclusions

The distribution of carbon in the different AHS size fractions was indicative of significant differences between samples in the structures of the humic substances. In December, the period of low water in the Rio Negro, humic material was preferentially composed of smaller molecules. In July (high water), larger molecules were present, while an intermediate composition was obtained in March. The low water level in December suggests that there was less influence of soil organic matter at this time, in contrast to the July high water period when AHS showed a greater influence of soil organics. These findings demonstrate the influence of seasonality on formation of aquatic humic substances, with the fractionation procedure confirming that AHS were composed of substances having different structures according to season.

The complexation capacity, for Hg(II) ions, of AHS in different size fractions indicated that, in general, the greater the carbon content, the lower the complexation capacity. It can therefore be inferred that the latter was not directly related to carbon content, but possibly to the type and availability of the functional groups present. Multivariate statistical analyses indicated that the samples could be grouped according to the month of collection and rather than to molecular size.

The results obtained in this study showed that AHS extracted from samples from the middle of the Negro River Basin have a change in the structural characteristics as a seasonal function, directly related to the complex of mercury ions, which shows the importance of this process in the mobility of mercury in the middle of the Negro River basin and the natural cycle of mercury in the Amazon region.

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