Article

Assessment of Anthropogenic Influences on Copper Complexation by Aquatic Dissolved Organic Matter using Synchronous Fluorescence

Fernando F. Sodré[#] and Marco T. Grassi[*]

Departamento de Química, Universidade Federal do Paraná, CP 19081, 81531-990 Curitiba-PR, Brazil

Neste trabalho foi avaliada a influência das atividades antrópicas sobre as características da matéria orgânica dissolvida (MOD) aquática empregando-se fluorescência sincronizada (SyF). Amostras dos rios Iraí e Iguaçu foram coletadas em pontos localizados à montante e à jusante de uma região urbanizada, respectivamente, durante o inverno e o verão. A MOD das amostras foi analisada por SyF, titulada com Cu^{2+} e os dados gerados foram tratados com um modelo não linear em, pelo menos, cinco regiões espectrais. Os resultados mostraram que durante o verão, o período de maior pluviosidade, a MOD de ambos os rios apresentou estruturas orgânicas simples tais como aminoácidos aromáticos e/ou compostos alifáticos conjugados como constituintes majoritários, surgidos, provavelmente, a partir de fontes difusas. Durante o inverno foi possível evidenciar que o aporte de esgoto bruto a partir da região urbanizada contribuiu para o aumento de MOD fluorescente incapaz de complexar o cobre.

Anthropogenic influences on the characteristics of aquatic dissolved organic matter (DOM) were evaluated in this work using synchronous fluorescence (SyF) scan spectroscopy. Samples from the Iraí and the Iguaçu Rivers were collected during the summer and winter at points located upstream and downstream from a heavily urbanized area in Brazil, respectively. DOM from all samples was titrated with Cu^{2+} and monitored by SyF quenching. A 1:1 non linear model was used for data treatment in, at least, five distinct regions of the spectra. Results revealed that during the rainy summer, DOM from both rivers exhibited simple organic structures such as aromatic amino acids or conjugated aliphatics as the major constituents, probably arising from non-point sources. However, during the dry winter it became clear that raw sewage discharges arising from the urbanized area contributed to an increase of fluorescent organic matter that is unable to complex copper.

Keywords: synchronous fluorescence, dissolved organic matter, fluorescence quenching, copper complexation, raw sewage discharges

Introduction

Organic matter is an important component responsible for several chemical and biological processes in natural waters.[1] Furthermore, dissolved organic matter (DOM) is known to play a key role on copper speciation in aquatic environments.[2] Many investigations have shown that in its free or labile form, copper can be toxic to aquatic biota.[3] This toxicity can be moderated or even completely eliminated through copper complexation by selected functional groups occurring in the DOM.[4]

The chemical significance of the DOM at a given place is determined by its chemical nature. The diversity and variability of organic materials within a specific stream are mostly dependent on the potential sources of carbon.[5] These sources can arise from in-stream processes (autochthonous sources) or from external ones (allochthonous sources) such as leachate from surrounding soils, stormwater runoff as well as from treated or raw sewage discharges.[6] Thus, it is expected that not all organic matter will interact to the same extent with trace metals in natural waters.

Xue *et al.*[7] obtained copper complexation parameters for selected freshwater systems in Switzerland by differential pulse cathodic stripping voltammetry and observed no obvious correlation between metal complexation and the dissolved organic carbon (DOC) content. This result indicates that copper-complexing ligands are specific organic compounds. In an earlier study,

*e-mail: mtgrassi@quimica.ufpr.br
[#]Present Address: Instituto de Química, Universidade Estadual de Campinas, CP 6154, 13084-862 Campinas-SP, Brazil

we investigated the copper-complexing capacities of two rivers by potentiometric titrations using an ion-selective electrode (ISE).[8] Results showed that for samples collected upstream from a heavily urbanized area the ligand concentration increased with DOC. On the other hand, for samples collected downstream from the same area no satisfactory correlation was observed between the two previously mentioned parameters. More recently, we have demonstrated that raw sewage discharges contribute to an increase in DOC levels at the sampling site located downstream from the heavily urbanized area, mainly during the lower precipitation period.[9] Therefore, it is possible that raw sewage discharges provide a pool of organic materials that do not interact with copper to the same extent as was observed for the samples collected upstream the urbanized region. However, it is difficult to develop a detailed understanding of the nature of this complexation because of the complexity and heterogeneity of natural water systems and because of the technique used in these studies.

Nearly all techniques used to evaluate the complexation of trace metals by DOM in natural waters are based on the direct measurement of the free or labile metal in order to determine the extent of the complexation by the organic matter.[10] However, these techniques present some limitations since metal ions may also be complexed by inorganic species in the sample and adsorbed to colloidal particulates that are initially present or that form as a result of coagulation of the organic matter.[11] Considering this, fluorescence techniques are unique among metal-complexing methods in that they measure only the organic matter that changes with complexation rather than measuring free or labile metal species.[11-13] Thus, fluorescence measurements provide data that are complementary to ISE results or data from methods that measure free metal ions in equilibrium with DOM.[14]

Fluorescence spectroscopy has been widely applied to molecular studies on DOM due to the presence of fluorescent material in this complex mixture of organic compounds.[15,16] The study of fluorophores in natural waters represents an important tool for improving knowledge of both the chemical nature of DOM and its possible sources.[16]

Trace metal complexation with DOM has been previously measured through the fluorescence quenching technique.[11-16] This technique is sensitive to low concentrations of organic matter, and thus is suitable for use under natural conditions.[17] The binding of paramagnetic metal ions, such as Cu^{2+}, Ni^{2+}, and Co^{2+} to natural organic matter results in the quenching of its fluorescence.[18] The complexation parameters are obtained by models analogous to Scatchard[19] through the evaluation of the relationship between concentrations of bound metal and total binding sites.[20-22]

Quenching techniques for the study of copper-DOM interactions were first applied using the fluorescence emission spectra.[11,12] However, it is possible to obtain spectra that are significantly more valuable when excitation and emission wavelengths are allowed to vary simultaneously.[23] Synchronous fluorescence (SyF) has been reported to have several advantages over conventional fluorescence emission spectroscopy in studying natural organic matter.[24] It is a powerful technique because of its ability to detect multiple fluorophores in a single spectrum[25] as well as to provide better sensitivity and improved peak resolution compared to the conventional emission fluorescence technique. SyF scan spectroscopy also allows differentiation of the fluorescence spectra of samples of different origin.[24] Moreover, it has already been demonstrated that Cu^{2+} also stimulates quenching of multiple fulvic acid fluorophores in SyF spectra.[17,18]

This study was undertaken due to the importance of aquatic organic matter in regulating the speciation of trace metals in freshwaters and the significance of a better comprehension of the interactions between copper and DOM in rivers under anthropogenic influences. Therefore, our main objectives were (*i*) to use SyF scan spectrometry to characterize the organic material in rivers located in a metropolitan region of Brazil, in order to examine seasonal and anthropogenic influences, (*ii*) use an approach based on the SyF quenching of DOM with copper and compare the titration profiles in different spectral ranges; (*iii*) identify differences among the samples regarding the copper interaction with distinct structures of organic fluorophores; and (*iv*) investigate the role of raw sewage discharges on copper complexation in urban freshwaters.

Experimental

River water samples were collected at two points located in the Metropolitan Region of Curitiba (MRC), in southern Brazil, as it is shown in Figure 1. The MRC is a somewhat humid region in a subtropical zone with an annual rainfall of about 1400 mm.[26] It has an average temperature of 13 °C in the coldest month. Even on the coldest days, the minimum temperature is usually slightly above 0 °C. During the summer, the average temperature is around 23 °C, with peaks around 35 °C on hot days. About 2.1 million inhabitants live in the MRC, while most of its urban area is located in the Upper-Iguaçu River Basin, which has a total basin area of 1000 km².[27]
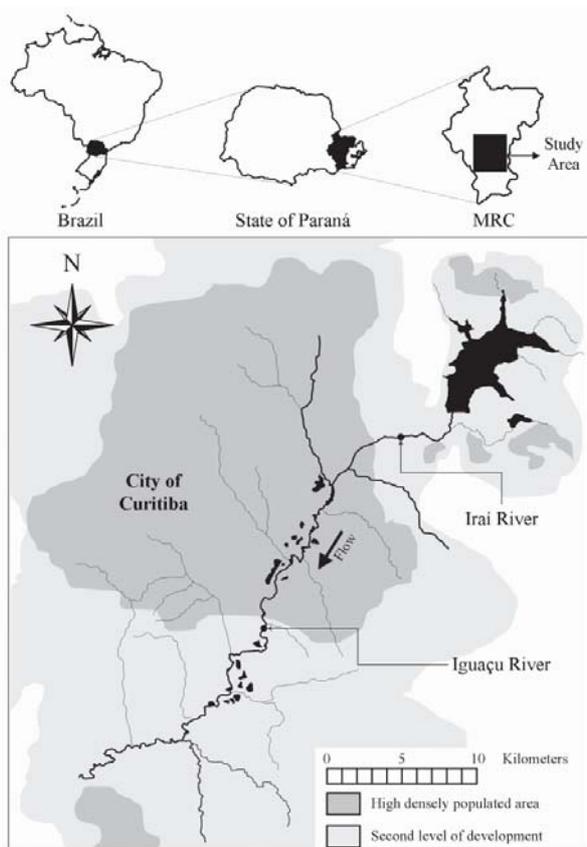
Figure 1. Location of the sampling points in the Metropolitan Region of Curitiba at the Upper-Iguaçu River Basin.

The sampling point on the Iraí River (25°26'40''S, 49°08'34''W, 890 m elevation) is located in a sparsely populated area and is subjected to a substantially lower human impact. It is considered a less polluted site where water quality is generally good, except for small discharges and some pollution from diffuse sources.[9] The sampling point on the Iguaçu River (25°32'16''S, 49°13'32''W, 865 m elevation) receives effluents drained from a very densely populated area. This river is a polluted anoxic body of water which receives discharges from sewage treatment works (STW) as well as raw sewage inputs.[9] This transition from a less polluted upstream river to a heavily polluted system downstream from the city of Curitiba provides a suitable scenario for assessing anthropogenic effects on DOM characteristics.

The samples were collected using clean techniques,[28] in August 2004 and January 2005, in order to represent typical winter and summer samples, respectively. Just after being transported to the laboratory, samples were filtered in 0.45 micron filter (Schleicher & Schuell) to separate the dissolved fraction and were preserved at 4 °C until the analyses. Aliquots of the dissolved fractions were submitted to total organic carbon determinations using a

Shimadzu TOC 5000 analyzer. For better understanding, samples from the Iguaçu and the Iraí rivers will be represented as IG and IR, respectively, while samples collected in summer or winter will be called S or W.

Synchronous fluorescence measurements were made directly on the dissolved fractions using a Hitachi F4500 spectrofluorometer equipped with a xenon lamp as the light source. All spectra were recorded in the 250-600 nm excitation wavelength range using the bandwidth of 18 nm between the excitation and emission monochromators ($\lambda_{em} = \lambda_{ex} + 18$ nm). Both monochromators were adjusted to 5 nm slit width. The scan velocity was 240 nm min^{-1} and the resolution for all spectra was 0.2 nm.

Fluorescence quenching experiments with copper were carried out according to a modification of a procedure described by Ryan and Weber.[11,12] 50 mL of the dissolved fraction was continuously stirred with a Teflon magnetic stir bar in a titration cell. The ionic strength of the solution was adjusted to 0.01 mol L^{-1} with KCl (Biotec). Appropriate aliquots of the 1.000 ± 0.002 g L^{-1} Cu^{2+} stock standard solution (CuCl$_2$ Tritisol, Merck) were added to the solution. After allowing for contact time, 2 mL of the solution were transferred from the titration cell to a quartz cuvette (Sigma) and fluorescence measurements were taken. Then, the solution in the cuvette was transferred back to the titration cell and subsequent additions of Cu^{2+} were carried out. Total added Cu^{2+} concentrations were 66 mmol L^{-1} and 308 mmol L^{-1} for Iguaçu and Iraí river samples, respectively. At the end of titrations, total volume of the samples did not exceed 51 mL. The ionic strength of both river samples was not estimated. However, typical conductivity values of 100 and 300 μS cm^{-1} are frequently determined in Iraí and Iguaçu rivers, respectively.

The contact time after each Cu^{2+} addition was optimized using a 5 mg L^{-1} soil fulvic acid solution using the fluorescence emission mode ($\lambda_{ex} = 330$ nm, $\lambda_{em} = 445$ nm). The solution was spiked with Cu^{2+} (1.0 mmol L^{-1}) and the fluorescence intensity at 445 nm remained constant after 30 min. Using this contact time we observed an average scattering below 7% during all the experiments. All titrations were carried out in duplicates under the ambient pH of the samples which varied from 6.8 to 7.2. During the experiments the pH was measured at the beginning and at the end of the titrations and an average variation lower than ± 0.1 was observed. Prior to each titration, the quartz cuvette was washed with alcohol and rinsed with Milli-Q type water (Millipore) to remove traces of organic matter, washed with purified HCl to remove metal residues and, finally, copiously rinsed with Milli-Q water.[21]

Data treatment involved the use of the well defined Ryan and Weber model.[11,12] Considering a 1:1 stoichiometric interaction the complexing reaction that fits the titration data can be expressed by a non-linear regression according to equation 1:

$$I = I_{ML} - 100/2KC_L \{[KC_L + KC_M + 1] - [(KC_L + KC_M + 1)^2 - 4K^2C_LC_M]^{1/2}\} + 100 \quad (1)$$

where I is the fluorescence intensity at any point in the titration, K is the conditional stability constant for the metal-ligand complex, C_L is the total ligand concentration, C_M is the total metal ion concentration, and I_{ML} is the lower limit of fluorescence intensity due to the fluorescence of the metal-ligand complex or fluorophores that do not participate in the complexation reaction.

The non-linear regression analysis was performed with Microcal Origin® software, using the Levenberg-Marquardt algorithm to minimize the sum of the mean-square errors between experimental and calculated values in order to obtain the parameters K, C_L, and I_{ML}.

Results and Discussion

Synchronous fluorescence characteristics of DOM

Synchronous spectra for both the Iraí and the Iguaçu Rivers in typical samples collected during summer and winter are portrayed in Figure 2. The spectra were normalized using the concentration of DOC determined in each sample. Thus, the fluorescence intensity, commonly expressed as arbitrary units, is now expressed per milligrams of carbon.

As shown in Figure 2, all SyF spectra differ from each other due to the presence of fluorescence peaks, with different intensities, at various excitation wavelengths. It is possible to observe that the IGW-DOM showed a larger spectrum with isolated peaks at 285 and 485 nm as well as higher fluorescence intensities in the range between 340 and 400 nm. The synchronous spectrum for the IGS-DOM showed a well-defined peak at 285 nm and some minor shoulders at 380 nm and 300-350 nm. The spectra from the Iraí River samples exhibited similar line shapes from 350 to 570 nm. However, a well-defined peak was noted in the IRS-DOM spectrum at 285 nm, in contrast with the IRW-DOM spectrum.

Many investigations show that there is a relationship between the location of the peaks in SyF spectra and certain characteristics of the organic matter.[29] Peaks at longer wavelengths suggest that the organic matter contains higher amounts of conjugated aromatic systems
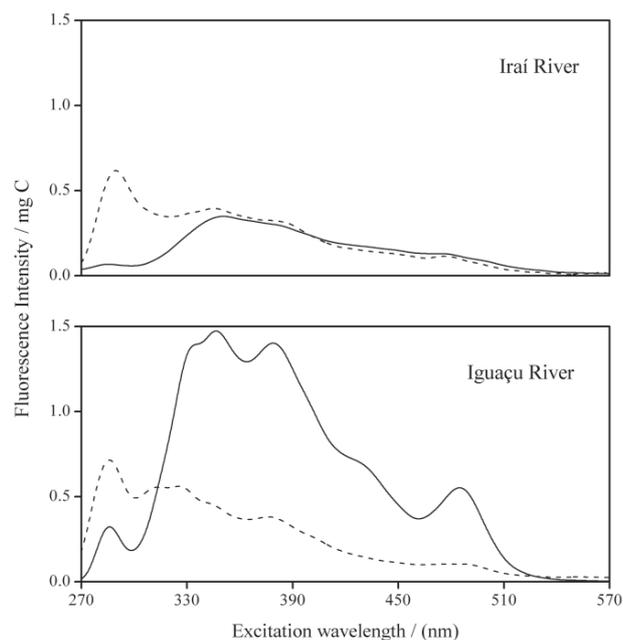

Figure 2. Synchronous (18 nm offset) fluorescence spectra for samples collected in Iraí and Iguaçu Rivers during the winter (solid lines) and the summer (dashed lines).

with electron-withdrawing functional groups. In comparison, higher fluorescence intensities at shorter wavelengths indicate simpler structural features with fewer aromatic structures, conjugated chromophores, and electron-donating groups such as hydroxyl, methoxyl, and amino.[30]

An attempt to differentiate untreated water samples according to their fluorescent materials was recently described by Peuravuori et al.,[31] where distinct chromophores were detected in isolated humic fractions as well as in natural DOM samples using SyF with a wavelength offset of 18 nm. According to these authors, the spectral region around 280-298 nm can be assigned mainly to aromatic amino acids and other volatile acids containing highly conjugated aliphatic structures; the region between 330-348 nm can be naphthalene with its derivatives; fluorescence peaks ranging from 355 to 373 nm can occur due to the presence of polycyclic aromatics with three to four fused rings; the spectral section around 400-418 nm corresponds to polycyclic aromatics with five fused rings; and the region from 460 to 478 nm probably reflects the influence of polycyclic aromatics with approximately seven fused benzene rings or humic-like organic structures.[32,33]

Based upon the results obtained by Peuravuori et al.[31] it is plausible to suppose that during the winter the DOM from both the Iraí and the Iguaçu Rivers contains polynuclear aromatics with two to five fused rings as major constituents. Additionally, IGW-DOM also presents humic

substances with higher amounts of fused benzene rings. During the summer, the opposite is true: both rivers contain higher amounts of less complex structures such as aromatic amino acids or conjugated aliphatics. Considering that in a sub-tropical climate summer is commonly a rainy period, these compounds probably arise in both rivers due to non-point sources, such as stormwater runoff, which actually plays an important role in contaminant loads for both rivers.[9]

A comparison between the SyF spectra from the Iguaçu River's DOM reveals higher amounts of organic fluorophores in the sample collected during the winter. In an earlier study[9] we have already shown that raw sewage discharges from the city of Curitiba are the major sources of pollution to the Iguaçu River waters during this season. Thus, it is believed that an increase of fluorophores could be also a consequence of sewage discharges. Iraí River waters, on the other hand, do not receive discharges from the city of Curitiba. For this reason, the two SyF spectra of the Iraí River's DOM show a great similarity, with the exception of the peak around 280 nm in the IRS-DOM spectrum.

Cu-DOM complexation measured by SyF quenching

Quenching experiments based upon the titration of the samples with Cu^{2+} were performed to better characterize the organic material as a function of the concentration of potential binding sites that can act as metal scavengers as well as to evaluate the extent of DOM-copper interactions. Figure 3 Shows SyF spectra for the IRW sample obtained during the titration of DOM with Cu^{2+}. It is possible to note that quenching occurs in the whole DOM spectrum but with slight differences in the degree of quenching at each wavelength. Despite the fact that the synchronous technique allows the identification of different organic structures, most studies estimate complexation parameters only for selected spectral bands based on the higher degree of fluorescence quenching.[22,34,35] This happens due to the fact that binding sites on DOM are not independent of each other and the polyelectrolyte nature of organic materials leads to a multi-site complexation of metals. However, as pointed out by Cabaniss,[25] the differences in the shapes of the SyF spectra suggest that this technique can be used for the qualitative study of metal binding sites in DOM as well as in isolated humic fractions.

Figure 4 portrays the fluorescence titration data for all samples in two distinct excitation/emission wavelengths pairs. The line passing through the points represents the best fit using equation 1.
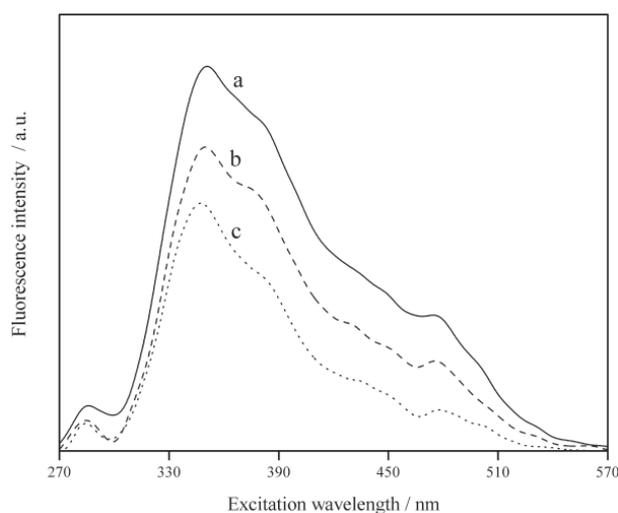

Figure 3. Synchronous (18 nm offset) fluorescence spectra for the Iraí River sample collected in winter as a function of added copper: (a) natural DOM, (b) Cu^{2+} 70 μmol L^{-1}; (c) Cu^{2+} 230 μmol L^{-1}.

Titrations with Cu^{2+} resulted in some significant differences when data for all samples are compared. It is possible to determine the difference in the degree of quenching at each wavelength. A large proportion of the quenching occurs at 485 nm for all samples. Additionally, the range of added copper concentration is different between the two rivers. After approximately 60 μmol L^{-1} of added Cu^{2+}, titration curves IGS and IGW in Figure 4 exhibited an increase in the measured signal after going through a minimum. This behavior is not usual when dealing with humic-like substances, and some aspects must be taken into account to explain it. Most of the studies dealing with metal complexation by humic-like substances are frequently applied to simulated systems, *i.e.* humic or fulvic fractions, and the inherent complexity of natural organic matter makes it difficult to describe metal behavior in real samples.[36] Ryan and Weber[12] observed that samples of the Great Bay Estuary (USA) also showed an increase of the fluorescent signal as scattered radiation was poorly rejected by the emission monochromator. According to the authors, the distinct behavior of those samples could be a consequence of the extremely low organic matter concentrations indicating a very small complexing capacity that may not be measured by fluorescence quenching techniques. However, in our experiments this condition was not achieved since Iguaçu River samples always presented higher amounts of organic carbon[9] as well as higher relative fluorescence when compared to the Iraí River ones.

Finally, the origin of the organic material in both rivers may be significantly different. Ryan and Weber[12] also suggest that an increase of the fluorescence intensity
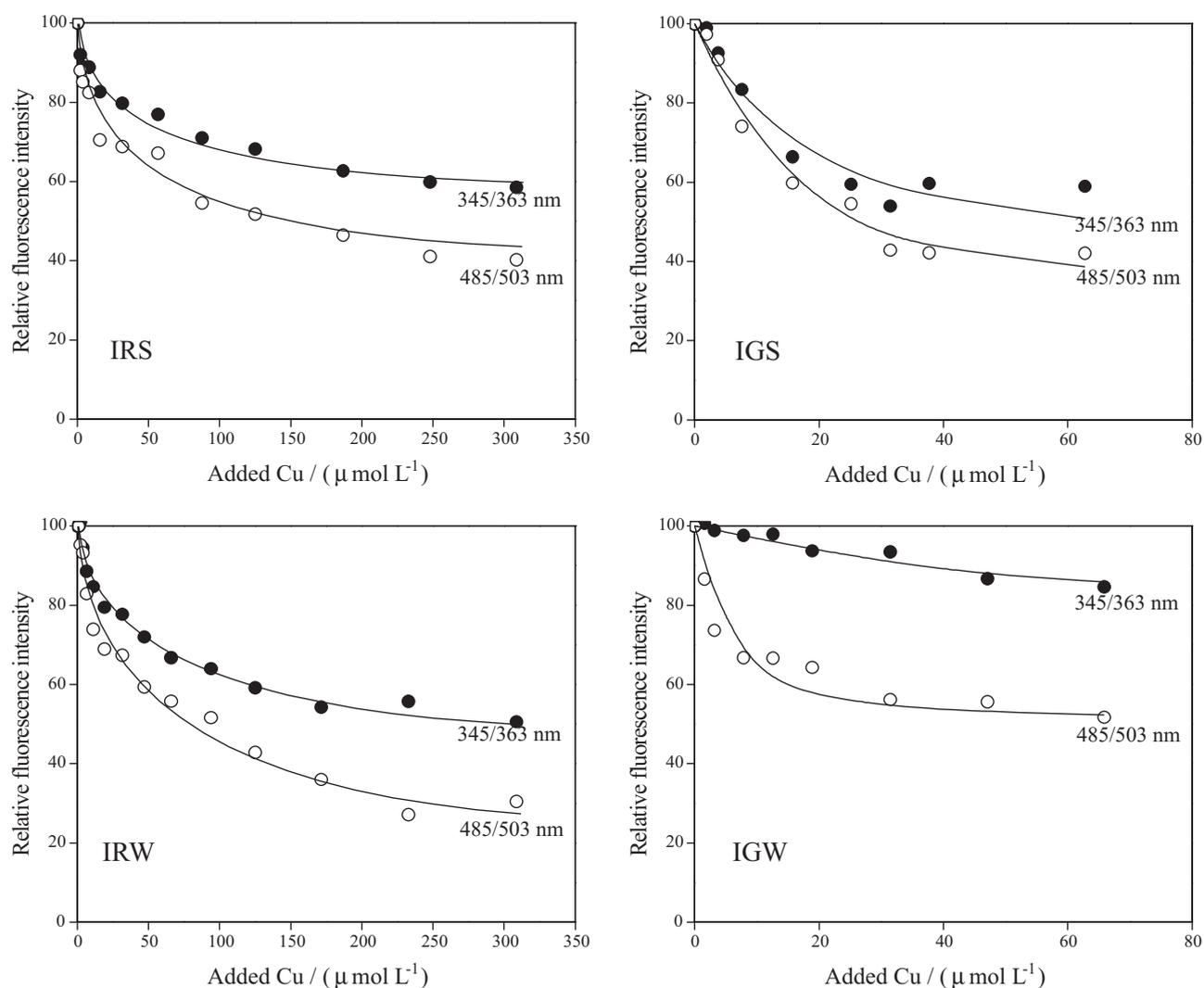
Figure 4. Synchronous fluorescence titration curves for the DOM from all samples for two different excitation wavelengths. The plotted line represents the curve adjustment performed with the 1:1 Ryan and Weber model.

during the titration of samples collected in the Great Bay Estuary could occurred due to the marine-organism origin of the organic material, as opposed to the terrestrial origin of DOM in other freshwater systems. As mentioned earlier, the rivers evaluated in this work strongly differ from each other, mostly as a consequence of raw sewage discharges from the city of Curitiba.[9] Therefore, it is possible that the DOM in both rivers also differs, in terms of its origins, due to the organic matter in the Iguaçú River arising downstream from the heavily urbanized area. To confirm this hypothesis we conducted an experiment based on a fluorescence index (FI) which has been used to discriminate various fulvic acids from different origins.[33] Unlike the UV-Visible absorption spectra, the fluorescence emission spectra have characteristics that may vary between environments. Microbially derived fulvic acids have fluorophores with a more sharply defined emission

peak occurring at lower wavelengths than fluorophores in terrestrially derived fulvic acids. McKnight *et al.*[37] show that the ratio of the emission intensity at a wavelength of 450 nm to that at 500 nm, obtained with an excitation of 370 nm, can be used as a index to distinguish sources of isolated aquatic fulvic acids. FI values of about 1.9 may be obtained for microbially derived fulvic acids whereas a value of ~1.4 for terrestrially derived fulvic acids. In our experiments, FI values obtained through spectra for all samples (not shown) revealed that the DOM from the Iraí River (FI 1.36 and 1.42 for summer and winter samples, respectively) has a predominant terrestrial origin, while the DOM from Iguaçu River samples (FI 1.73 for both seasons) has a more microbial origin.

Despite the different ranges of total added Cu^{2+} concentrations during the titrations, the complexing parameters obtained for all samples can be compared

with each other.[35,38] Table 1 gives the parameters obtained for all samples at different excitation wavelengths according to the spectral ranges suggested by Peuravuori *et al.*[31] Few C_L values could not be obtained from the analysis of the quenching profiles due to a limitation of the non-linear method, particularly when the concentrations of the binding sites are relatively low.[35,39,40] However, when C_L is fixed to markedly different values, K and I_{ML} values as well as overall adjustment of the data were not affected. It is also worth mentioning that due to the heterogeneity of DOM at natural conditions, our results should be interpreted as qualitative-possible average sites, and not as rigorously defined chemical components. Also, some considerations related to the composition of the samples must be taken into account. Certainly, both river samples contain considerable amounts of metals that may compete with Cu^{2+} as well as anions or non-organic binding sites. The complexing capacity of DOM, measured in different excitation wavelengths, represent the ability of Cu^{2+} ions in occupy active binding sites on organic material that were originally unoccupied. Furthermore, it is possible that during the titration the added Cu^{2+} replaces more weakly bound metal ions. However, metal ions such as Al^{3+} and Fe^{3+} could compete effectively with Cu^{2+} for binding sites during the entire titration.[12] Despite the fact that the complexation of copper by DOM evaluated by fluorescence implies that only fluorescent organic structures will be considered in the generation of complexing parameters, negatively-charged minerals or even some anions may also coordinate with Cu^{2+} or other cations in the sample.

From data related to the two Iraí River samples in Table 1, it is possible to observe that there is a slight correlation between C_L and log K values. As the complexity of the organic structures becomes higher, *i.e.* excitation wavelengths become longer, the concentration of binding sites also increases and the stability of Cu-MOD complexes (log K) tends to decrease. This behavior probably indicates that

potential binding sites such as carboxylic or phenolic groups are concentrated in simpler structures whereas high concentrations of negatively-charged sites are associated to more complex structures. For the IGS sample, the opposite is observed: as the complexity of DOM increases C_L and log K values also become higher, indicating that more stable complexes between copper and DOM are preferably formed in humic-like substances. Since few values were obtained for C_L for the IGW samples, it is not possible to apply the same considerations to this sample. However, note that the IGW sample also presented increased values for log K as well as a high value of C_L for the spectral range attributed to more complex organic structures.

Another interesting complexing parameter obtained from the non-linear model shown in equation 1 is the I_{ML} value, which can be described as the lower limit of the fluorescence when all the available ligands are bound and their fluorescence quenched. This limiting fluorescence could be a consequence of the nature of the organic materials in a sample that shows fluorescence but does not bind and, therefore, does not quench.[11,12]

Figure 5 shows the total fluorescence quenching ($TQ = 100 - I_{ML}$) for all samples in different excitation wavelengths of the SyF spectra. It is possible to observe that, for all samples, TQ increases as the wavelengths become higher. However, when the two aquatic systems are compared, a remarkable difference can be noticed. While the two samples from the Iraí River seem to behave equally, the Iguaçu River samples strongly differ from each other. During the summer, all organic structures may contribute to copper complexation, since total TQ does not strongly vary. During the winter, however, there are extremely low values for TQ in the middle region of the SyF spectrum.

Low values of TQ have also been reported in the literature for natural organic matter from different origins.[17,40] Milne and Zika[41] reported higher I_{ML} for some marine fluorophores and concluded that they are unable to complex copper. It is interesting to point out that the middle region in the IGW-DOM spectrum exhibited higher

Table 1. Conditional stability constant (K) and ligand concentration (C_L) for samples collected in Iraí and Iguaçu Rivers during summer and winter seasons

Sample	Complexation parameters									
	λ_{exc} 285 nm		λ_{exc} 345 nm		λ_{exc} 380 nm		λ_{exc} 430 nm		λ_{exc} 485 nm	
	log K	C_L	log K	C_L	log K	C_L	log K	C_L	log K	C_L
IRS	5.49 (0.12)[a]	*[b]	4.60 (0.13)	28.5 (0.8)	4.60 (0.20)	28.5 (1.5)	4.39 (0.11)	42.8 (0.5)	4.82 (0.21)	40.7 (0.2)
IRW	5.02 (0.16)	*	4.96 (0.11)	30.4 (0.7)	4.62 (0.19)	36.0 (0.9)	4.74 (0.19)	44.0 (0.3)	4.50 (0.18)	43.0 (0.8)
IGS	4.68 (0.21)	32.0 (0.3)	5.08 (0.19)	25.2 (1.0)	4.92 (0.20)	27.9 (0.9)	5.57 (0.24)	21.6 (1.1)	5.26 (0.17)	61.2 (1.9)
IGW	NQ[c]	NQ	5.33 (0.24)	*	5.37 (0.26)	*	5.13 (0.20)	*	5.66 (0.30)	65.9 (2.8)

[a]The number indicated in parentheses is the standard deviation considering the titration duplicates. [b]No reasonable values were obtained, but fixed concentration values between 10^{-8} and 10^{-3} mol L^{-1} showed no influence on the other calculated parameters. [c]Quenching does not occur.
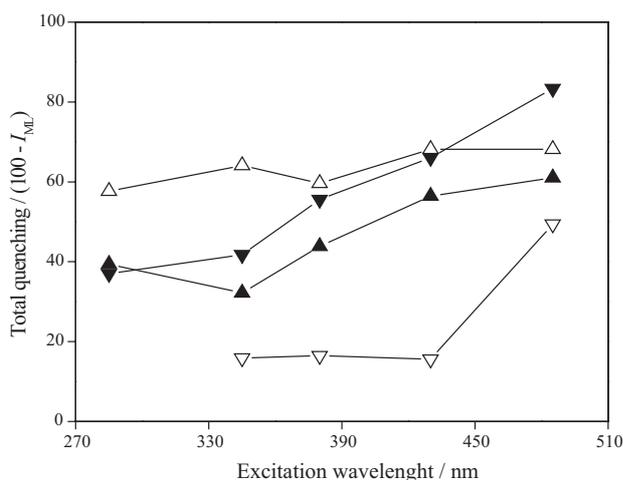
Figure 5. Total fluorescence quenching as a function of excitation wavelength obtained through synchronous (18 nm offset) fluorescence for all samples. Solid symbols represent the Iraí River samples and open symbols the Iguaçu River ones. Triangles that point up are related to samples collected in summer whereas down triangles represents samples from winter.

fluorescence values among all samples as can be seen in Figure 2. This behavior indicates that, in fact, raw sewage discharges from urbanized areas contribute to the increase of specific organic materials that do not show ability to complex metals in aquatic environments.

Conclusions

This work showed that synchronous fluorescence can be applied to the identification anthropogenic influences on the characteristics of aquatic organic matter. A simple characterization of samples collected upstream and downstream from the city of Curitiba, in Brazil, revealed that during the rainy summer the major constituents of DOM in both rivers arise, probably, through non-point sources. During the dry winter, on the other hand, there is an increase of organic structures related to raw sewage discharges from the urbanized area.

Quenching experiments using the 1:1 complexation model indicated that there are differences between all samples. These differences are associated with the extent of the interaction between DOM and copper. DOM from the Iraí River samples, collected during the summer and the winter, presents stable binding sites in simpler structures and higher concentrations of sites in more complex structures. On the contrary, DOM from the Iguaçu River shows high quantities of more stable complexes associated to humic-like substances. Finally, it was demonstrated that raw sewage discharges provide a pool of organic materials

that are not able to interact with copper in the Iguaçu River during the dry winter.

References

1. Keil, R. G.; Montlucon, D. B.; Prahl, F. G.; Hedges, J. I; *Nature* **1994**, *370*, 549; Campbell, P. G. C.; Twiss, M. R.; Wilkinson K. J.; *Can. J. Fish. Aquat. Sci.* **1997**, *54*, 2543; Thomas, J. D.; *Freshwater Biol.* **1997**, *38*, 1; Hader, D. P.; Kumar, H. D.; Smith, R. C.; Worrest, R. C.; *J. Photochem. Photobiol., B* **1998**, *46*, 53; Kuchler, I. L. ; Miekeley, N.; Forsberg, B. R.; *J. Braz. Chem. Soc.* **2000**, *11*, 286.

2. Sunda, W. G.; Lewis, J. A. M.; *Limnol. Oceanogr.* **1978**, *23*, 870; Florence, T. M.; *Talanta* **1982**, *29*, 345; Bruland, K. W.; *Limnol. Oceanogr.* **1989**, *34*, 269.

3. Pagenkopf, G. K.; Russo, R. C.; Thurston, R. V.; *J. Fish. Res. Bd. Can.* **1974**, *31*, 462; Sunda, W.; Guillard, R. R. L.; *J. Mar. Res.* **1976**, *34*, 511; Howarth, R. S.; Sprague, J. B.; *Water Res.* **1978**, *12*, 455; Meylan, S.; Behra, R.; Sigg, L.; *Environ. Sci. Technol.* **2003**, *37*, 5204.

4. Diks, D. M.; Allen, H. E.; *Contam. Toxicol.* **1983**, *30*, 37; Takács, M.; Alberts, J. J.; Egeberg, P. K.; *Environ. Int.* **1999**, *25*, 315; McGeer, J. C.; Szebedinszky, C.; McDonald, D. G.; Wood, C. M.; *Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol.* **2002**, *133*, 147.

5. Koetsier, P.; McArthur, J. V.; Leff, L. G.; *Freshwater Biol.* **1997**, *37*, 79.

6. Lampert, W.; *Limnol. Oceanogr.* **1978**, *24*, 831; Bilby, R. E.; Bisson, P. A.; *Can. J. Fish Aquat. Sci.* **1992**, *49*, 540; Vega, M.; Pardo, R.; Barrado, E.; Debán, L.; *Water Res.* **1998**, *32*, 3581; Kalbitz, K.; Solinger, S.; Park, J.-H.; Michalzik, B.; Matzner, E.; *Soil Sci.* **2000**, *165*, 277; Prestes, E. C.; Anjos, V. A.; Sodré, F. F.; Grassi, M. T.; *J. Braz. Chem. Soc.* **2006**, *17*, 53; Shon, H. K.; Vigneswaran, S.; Snyder, S. A.; *Crit. Rev. Environ. Sci. Technol.* **2006**, *36*, 327.

7. Xue, H. B.; Oestreich, A.; Kistler, D.; Sigg, L.; *Aquat. Sci.* **1996**, *58*, 69.

8. Grassi, M. T.; Sodré, F. F.; *J. Phys. IV* **2003**, *107*, 1283.

9. Sodré, F. F.; Anjos, V. E.; Prestes, E. C.; Grassi, M. T.; *J. Environ. Monit.* **2005**, *7*, 581.

10. Donat, J. R.; Lao, K. A.; Brluland, K. W.; *Anal. Chim. Acta* **1994**, *284*, 547; Batley, G. E.; Apte, S. C.; Stauber, J. L.; *Aust. J. Chem.* **2004**, *57*, 903.

11. Ryan, D. K.; Weber, J. H.; *Anal. Chem.* **1982**, *54*, 986.

12. Ryan, D. K.; Weber, J. H.; *Environ. Sci. Technol.* **1982**, *16*, 866.

13. Seitz, W. R.; *Trends Anal. Chem.* **1981**, *1*, 79.

14. Ryan, D. K.; Thompson, C. P.; Weber, J. H.; *Can. J. Chem.* **1983**, *61*, 1505.

15. Sierra, M. M. D.; Giovanela, M.; Donard, O. F. X.; Belin, C.; *Quim. Nova* **1996**, *19*, 294.

16. Luster, J.; Lloyd, T.; Sposito, G.; Fry, I. V.; *Environ. Sci. Technol.* **1996**, *30*, 1565.

17. Lombardi, A. T.; Jardim, W. F.; *Chem. Speciation Bioavailability* **1997**, *9*, 27.

18. Waite, T. D.; Morel, F. M. M.; *Anal. Chim. Acta* **1984**, *162*, 263.

19. Scatchard, G.; *An. NY. Acad. Sci.* **1949**, *51*, 660.

20. Saar, R. A.; Weber, J. H.; *Anal. Chem.* **1980**, *52*, 2095.

21. Oliveira, C. R.; Lombardi, A. T.; Jardim, W. F.; *Chem. Speciation Bioavailability* **1995**, *7*, 125.

22. Hays, M. D.; Ryan, D. K.; Pennell, S.; *Appl. Spectrosc.* **2003**, *57*, 454.

23. Lloyd, J. B. F.; *Nature Phys. Sci.* **1971**, *231*, 64.

24. Miano, T. M.; Senesi, N.; *Sci. Total Environ.* **1992**, *117/118*, 41.

25. Cabaniss, S. E.; *Environ. Sci. Technol.* **1992**, *26*, 1133.

26. http://www.simepar.br, accessed in October 2006.

27. SUDERHSA; *Qualidade das Águas Interiores do Estado do Paraná: 1987-1995*, Suderhsa: Curitiba, 1997.

28. U.S.EPA; *Method 1669: Sampling Ambient Water for Determination of Trace Metals at EPA Water Quality Criteria Level*, U.S. EPA: Washington DC, 1996.

29. Guibault, G.; *Practical Fluorescence: Theory, Methods and Techniques*, Marcel Decker Inc.: New York, 1973; Miano, T. M.; Sposito, G.; Martin, J. P.; *Soil Sci. Soc. Am. J.* **1988**, *52*, 1016; Senesi, N.; *Anal. Chim. Acta* **1990**, *232*, 77; Kister, J.; Pieri, N.; Alvarez, R.; Díez, M. A.; Pis, J. J.; *Energy Fuels* **1996**, *10*, 948; Lombardi, A. T.; Jardim, W. F.; *J. Braz. Chem. Soc.* **1997**, *8*, 339; Lombardi, A. T.; Jardim, W. F.; *Water Res.* **1999**, *33*, 512.

30. Pullin, M. J.; Cabaniss, S. E.; *Environ. Sci. Technol.* **1995**, *29*, 1460.

31. Peuravuori, J.; Koivikko, R.; Pihlaja, K.; *Water Res.* **2002**, *36*, 4552.

32. Westerhoff, P.; Anning, D.; *J. Hydrol.* **2000**, *236*, 202.

33. Chen, J.; LeBoeuf, E. J.; Dai, S.; Gu B.; *Chemosphere* **2003**, *50*, 639.

34. Esteves da Silva, J. C. G.; Machado, A. A. S. C.; Pinto, M. S. S. D. S.; *Fresenius J. Anal. Chem.* **1997**, *357*, 950.

35. Esteves da Silva, J. C. G.; Machado, A. A. S. C.; Oliveira, C. J. S.; Pinto, M. S. S. D. S.; *Talanta* **1998**, *45*, 1155.

36. Town, R. M.; Filella, M.; *Aquat. Sci.* **2000**, *62*, 252.

37. McKnight, D. M.; Boyer, E. W.; Westerhoff, P. K.; Doran, P. T.; Kulbe, T.; Andersen, D. T.; *Limnol. Oceanogr.* **2001**, *46*, 38.

38. Saito, T.; Nagasaki, S.; Satoru, T.; *Radiochim. Acta* **2002**, *90*, 545.

39. Esteves da Silva, J. C. G.; Machado, A. A. S. C.; *Chemom. Intell. Lab. Syst.* **1995**, *27*, 115.

40. Holm, T. R.; Barcelona, M. J.; *Abstracts of the Ground Water Geochemistry Conference*, Colorado, USA, 1998.

41. Milne, P. J.; Zika, R. G.; *Mar. Chem.* **1989**, *27*, 147.