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Article

Solar Radiation Effect on the Complexation Capacity of Aquatic Humic Substances with Metals

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Este estudo teve como principal objetivo avaliar o efeito da radiação solar sobre a capacidade de complexação das substâncias húmicas aquáticas (AHS) com as espécies metálicas Al^{3+}, Cr^{3+}, CrO_4^{2-} e Ni^{2+}. Microcosmos foram montados com AHS extraídas do Rio Preto com concentração de 5,0 mg L^{-1} de carbono orgânico dissolvido e diferentes concentrações de metais, sendo expostos à radiação solar *in situ*. As constantes de estabilidade condicional foram de $2,0 \times 10^{-2}$, $1,1 \times 10^{-5}$ e $2,6 \times 10^{-2}$ para os complexos AHS–Cr^{3+}, AHS–Ni^{2+} e AHS–CrO_4^{2-}, respectivamente. Os resultados mostraram que a radiação solar foi responsável por um decréscimo na capacidade de complexação de 15-26% para AHS–Al^{3+}, 15-72% para AHS–Cr^{3+}, 12-18% para AHS–CrO_4^{2-} e de 13-42% para AHS–Ni^{2+}. Conclui-se que a radiação solar altera a disponibilidade de metais previamente complexados às AHS, tornando-os disponíveis no corpo aquático.

This study had as main objective to assess the effect of solar radiation on the capacity of the aquatic humic substances (AHS) to complex Al^{3+}, Cr^{3+}, CrO_4^{2-} and Ni^{2+}. Microcosms were prepared with AHS from Preto River containing 5.0 mg L^{-1} of dissolved organic carbon and different concentrations of metal species, being exposed to solar radiation, *in situ*. Conditional stability constants obtained for the AHS–Cr^{3+}, AHS–Ni^{2+} and AHS–CrO_4^{2-} complexes were 2.0×10^{-2}, 1.1×10^{-5} and 2.6×10^{-2}, respectively. The results showed that solar radiation is responsible for decreasing capacity complexation in 15-26% for AHS–Al^{3+}, 15-72% for AHS–Cr^{3+}, 12-18% for AHS–CrO_4^{2-} and 13-42% for AHS–Ni^{2+}. We can conclude that solar radiation alters metals' availability previously complexed to AHS, making them available in the aquatic body.

Keywords: aquatic humic substances, metal complexation, solar radiation, fluorescence

Introduction

Aquatic humic substances (AHS) have been intensively studied because they play an essential role in biogeochemical processes.[1,2] They can participate in the complexation reactions with metal ions thereby influencing the transport and availability of them in the environment,[2-4] may suffer interactions with organic compounds and thus, reduce or increase its toxicity to the biota,[4,5] can act in redox[6] and photochemical reactions.[7]

Aquatic HS are products of the decomposition of vegetal and animal remains from microbiological transformations. They are normally dark colored organic macromolecules with a high molecular weight, a complex structure consisting of aromatic and aliphatic compounds, which have a large number of functional groups containing oxygen, e.g., carboxylates (–COOH), phenolics (aromatics–OH), alcoholics (–OH), quinones (aromatics=O), among others.[1,2] Depending on the water solubility, AHS can be operationally separated into three fractions: fulvic acids that are soluble in all pH range, humic acids that are soluble in alkaline pH and insoluble in acid pH, and humin that is insoluble in all pH range. It is worth noting that the AHS comprise about 40-60% of the dissolved organic carbon (DOC) in aquatic environments[2] and, due to the heterogeneity of pre-existing materials in the water bodies, the composition of AHS varies from one to another environment according to the source of organic matter, pH, temperature and weather conditions like rainfall and mainly to the photochemical processes.[8,9]

The ability of AHS in complexing metal species is due to aromatic and aliphatic groups, which contains

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oxygen atoms responsible for the interactions with cationic species such as metals.[10] Studies have covered the AHS complexation with different metals under different pH conditions, ionic strength and concentration of metals and AHS.[11-15] A recent study demonstrated that solar radiation can also be an important factor in the complexation capacity of AHS with metals.[8,16] These authors showed that radiation alters the complexation capacity that the AHS had when not exposed to radiation previously.[16-19] The role of solar radiation on the complexation capacity is not well understood mainly in tropical regions of the world where solar radiation is intense. Most studies dealing with the capacity complexation of AHS are made with Cu^{2+}. On the other hand, so far metals such as Al, Cr and Ni are poorly studied despite their environmental relevance.

Studies of the complexation capacity of AHS can be made using various analytical techniques such as polarography, voltammetry, ion selective electrode, ultrafiltration and molecular fluorescence.[20-22] Among them molecular fluorescence has been one of the techniques used in many works to characterize AHS,[23-26] as well as studies of complexation with metal ions.[27-29] The fluorescence has advantages over others, such as nuclear magnetic resonance (NMR) which is also widely used in AHS studies.[30-32] Using fluorescence is possible to work with liquid samples and thus eliminating physical and chemical treatments which could alter AHS structure, and also enables the use of small samples, as well as having good sensitivity, robustness and its low cost. The fluorescence analysis of humic substances generate information about the structure, conformation and heterogeneity.[33,34] The fluorescence excitation-emission matrix (EEM) is a tool that allows to obtain better information on the existence of fluorophores and consequently on the chemical structure of AHS.[35]

Several authors have demonstrated that metals are responsible to quench the fluorescence signal,[36-38] but in some cases it is possible to increase it.[39] In cases of quenching of the fluorescence signals is possible to determine the conditional stability constant (K_C) of AHS-metal complex using the Stern-Volmer model.[40-43] The K_C provides information as to whether or not favoring the formation of AHS-metal complex, where higher values indicate K_C favoring the complexation of metals in AHS. The Stern-Volmer model allows the calculation of K_C from experiments where the metals are being gradually added to the AHS and the fluorescence signal is begin followed after each addition. Due to the aforementioned, the goal of this work is to evaluate the effect of solar radiation in the complexation capacity of AHS with metals species, such as Al^{3+}, Cr^{3+}, CrO_4^{2-} and Ni^{2+}.

Experimental

Collecting water samples and isolation AHS

Water samples were collected during rainy season from Preto River, São José do Rio Preto city, São Paulo state, Brazil, S 20º 48’ 29.2” and W 049º 22’ 24.1” (Figure 1). The aquatic body chosen in this research is employed for public water supply and it has been considered in previous works,[36] where AHS from rainy season show higher capacity complexation with metals. The water in this region is tropical hot, with rainy summers and temperatures of about 27 °C.

Nearly 400 L of water samples were collected using larger flasks pre-cleaned and acclimatized with water from the river 30 cm below the surface. Temperature, pH, conductivity, turbidity and dissolved oxygen were measured *in situ* using multiparameter equipment previously calibrated in the laboratory and, when necessary, in field (Hanna, HI991300 and Hanna, HI9146-04, Romania). Water samples were filtered in qualitative paper for the removal of small branches and leaves and the pH was adjusted to approximately 2 with 6 mol L^{-1} HCl. AHS extraction were made using the method suggested by the International Humic Substances Society (IHSS).[44] The pH was adjusted to the original 6.8 pH of the sample with 1.0 mol L^{-1} HCl.

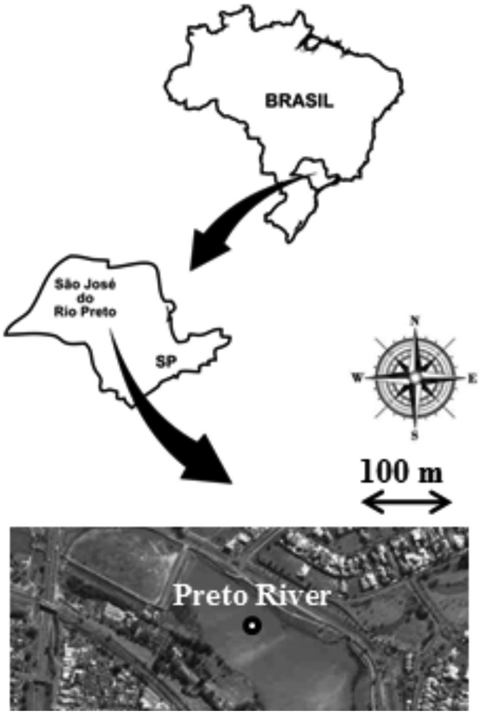

Figure 1. Map of the sample site, Preto River, São José do Rio Preto, São Paulo State, Brazil.

Molecular fluorescence measurements

All fluorescence measurements were made using a spectrofluorimeter (Varian, Cary Eclipse, Australia) equipped with Xe lamp. In the experiments of complexation capacity the fluorescence spectra were obtained in two measurement modes: emission mode and synchronous mode. In both modes, a pre-scan of AHS sample was previously done, which indicated the best excitation wavelength. Emission mode was scanned from 350 to 650 nm with an excitation wavelength fixed at 332 nm. Synchronous mode spectra were obtained from 240 to 700 nm using a wavelength interval of 18 nm. All spectra were obtained with slits set at a 10 nm bandwidth and measurements were performed in triplicate. In the experiments that evaluated the effect of solar radiation on the complexation capacity, the fluorescence spectra were obtained only in emission mode from 350 to 650 nm with excitation fixed at 332 nm and slit of 10 nm bandwidth. For the AHS sample extracted from Preto River was made fluorescence spectra in emission and synchronous modes and also in excitation-emission matrix (EEM) mode. In this EEM, the spectra was obtained by colleting a series of scans, 400-600 nm for emission and 300-500 nm for excitation in increments of 5 nm.[24]

Quantification of dissolved organic carbon (DOC)

Dissolved organic carbon was measured using a total organic carbon analyzer (Shimadzu, TOC-VCSN, Japan) after filtering the water samples.

AHS-metal complexation capacity and determination of the conditional stability constant (K_C)

Complexation capacity studies were made individually with Al^{3+}, Cr^{3+}, CrO_4^{2-} and Ni^{2+} metal ions. The metal solutions were prepared using reagents from pure analytical grade ($AlCl_3.6H_2O$, $NiCl_2.6H_2O$ and K_2CrO_4 from Synth, Diadema, Brazil; $CrCl_3.6H_2O$, from Sigma/Aldrich) in ultrapure water (Millipore, Direct-Q, France). First, an experiment was done to evaluate the equilibrium time to reach complexation capacity of metals and AHS. So, 50.0 mL of AHS containing 5.0 mg L^{-1} of DOC was transferred to a magnetically stirred beaker with an aliquot of metal for a pre-determined period of time, being fluorescence signals in the emission and synchronous mode obtained for all samples. DOC concentration of 5.0 mg L^{-1} was chosen based on the DOC found in the Preto River (3.3 mg L^{-1}) and previous study made in laboratory about complexation capacity.[45] The equilibrium time was determined when any changes in the fluorescence signal would be observed (30 min for Al^{3+} and 20 min for the other ions).

Complexation capacity studies were made with 50.0 mL of AHS containing 5.0 mg L^{-1} of DOC, kept on agitation, followed by the addition of known metal concentrations. Fluorescence signals in the emission and synchronous mode were measured after each metal addition. The addition of metals was only stopped when the fluorescence signal was stabilized. The pH was always maintained at about 6.0 and 7.0 to avoid precipitation of metals. Analysis of the coagulation process for each metal studied was evaluated employing a program for chemical simulation equilibrium (CHEAQS Pro),[46] which varied according to the composition of the samples.

In all experiments with metal addition where a quenching of the fluorescence signal was observed, conditional stability constants were calculated according to the Stern-Volmer model.[41] In this model, a graph of 1/Metal *versus* Io/ΔI was constructed where Io is the fluorescence intensity of the ligand when no metal is present and ΔI the initial fluorescence fraction which corresponds to the available fluorescent structure for complexation (ΔI = Io – I, where I = fluorescence intensity of the ligand due to added metal) and from the linearity of the graph (equation 1), the angular coefficient values ($1/f\,K_C$) and linear coefficient (1/f) were used to determine K_C.

$$\frac{Io}{\Delta I} = \frac{1}{f} + \frac{1}{(fK_C[M])} \qquad (1)$$

Solar radiation effect on the complexation capacity of AHS

The effect of solar radiation on the complexation capacity of Al^{3+}, Cr^{3+}, CrO_4^{2-} and Ni^{2+} metals was assessed with microcosm experiments. These experiments were made by exposing samples containing 5.0 mg DOC L^{-1} AHS and different concentrations of metals as a strategy to maintain the same conditions of temperature and solar radiation intensity that occurs in the aquatic body. Polyethylene terephthalate (PET) bottles were filled up with AHS and metals and they submerged at a depth of approximately 30 cm in the Preto River waters. A control group was kept in the laboratory (not exposed to radiation) plus two groups in the field exposed to solar radiation under light conditions (exposed in the field to solar radiation) and under dark conditions (exposed in the field but with the whole bottle wrapped in aluminum foil). A group containing only AHS with 5.0 mg L^{-1} of DOC without metals was also kept under each of the laboratory and field conditions (light and dark). The concentrations of metals used in field experiments were those previously tested in laboratory in the experiments to assess the complexation capacity of same AHS. A total of 40 samples containing AHS with 5.0 mg L^{-1} of DOC received known concentrations of each metal, being 0.4, 1.6 and 5.9 mg L^{-1} for Al^{3+}, 5.9, 13.7 and 43.7 mg L^{-1} for Cr^{3+}, 5.9 and 43.7 mg L^{-1} for CrO_4^{2-} and 0.5, 17.7 and 377.4 mg L^{-1} for Ni^{2+}. All these samples were kept in laboratory and field conditions under light and dark conditions.

At zero, 5 and 10 h of solar exposition (8:00 a.m., 1:05 p.m. and 6:15 p.m. respectively), the samples of each group were collected in field and transported to the laboratory conditioned in polyethylene boxes with ice. The distance from the locality in the field where the samples were exposed to the laboratory is 2 km, a trip made by car in 5 min. In the laboratory, the fluorescence spectra were obtained in emission mode as described previously.

A spectrum scan of UV-Vis at wavelengths of 200 and 800 nm of the PET bottle contents was obtained using a UV-Vis spectrophotometer. Water temperature was monitored using a thermometer.

Results and Discussion

The Preto River waters show quality where values found were pH 6.8, temperature 27.0 °C, dissolved oxygen 7.2 mg L^{-1}, conductivity 40.0 µS cm^{-1}, dissolved organic carbon 3.3 mg L^{-1} and turbidity 13.1 FTU.

Fluorescence spectra

Molecular fluorescence spectra obtained in emission and synchronous modes from AHS extracted from the Preto River, shows a maximum fluorescence signal at 436 nm in the emission spectrum (Figure 2a), which indicates the predominant structure of aliphatic compounds.[30] In the synchronous mode (Figure 2b), a maximum fluorescence intensity was observed in the range of 390-430 nm, being associated to aromatic polycyclic hydrocarbon having five conjugated rings, as well as fulvic acids.[30,47] EEM spectrum (Figure 2c) shows a maximum fluorescence intensity in $\lambda_{exc}/\lambda_{emi}$ 250/425 nm and in $\lambda_{exc}/\lambda_{emi}$ 340/430 nm, which correspond to fulvic and humic acids, respectively, according to the literature.[25,48,49] It stands out that maximum fluorescence intensity in $\lambda_{exc}/\lambda_{emi}$ 250/425 nm attributed to fulvic acids have a greater intensity. Therefore, it can be verified that AHS extracted from the Preto River waters have the characteristics of a recently degraded organic matter due to a predominance of aliphatic compounds in their structure, as seen from the spectra. These results are in agreement with the period of AHS isolation.

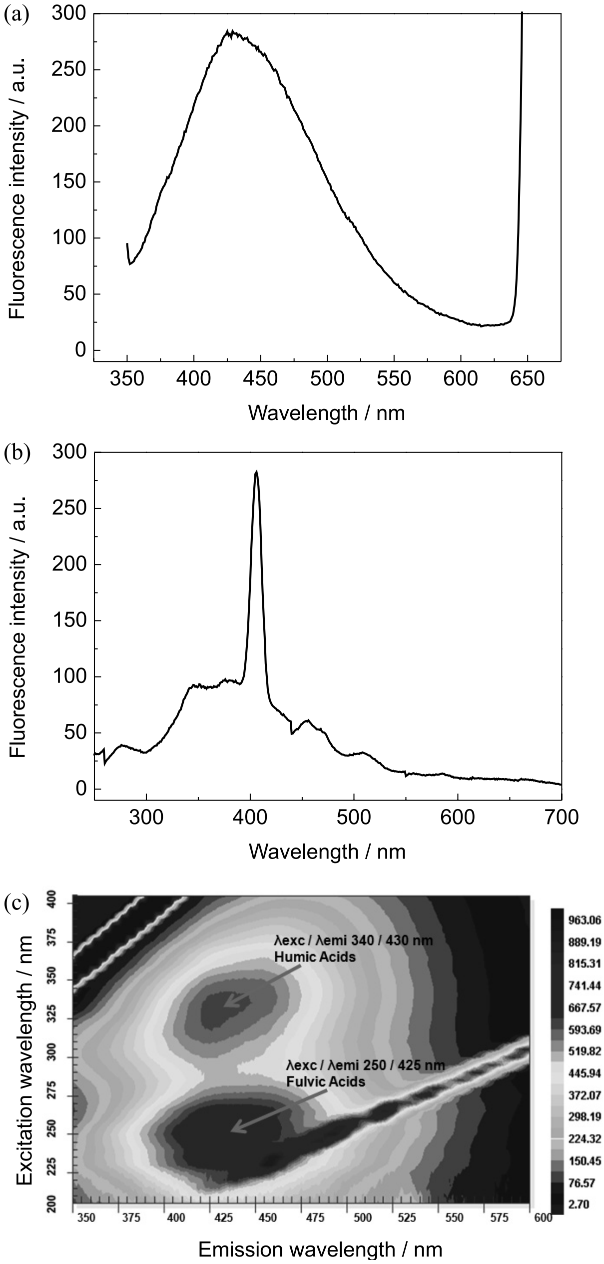

Figure 2. Fluorescence spectra in the emission (a), synchronous (b) and excitation-emission matrix (EEM) (c) modes for the AHS extracted from the Preto River waters.

Complexation capacity of AHS from the Preto River with Al^{3+}, Cr^{3+}, CrO_4^{2-} and Ni^{2+}

AHS are compounds that have the capacity of emitting fluorescence when excited by electromagnetic radiation due to their organic composition with conjugated aromatic rings. The AHS interaction with metal species cause, frequently, a quenching of initial fluorescence intensity.[37,38] With some metals this interaction can also promote an increase of fluorescence signal, as noted for aluminum.[39] Complexation capacity studies made in this work showed

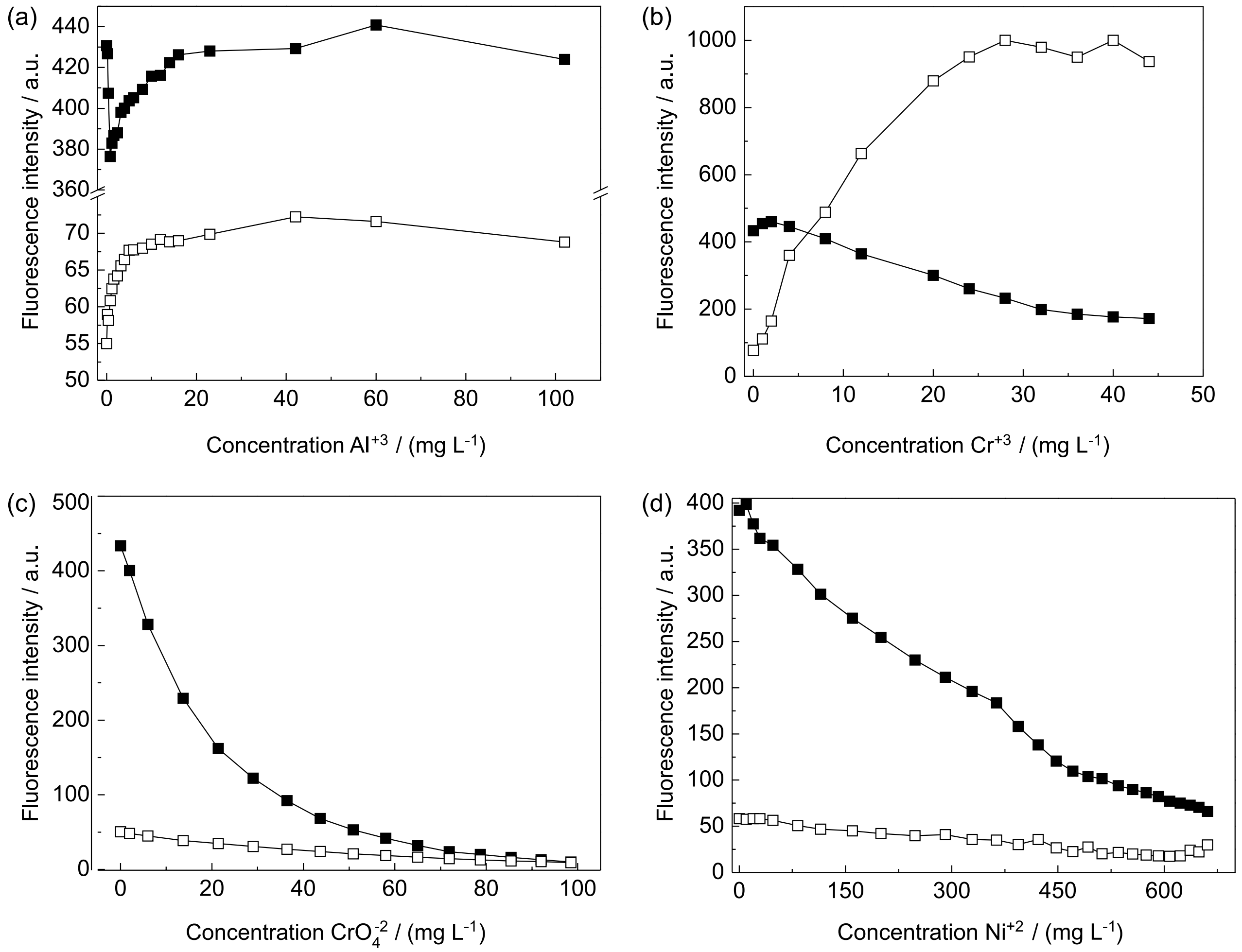

Figure 3. Fluorescence maximum intensity curves obtained in the modes emission (■) and synchronized (□) for the AHS−Al^{3+} (a), AHS−Cr^{3+} (b), AHS−CrO_4^{2-} (c) and AHS−Ni^{2+} (d) complexes.

that fluorescence intensity grows with an increase of the quantities of Al^{3+}. On the other hand, when adding Cr^{3+}, CrO_4^{2-} and Ni^{2+}, there was a quenching of fluorescence intensity. This behavior is shown in Figures 3a, 3b, 3c, and 3d.

The first fact that should be observed is that with the first additions of Al^{3+}, the emission mode fluorescence intensity decreased. Adding more Al^{3+}, the fluorescence signal increased until it stabilized where further additions had no effect. A similar fluorescence increase behavior was observed with spectra obtained in the synchronous mode. Sharpless and McGown[39] found similar behavior when assessing the complexation capacity of Al^{3+} with humic substance of aquatic source. According to these authors,[39] a decrease in intensity of the fluorescence signal with metal addition is common when humic substances are of terrestrial origin. Another fact that is worth mentioning is that the maximum emission fluorescence signal (431 nm for AHS without metal addition) is a bit shifted towards lower wavelengths (422 nm) in the emission spectrum. Elkins and Nelson[27] observed a 142% increase of the emission fluorescence intensity when Al^{3+} was added to the fulvic acid extracted from a river. These authors also evidenced a shift in the emission wavelength from 440 to 424 nm, and the excitation wavelength from 324 to 344 nm. These shifts were associated to a strong Al^{3+} interaction and high density charge which ends up significantly altering the electronic structure of the fulvic acid. In the synchronous spectrum, the maximum fluorescence signals where intensity increased referred to wavelengths of 366 and 406 nm, where 5 aromatic ring compounds can be present. Stability of the AHS−Al^{3+} complex was reached when final concentration reached 20 mg L^{-1} of Al^{3+} (Figure 3a). According to the results obtained employing CHEAQS Pro, from 70 to 91% of Al^{3+} is complexed with organic matter in the concentrations employed. Other species in low concentrations are present, such as $[Al(OH)_2]^+$, $Al(OH)_{3(aq)}$ and $[Al(OH)_4]^-$.

No fluorescence signal shift was observed with CrO_4^{2-} for lower or higher wavelengths, with the maximum emission spectrum fluorescence signal occurring at 429 nm.

In the synchronous spectrum there were three maxima fluorescence signals at 280, 368 and 406 nm. It should be noted that maximum fluorescence intensities at 280 and 368 nm gradually decrease with the addition of metal until a near total quenching, suggesting that CrO_4^{2-} must first act on compounds of the aromatic amino acid type and volatile acids of aliphatic structure (280 nm), followed by those with chromophores groups that have from 3 to 4 conjugated aromatic rings (368 nm). Stability of the AHS−CrO_4^{2-} complex was reached when a quantity close to 90.0 mg L^{-1} of CrO_4^{2-} was added (Figure 3c).

In studies with Cr^{3+}, a slight increase of the fluorescence intensity in the emission mode at 429 nm can be first seen and afterwards a gradual decrease followed by stability. In the synchronous mode, an increase of the fluorescence intensity was observed at a wavelength of 406 nm. Stability of the AHS−Cr^{3+} complex was reached when 32.0 mg L^{-1} of Cr^{3+} was added.

No wavelength shifts in the spectra were observed with Ni^{2+}. A quenching of fluorescence with the addition of Ni^{2+} was observed in both emission and synchronous spectra. Three maximum fluorescence signals were seen in the synchronous spectrum, one at 280 nm, other at 364 nm and the last one at 405 nm. As discussed previously, the fluorescence signal at 364 nm is in a region where there are 3 to 4 aromatic ring structures, and the signal at 405 nm corresponds to 5 aromatic rings.[24] It was observed that similarly to CrO_4^{2-} ion the fluorescence signal at 280 nm was quenched with the addition of Ni^{2+}. Stability of the AHS−Ni^{2+} complex was reached when close to 600.0 mg L^{-1} of Ni^{2+} was added (Figure 3d).

The calculation to find the conditional stability constant (K_C) cannot be applied to the AHS-Al^{3+} complex because the Stern-Volmer model is only applicable when the fluorescence signal is quenched. The knowledge of the conditional stability constant is intimately related to the formation stability of the AHS−metal complex and, consequently, to its important role in the sequestration and maintenance of the availability of the metal. Conditional stability constants were calculated by applying the Stern-Volmer model to the AHS−CrO_4^{2-}, AHS−Ni^{2+} and AHS−Cr^{3+} complexes for emission mode spectra. Table 1 shows the linear regression coefficient values for the complexes and the conditional stability constant values.

Note that the AHS−Ni^{2+} complex shows a lower conditional stability constant value, which allows the inference that this complex is the most unstable and the metal is more liable for to be uncomplexed from the AHS structure and, therefore, become more available in an aquatic environment. These results are in agreement with values previously obtained employing CHEAQS Pro,[46] where around 99% of Ni^{2+} is present in free form not bound to organic nor inorganic compounds. Similar results were found by Silva *et al.*[12]

Table 1. Linear regression coefficient values for linearity curves obtained from Stern-Volmer model for the AHS−CrO_4^{2-}, AHS−Ni^{2+} and AHS−Cr^{3+} complexes and conditional stability constant values

Complex	R^2	K_C
AHS−CrO_4^{2-}	0.99	2.6 10^{-2}
AHS−Ni^{2+}	0.99	1.1 10^{-5}
AHS−Cr^{3+}	0.99	2.0 10^{-2}

Regarding to chromium complexes, no statistical differences ($p = 0.05$) between the complexes AHS−Cr^{3+} and AHS−CrO_4^{2-} were observed. One hypothesis to explain this behavior can be associated with the capacity of AHS to reduce metals or the increase of semiquinones radicals' generation by radiation. For the first case AHS could reduce Cr(VI) to Cr(III) form and subsequently complex the reduced form. Romão *et al.*[50] demonstrated that AHS can reduce Cr(VI) in acid pH (2.5), being that this behavior was not observed for pH 6.0. Probably this mechanism can be disregarded for this work. Similar K_C results were found by Merdy *et al.*[51]

Solar radiation effect on the complexation capacity of AHS

Some studies have already shown that solar radiation is able to degrade natural organic matter[19,48] and to interfere in the interaction of these metal species. However, few studies with the same AHS thematic have been addressed in the literature. Experiments performed in this work were carried out simulating the environmental conditions of an aquatic body during the day, such as exposure to solar radiation, temperature and movement, among others. PET bottles containing the AHS and metal ion species were taken to the field where they remained under the same conditions of the aquatic body. An absorbance spectrum of the PET bottle material was obtained and it was noted that the PET bottle absorbed wavelengths below 330 nm, allowing the passage of visible radiation.

During the experiment, parameters such as water temperature and ultraviolet radiation intensity were monitored. Water temperature was taken at 8:00 a.m. (24 °C) when the bottles were placed in the waters. Temperature was again monitored at 1:05 p.m. (29 °C) when a group of samples were withdrawn, and again at 6:15 p.m. (30 °C) when remaining bottles were withdrawn.

A control for each experiment was kept in the laboratory for comparative purposes. AHS without metal additions were exposed in the field under light and dark conditions, as it was made with the control in the laboratory. Figures 4a and 4b have the emission and synchronous fluorescence spectra which show the behavior of AHS without metal ion addition. It may be seen that for the AHS samples exposed to solar radiation there was a decreased fluorescence intensity of 13% (emission mode) after a 10 h exposition. No significant differences were observed between laboratory control samples and those kept under dark conditions in the field ($p = 0.05$), as was expected (Figure 4a). This result confirms that observed alterations must be attributed to solar radiation. These results were considered for the experiments of effect of solar radiation in the complexation capacity with metals. In the synchronous mode (Figure 4b), the same behavior was observed, a decreased fluorescence intensity of 16% for samples kept under light conditions. AHS DOC concentrations kept under light (7.41 mg L^{-1}) and dark (7.45 mg L^{-1}) conditions were also monitored, with no difference ($p = 0.05$) being observed during the exposition to solar radiation.

A decrease of fluorescence intensity due to exposure to natural or artificial radiation was observed by other authors by dissolved organic matter.[19,49,52] Gonsior *et al.*[19] verified a fluorescence intensity reduction of 24% for the Preto River water, in California, and verified a greater degradation in compounds that contain fluorescent chromophore groups. They also observed that a concentration of DOC remained constant during radiation. On the other hand, in a laboratory experiment with natural radiation,[52] it was verified that DOC concentration decreased close to 70% for AHS extracted from Amazon River waters. An interesting fact is that for the more aromatic AHS there was a greater intensity loss which was attributed to a greater generation of free radicals, while for AHS with aliphatic characteristics degradation was lower, though faster. In this study, the AHS had predominantly aliphatic characteristics what can be associated to solar radiation exposed for 10 hours, resulting in a rapid AHS alteration.

A decrease of fluorescence intensity as exposure to solar radiation was also observed in the group with AHS and metals. Table 2 shows the fluorescence intensity values for AHS−Al^{3+}, AHS−Cr^{3+}, AHS−CrO_4^{2-} and AHS−Ni^{2+} complexes according to the time exposed to solar radiation, as well as the observed percentages of decrease after discounting the decrease value of the AHS chromophores groups as presented in Figure 4 discussion.

A decrease of observed intensity for the different concentrations of the same AHS−metal group at 0 h (Table 2) is associated to the fact that the measurements were taken after the complexation time, which is necessary for the complex to stabilize (30 min for Al^{3+} and 20 min for the other ions). This explains why the maximum fluorescence intensity decreases as ion concentrations increase. Decrease percentages were calculated considering fluorescence intensities of the samples exposed for 10 h to solar radiation and the fluorescence intensities of samples exposed for 10 h to solar radiation under dark conditions. As it can be seen in Table 2, the groups exposed to solar radiation under dark conditions did not have significant losses of the maximum fluorescence intensity. This reinforces the observed behavior, enabling an association of intensity decrease to the effect of solar radiation.

Results showed in this work demonstrated that solar radiation decreased AHS−ion complexation capacity, identified by the fluorescence intensity decreases, making the metallic ions more available to be transported in the environment (Table 2). The differences between the decrease percentages were expected since each metal ion has distinctive characteristics such as load density,

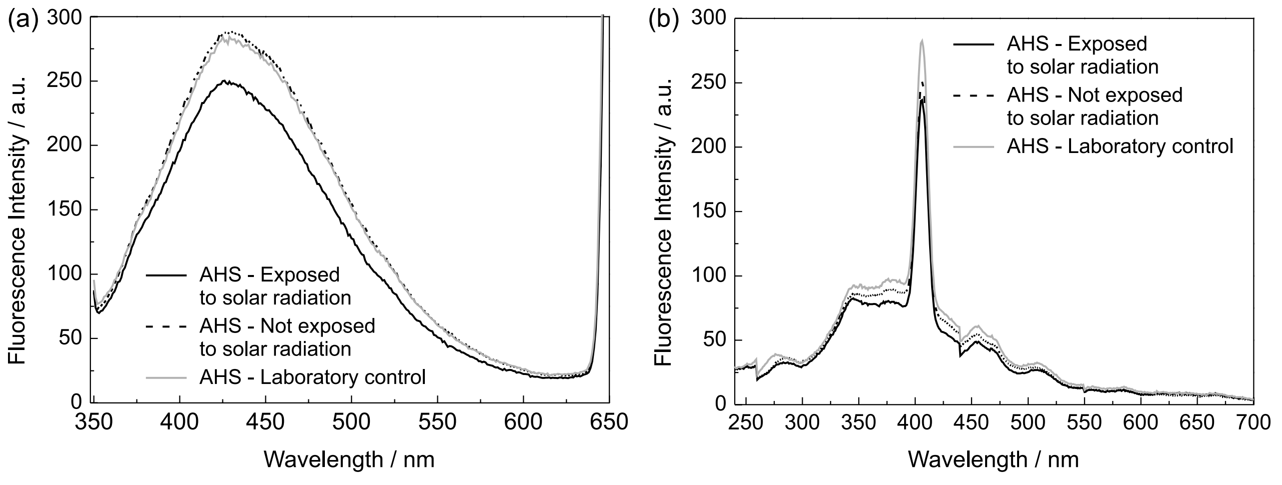

Figure 4. Fluorescence spectra in emission (a) and synchronous (b) modes for AHS (DOC of 5 mg L^{-1}) samples exposed to light (clear conditions), not exposed to light (dark conditions) and laboratory control.

Table 2. Fluorescence maximum intensity values obtained in 430 nm, for AHS–Al^{3+}, AHS–Cr^{3+}, AHS–CrO$_4^{2-}$ and AHS–Ni^{2+} complexes at different times of solar radiation exposition, decrease percentages due to radiation and the values of the group exposed to radiation in darkness

Concentration / (mg L^{-1})	Exposed to radiation (in light)			Percentages of decrease / %	Exposed to radiation (in dark condition)
	0 h[a]	5 h	10 h		
AHS–Al^{3+}					
0.4	294 ± 1.5	262 ± 1.3	250 ± 1.3	26	338± 1.7
1.6	237 ± 1.2	240 ± 1.2	232 ± 1.2	15	272 ± 1.4
5.9	151 ± 0.8	148 ± 0.7	137 ± 0.7	23	178 ± 0.9
AHS–Cr^{3+}					
5.9	249 ± 1.2	b	232 ± 1.2	15	274 ± 1.4
13.7	171 ± 0.9	b	42 ± 0.2	72	154 ± 0.7
43.7	116 ± 0.6	b	39 ± 0.2	63	104 ± 0.5
AHS–CrO$_4^{2-}$					
5.9	235 ± 1.2	217 ± 1.1	187 ± 0.9	12	212 ± 1.1
43.7	54 ± 0.3	48 ± 0.2	40 ± 0.2	18	49 ± 0.2
AHS–Ni^{2+}					
0.5	285 ± 1.4	259 ± 1.3	243 ± 1.2	13	280 ± 1.4
17.7	246 ± 1.2	203 ± 1.0	120 ± 0.6	42	209 ± 1.0
377.4	173 ± 0.9	157 ± 0.8	118± 0.6	29	167 ± 0.8

[a]After addition of metal. [b]Samples not collected.

atomic radius, among others. Recent studies show an association of metals having a concentration of radical semiquinones.[53] AHS exposed to solar radiation produce radical semiquinones.[54] An increase of the concentrations in radical semiquinones and hidroquinones causes a displacement of the metal ions of the humic substances structure, due to the greater formation of oxygen radicals which are reactive species in the structure. Decreases of fluorescence intensities are therefore expected as a greater formation of radical semiquinones due to the intensity of the solar radiation favors oxygen reactive species which act on the AHS structure, consequently on the complexation capacity of these with metallic ions.[53] EPR measurements could prove if radical semiquinones were present as previously demonstrated by Jerzykiewicz *et al.*[53] These authors observed a decrease of concentrations of radical semiquinones when Ni^{2+} and Cu^{2+} are present. On the other hand, metallic ions such as Zn^{2+}, Cd^{2+} and other diamagnetic ions increase the effect of metal coordination by increasing the concentration of the radical semiquinones.

Conclusions

In this study it could first be verified that AHS extracted from the Preto River has the characteristics of recently degraded organic matter as proved by the presence of predominantly aliphatic structures and, therefore, with a low humification degree. Based on conditional stability constant values obtained for the AHS–Cr^{3+}, AHS–Ni^{2+} and AHS–CrO$_4^{2-}$ complexes, it can be seen that AHS really come from an organic matter that does not have a good enough capacity to complex metallic ions efficiently, with K_C values considered low. We can conclude that solar radiation affects the capacity of AHS to complex metallic ions, being observed a decrease of 15-26% for AHS–Al^{3+}, 15-72% for AHS–Cr^{3+}, 12-18% for AHS–CrO$_4^{2-}$ and 13-42% for AHS–Ni^{2+}. These results are important because they allow inferring that with an aquatic body the solar radiation enables ions to be more available which, consequently, could increase toxicity in an aquatic body. In conclusion, radiation effect will be more pronounced depending on the size and charge of each metallic ion, which will affect its susceptibility of binding to the active site (chromophores group) of the AHS structure.

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