http://dx.doi.org/10.5935/0103-5053.20130277

J. Braz. Chem. Soc., Vol. 25, No. 1, 119-125, 2014.
Printed in Brazil - ©2014 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Fractionation of Aquatic Humic Substances and Dynamic of Chromium Species in an Aquatic Body Influenced by Sugarcane Cultivation

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Este estudo teve como objetivo principal avaliar a influência das SHA obtidas a partir de uma área de cultivo de cana de açúcar sobre a influência das espécies de crômio (Cr(III) e Cr(VI)). Foi estudada a capacidade de complexação (CC) das SHA com as espécies de crômio e estas foram caracterizadas por espectrofotometria de UV/Vis, fluorescência molecular, análise elementar e infravermelho. SHA com e sem fracionamento apresentou maior grau de aromaticidade e predominância de ácidos húmicos, e as razões E_4/E_6 e E_2/E_4 sugerem a presença de anéis aromáticos e maior contribuição das plantas e lignina. As maiores CC foram observados para a SHA fracionadas, sendo as frações com tamanho molecular < 10 kDa e 10-30 kDa as que apresentaram as maiores capacidades para complexar os íons Cr(III) e Cr(VI), respectivamente. Estes resultados corroboram com os dados de C/H/N e UV/Vis, permitindo concluir que as SHA sem fracionamento têm em sua estrutura maior grupos aromáticos e uma predominância de ácidos húmicos.

This study had as main objective to evaluate the influence of AHS obtained from an area under sugarcane cultivation on the dynamics of chromium species (Cr(III) and Cr(VI)). Was studied complexing capacity (CC) with the AHS of chromium species and these were characterized using UV/Vis spectrophotometry, molecular fluorescence, elemental analysis and infrared. AHS with and without fractionation showed a greater aromaticity degree and a predominance of humic acids, and the E_4/E_6 and E_2/E_4 ratios suggested aromatic rings and a greater contribution from plants, which indicates lignin structures. The highest CCs were observed for the AHS fractionated, being fraction with molecular-size < 10 kDa and 10-30 kDa showed the highest ability to complex Cr(III) and Cr(VI) ions, respectively. These results are corroborating with C/H/N and UV/Vis data, where we can conclude that the AHS without fractionation had the greatest aromaticity and a predominance of humic acids in their structure.

Keywords: aquatic humic substances, complexation, characterization

Introduction

Humic substances (HSs) are derived from the microbial degradation of plant and animal remains, which play an important role in soil fertility and processes in aquatic environments.[1,2] These substances correspond around 75% of soil organic matter and 50% of organic carbon in surface water.[3,4] Changes in soil composition and the water cycle caused by natural or anthropogenic sources can interfere with the amount and chemical nature of organic matter in these natural ecosystems, which consequently alters HS structure.[3,5-10] The heterogeneity of HS functional groups are environmentally important because they can interact with metal ions in the environment, which results in complexation and/or reduction reactions and consequently alters metal availability. Studies examining the structural and functional properties of aquatic HS (AHS) are important for understanding their mechanisms and processes in natural ecosystems.[11,12] Also highlighted, solar radiation plays a key role in altering the availability of metals previously complexed to AHS.[13]

To evaluates the role of AHS in chemical processes within water bodies, several researchers have employed various analytical characterization techniques, especially elemental analysis (Carbon/Hydrogen/Nitrogen), which assesses the level of aromatic ring condensation in HS and compares the origins of humic materials that comprise HS using H/C, O/C and C/N ratios.[14-16]

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UV/Vis spectroscopy is used to evaluate the level of HS aromaticity,[16-21] while spectrophotometry in the infrared region provides considerable information on the main functional groups in the HS structure.[3,22,23] Molecular fluorescence provides criteria for distinguishing and classifying HS origins.[16,21,22,24,25]

As mentioned above, AHS can influence the transport, accumulation and concentration of metal ions, as well as anthropogenic organic compounds (e.g., pesticides and herbicides,[26] in the planktonic food chain by altering turbidity and interactions with nutrients, as well as modifying primary production, because it is a carbon source in the food chain and can alter the photic zone.[3,25,27] Thus, AHS may act as sink for removing toxic metals in aquatic environments, given the abundance of organic functional groups in their structure.[28]

Studies have been conducted on the complexing ability of AHS with metals, especially copper.[7,13,14,29-32] Additional environmentally interesting metals have been given little attention, particularly chromium species, wherein the trivalent form is less toxic than the hexavalent form, and the latter is anionic. Some studies have shown that AHS can reduce hexavalent chromium at pH values below 4.0, and this process depends on temperature and HS concentration.[32-34] It should be noted that HS complexation with hexavalent chromium has been observed in certain studies,[35,36] and according to the supramolecular theory[37] and the most recent publication by Mazzei and Piccolo,[38] non-covalent interactions can be developed between HS and anionic compounds, such as glyphosate. Thus, an interaction between anionic chromium species (the hexavalent form) and HS is expected, as observed by Santos *et al.*[39] and Melo *et al.*.[13]

In this context, the Preto River, located in the northwestern region of the state of São Paulo and part of the Turvo/Grande watershed, wherein studies have just been initiated, was interesting for this study. This region is the largest area with sugarcane cultivation in the state of São Paulo, which it is responsible for 52% of the sugarcane production in Brazil.[40] According to São Paulo State Environmental Agency (CETESB) total chromium has been found in this region in values above Brazilian Law for drinking water (50 μg L^{-1}).[41] According to Bertolo *et al.*[42] high values of chromium in this region comes from natural sources, associated to diopside mineral found in sandstone and high values of pH (8.5-10.7) in soil in this region that favor desorption and mobilization of metal to the water. Based on these factors, the objective for the study herein was to characterize and study the influence of AHS with and without fractionation from areas with sugarcane cultivation on the dynamics of chromium species in the environment.

Materials and Methods

The reagents used were in had high purity levels (Sigma-Aldrich), and ultrapure water was generated by a Millipore water purification system (Direct-Q). The experiments were performed in triplicate with errors below 5%.

Sample collection

The study area comprised a predominantly agricultural body of water, which was used for various purposes, including a public water supply. This region has a tropical climate, 25 ºC average temperature and well-defined seasons. Water samples from the Preto River (S20º48'40.94" W49º21'13.62") in the northwestern region of the state of São Paulo and transported to the laboratory, where in they were filtered through semi-qualitative paper to remove suspended solid particulate material and then acidified with HCl 6.0 mol L^{-1} to a pH near 3 for AHS extraction, as suggested by the International Humic Substances Society (IHSS)[43] and previously described by Tadini *et al.* (2012).[44] Preto river has values of pH, conductivity, turbidity and dissolved oxygen of 5.72, 16.8 μS cm^{-1}, 29.12 FTU, 4.5 ppm, respectively.

HS extraction and fractionation

The method used for HS extraction was suggested by the IHSS.[43] The AHS were fractionated in accordance with the method proposed by Burba *et al.*[45] and adapted by Pantano *et al.*,[46] which used four tangential flow ultrafiltration system (TFUS). According to Tadini *et al.*[44] the procedure for fractionation of AHS included pumping 200.0 mL of the AHS sample with 15 mg L^{-1} of dissolved organic carbon (DOC) through the TFUS using a peristaltic pump, which passed through a series of four filters equipped with different size membranes that facilitated collection of individual fractions with different molecular sizes (where F_1, < 10 kDa; F_2, 10-30 kDa; F_3, 30-50 kDa; F_4, 50-100 kDa; and F_5, >100 kDa).

Determination of AHS complexing capacity (CC)

The complexation capacity of the samples (AHS without fractionation and AHS fractions of different molecular size) were determined using a TFUS equipped with a membrane of the 1.0 kDa (regenerated cellulose, 76 mm, NMWL: 1.000). The samples (100.0 mL of solution contain 10.0 mg L^{-1} of DOC, pH around 5.8) were pumped through the TFUS system. At experimentally determined time intervals, 2.0 mL AHS samples were collected

followed by quantification of the total and hexavalent chromium. After each collection, the AHS samples were titrated by receiving known concentrations of a standard monoelementar chromium solution (Cr(III) or Cr(VI)) which ranged from 9.8 μg L^{-1} at 7.8 mg L^{-1}, with a volume increase bellow than 3% of total volume. It is noteworthy that despite the species hexavalent chromium (Cr(VI)) does not exist naturally, but the anionic form, will be used the term "Cr(VI)" representing the species $Cr_7O_4^{-2}$.

Quantification of total and hexavalent chromium

Total chromium in the samples was quantified in accordance with recommendations for the method 3500-Cr B[47] using a graphite furnace atomic absorption spectrophotometer (GFAAS) with Zeeman background correction (Varian, model AA280Z, California, USA). The instrumental conditions used for quantification were adapted from suggestions by the manufacturer and Pereira *et al.*[48] An 8 μL sample volume was injected, and the universal chemical modifier was used, which consisted of a solution with 1500.0 mg L^{-1} Pd and 1000.0 mg L^{-1} $Mg(NO_3)_2$.[49,50] Accuracy was verified with a standard metal solution and analyzed after every 10 samples. The solutions for measuring total chromium were prepared by diluting certified standard solutions (Sigma Aldrich, St. Louis, USA). The Laboratory for Research in Environmental Sciences where this study was developed has participated in Proficiency Testing from the Brazilian Agricultural Research Corporation (EMBRAPA) and National Institute of Metrology, Quality and Technology (INMETRO) that quantifies metals in plant tissue and water samples. The results were considered satisfactory ($z \leq 2$).

The method employed for quantifying hexavalent chromium was 3500 D,[47] which is specific for quantifying hexavalent chromium. The level of hexavalent chromium was determined colorimetrically by reaction with diphenylcarbazide in an acidic medium, which produced a compound with a reddish-violet color. Trivalent chromium was obtained for difference between total and hexavalent chromium.

Characterization of the AHS

UV/Vis spectrophotometry

UV/Vis spectra of the AHS samples and their different molecular size fractions contain 1.0 mg L^{-1} of DOC were recorded on a Thermo Scientific Evolution 300 (Thermo Scientific, Massachusetts, USA) in 1.0 cm quartz cuvette and from 200 to 700 nm. pH and ionic strength were controlled following recommendations described by Peauravuori and Pihlaja.[51] HS aromaticity was assessed using the E_4/E_6 (absorbance at 465 nm and 665 nm), E_2/E_3 (absorbance at 254 nm and 365 nm) and E_3/E_4 correlations (absorbance at 300 nm and 400 nm).[51]

Molecular fluorescence

Molecular fluorescence spectra for the AHS samples (10.0 mg L^{-1}) were obtained in three modes: emission, synchronous and excitation-emission matrix (EEM) measurements were performed on a spectrofluorimeter (Varian, Cary Eclipse, Australia). Emission mode scanning was performed from 350 to 650 nm with an excitation wavelength at 332 nm. The synchronized mode spectra ranged from 300 to 600 nm with an 18 nm $\Delta\lambda$ (adapted).[23] The EEM mode (three dimensional spectra) wavelengths were 350-600 nm for emission and 300-450 nm for excitation (adapted).[25]

Spectroscopy in the infrared region

Infrared characterization was performed using KBr pellets at a 100:1 ratio (KBr/sample). The spectra produced ranged from 4000 to 400 cm^{-1} with a 4 cm^{-1} resolution and 100 scans using a PerkinElmer spectra FTIR spectrum device (PerkinElmer, Massachusetts, USA).[12,44]

Elemental analysis

Elemental analyses for the AHS without fractionation were performed by the Center for Chemical Instrumental Analysis in the Institute of Chemistry of São Carlos with a CE Instruments model EA 1110 elemental analyzer.

Quantification of dissolved organic carbon (DOC)

Dissolved organic carbon in the AHS samples without fractionation, and their different molecular size franctions (< 10, 10-30, 30-50, 50-100 and > 100 kDa) was quantified using a total organic carbon analyzer (Shimadzu, TOC - VCSN, Tokyo, Japan).

Decomposition of the AHS samples

The AHS without fractionation and water samples from the Preto River were decomposed in accordance with recommendations from the 202.2 EPA method,[50] which comprises decomposition through a mixture of concentrated HNO_3 and H_2O_2 (30% v/v). This procedure was performed in triplicate using deionized water as a blank.

Results and Discussion

Characterization of aquatic humic substances

The Figure 1 shows the results obtained by spectroscopy characterization of different molecular size fractions (F_1, F_2,

F_3, F_4 and F_5) and the AHS without fractionation from a typical region of sugarcane cultivation exhibited higher aromaticity compared with additional locations described in various published studies.[16,17,46,51-55] Melo *et al.*[13] studied the effect of solar radiation on capacity complexation of aquatic humic substances from Preto River three years before this study to be made and theses authors showed that this AHS present a mix of humic and fulvic acids. We attributed this difference to expansion of sugarcane cultivation in the region.

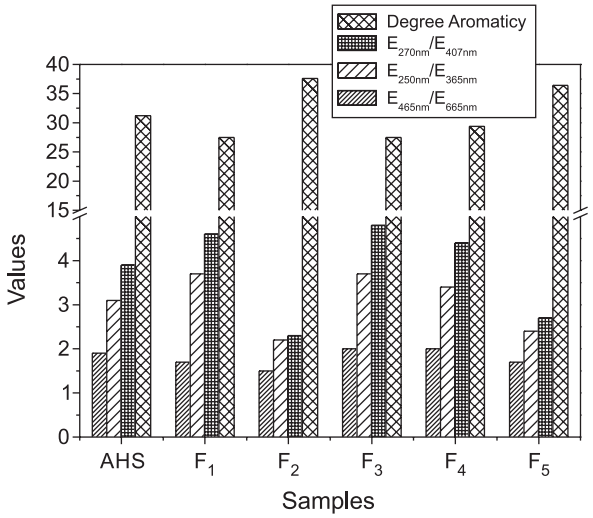

Figure 1. Different ratio ($E_{250}/E_{365\ nm}$, $E_{270}/E_{407\ nm}$ e $E_{465}/E_{665\ nm}$) to AHS samples and their different molecular sizes fractions, where F_1, < 10 kDa; F_2, 10-30 kDa; F_3, 30-50 kDa; F_4, 50-100 kDa; and F_5, > 100 kDa obtained by UV/Vis.

Degree aromaticity was calculated employing the equation suggested by Peuravuori and Pihlaja[51]: $DA = 52{,}509 - 6{,}780.\ E_{250}/E_{365}$. From the E_4/E_6 and E_2/E_4 ratios, aromatic rings and a greater contribution from plants can be inferred, which indicates lignin structures in AHS from the Preto River. This result may be related to the land use and occupation, where in the structure of sugarcane bagasse comprises 40% glucose in cellulose polymers, 30% hemicellulose and 18% lignin, and the remainder (12%) is wax, proteins and additional compounds.[56,57] It is worth noting that studies in the literature report a lower value for the E_4/E_6 ratio, a greater level of humification and consequently a larger number of aromatic groups in AHS structures.[15,19,54,55,58] The results of this study support the following descending order of aromaticity: $F_2 > F_5 > AHS > F_4 > F_1 \sim F_3$.

Using gel permeation chromatography, Handerson and Hepburn[59] showed that humic fractions with large molecular weight and low E_4/E_6 ratios are primarily composed of aliphatic compounds, while those with smaller molecular weights featured high levels of aromatic groups.[22] According to certain authors,[4,54,60] the level of aromaticity decreases with the molecular size of HS fractions. In this study, the < 10 kDa and 30-50 kDa fractions showed the lowest levels of aromaticity and highest values for the E_2/E_3 ratio, which indicates that these fractions contained a smaller number of condensed aromatic rings compared with the number of aliphatic groups in their structure because of the lower humification levels.[4,46,60] The results obtained by molecular fluorescence and discussed by Tadini *et al.*[44] confirm that the AHS extracted from an river typically from a sugarcane area are rich in humic acid.

Studies in the literature report that infrared spectroscopy may be used to characterize AHS and as discussed by Tadini *et al.*[44] the spectrum IR indicated more presence of aromatic groups.[12,22,61-65] Table 1 shows characteristics of the mainly peaks obtained employing Infrared to the different molecular size fractions (F_1, F_2, F_3, F_4 and F_5) and the AHS without fractionation date obtained of the publication of Tadini *et al.*[44] The spectrum show a band near 3450 cm^{-1} which may be attributed to OH stretching in phenolic groups, carboxylic acids and/or amines, and band in region of 1639 to 1636 cm^{-1} it can be attributed vibrations of carbonyl of carboxylates and/or ketones groups[2,12,14,55,60] and a band near 1385 cm^{-1} only present in the AHS without fractionation and it can be associated with carboxylate and/or alcohol group stretching in its structure.[2,12,66,67]

Table 1. Main characteristics of the FT-IR spectra the AHS different molecular size fractions and the AHS without fractionation

Band of AHS / cm^{-1}		Assignment
Without fractionation	Different molecular size fractions	
3450	3450	O–H stretch (phenolic, carboxylic and/or amines groups)
1636-1639	1636-1639	C=O vibrations (carbonyl and/or carboxylic acids)
1380	[a]	C–H bound (carboxilate and/or alcohol group)

[a]Did not show the band in these samples (spectrum infrared obtained in the publication Tadini *et al.*[44]).

Values from elemental analysis of AHS extracted from the Preto River without fractionation were 30.3% carbon, 1.8% nitrogen, 4.1% hydrogen and 2.6% sulfur, and these values were lower than those described in the literature.[12,15,22,23,61] Senesi *et al.*[61] demonstrated that fulvic acid (FA) extracted from Suwannee River, Fargo, Georgia, USA, and another sample extracted from North Sea, Norway and the terrestrial sample isolated from soil near Joliet, Illinois, USA, and peat obtained at Belle Glade Research Station, Everglades, Florida, USA. These

samples were obtained from the IHSS and extracted from a region with temperate climate. According to these authors this FA had low carbon concentrations compared with FA extracted from soils in the same region; however, hydrogen content had the opposite behavior, which allowed to infer the following order of hydrogen concentrations: river > soil > peat. Study conducted by Araújo and co-authors[14] with SHA extracted from water samples from the River Itapanhaú, Bertioga, SP, Brazil, and its fractions of different molecular sizes (F_1: < 5; F_2: 5-10; F_3: 10-30; F_4: 30-50; F_5: 50-100 e F_6: > 100 kDa) indicated that fractions F_1 and F_6 had the lowest ratios of H/C (0.46 and 1.0, respectively), indicating a higher aromaticity in their structures, while the atomic ratio C/N showed a similar behavior for the fractions F_2, F_3, F_4 and F_5 indicating similarity in the degree of humification. Thus, the authors concluded that there was no significant difference in the degree of humification between all fractions and fractions F_1 and F_6 contained higher aromaticity corroborated with dates obtained for atomic ratio H/C.

The C/H ratio supports the inference of AHS structure saturation, where the smaller C/H ratios had a greater number of aromatic structures and therefore a higher level of aromaticity.[15] The C/H ratio for AHS from an area with sugarcane cultivation was 7.4, which indicates higher levels of aromatic structures and consequently greater aromaticity, which corroborates the E_2/E_3 ratios from the AHS samples (Figure 1). Therefore, the AHS extracted from the Preto River without fractionation had greater aromaticity compared with other studies in the literature.[16,17,46,51,55]

Complexity of aquatic humic substances with species chromium

Chromium species variation as a function of pH was evaluated using software for simulating chemical equilibrium in aquatic environments (HYDRA - MEDUSA)[68] prior to the CC experiments. The results indicated that the Cr(III) ion is the predominant species at pH 5.0, whereas for pH values greater than 7.0, Cr(III) oxides and hydroxides begin to dominate. Therefore, the solutions containing AHS without fractionation and its fractions were maintained around pH 5.8 for CC studies with the ions Cr(III) and Cr(VI).

Figure 2 shows the CC values for AHS samples without fractionation and its different molecular size fractions. These values were similar than those found by Van den Bergh *et al.*[36] with AHS extracted from rivers in Germany (0.04 to 0.92 mmol Cr(III) g^{-1} carbon) and lower to those generated by Santos *et al.*[39] from HS samples extracted from peat with the CC value 4.63 ± 0.06 mmol Cr(VI) g^{-1} carbon. Comparing the CC for AHS from the Preto River with the species Cr(III) and Cr(VI), most AHS fractions had similar CC values for the chromium species, except the 50-100 kDa fraction.

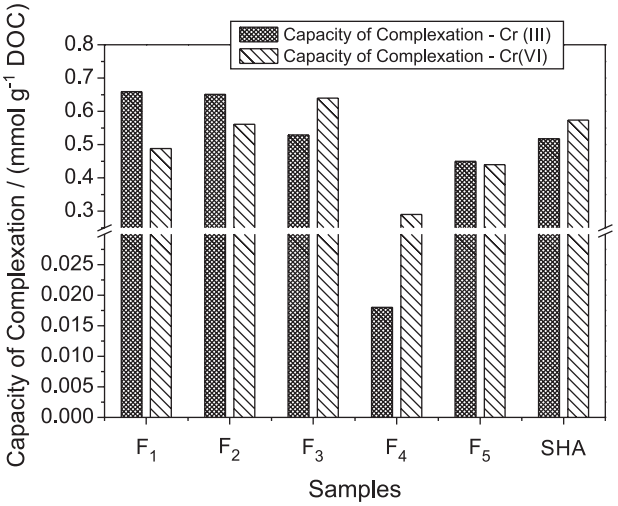

Figure 2. Capacity complexation and exchange constants for the AHS different molecular size fractions and the AHS without fractionation to the chromium species Cr (III) and Cr (VI), where F_1, < 10 kDa; F_2, 10-30 kDa; F_3, 30-50 kDa; F_4, 50-100 kDa; and F_5, > 100 kDa.

The total chromium concentration in the AHS extracted from Preto River and water samples colleted from Preto River were 1.0 ± 0.6 μg L^{-1} and 59 ± 1 μg L^{-1}, respectively. The concentration of total chromium in this aquatic body was calculate and concluded that approximately 2% of total chromium was complexed to humic substances indicating that most of the metal was free to interact with the biota, as well as to be transported and deposited in the sediment or to bind inorganic particulate material. Melo *et al.*[13] concluded that solar radiation making metal more available to be transported to environment because the capacity complexation decrease until 72% compared to humic substance no exposed to solar radiation.

Conclusion

Aquatic humic substances with and without fractionation from an area influenced by sugarcane cultivation showed higher quantity of aromatic groups and indicated to be originated from plants. Aromaticity degree decreased according to following order: $F_2 > F_5 > AHS > F_4 > F_1 \sim F_3$. This results are in agreement with characterization of AHS, where C/H/N results support the inference that the AHS without fractionation have more aromatic structures. We can concluded that SHA-Cr(VI) complex and fractions of SHA-Cr(III) presented more stability than compared to other studies and that this AHS present lower complexation

capacity indicating metal are more available to interact with biota.

Acknowledgments

We would like to thank the São Paulo Research Foundation (Fundação de Amparo a Pesquisa do Estado de São Paulo) for providing financial aid and scholarships (Processes 2010/13879-2, 2005/51242-8 and 2010/09271-9).

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Submitted: August 5, 2013
Published online: November 26, 2013

FAPESP has sponsored the publication of this article.