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Interaction of pyrene fluoroprobe with natural and synthetic humic substances: Examining the local molecular organization from photophysical and interfacial processes

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Abstract

The direct and indirect interaction mechanisms of pyrene with (i) various molecular weight fractions of a synthetic humic-like substance (SyHA) and (ii) extracts of natural humic acids (NHA) from Moselle River suspended matter, were investigated using quenching fluorescence and surface tension measurements. Humic materials were characterized in a previous study. The Stern-Volmer associative constants were determined from the quenching technique. Surface tension measurements revealed an increase in surface activity as a function of concentration for each humic fraction independently of the pyrene presence in solution, even during the formation of humic micelles. The results obtained suggest the possibility of specific intermolecular interactions occurring during pyrene entrapment within humic acids. In addition, we show that molecular weight, aliphatic chains (especially those containing nitrogen groups) and acidity are determinant characteristics for pollutant entrapment capacity at concentrations below the critical micellar concentration (CMC) of humic substances.

Key words: natural - synthetic humic substances - fluorescence quenching - pyrene - surface tension.

Introduction

Understanding the fate and the transport of anthropic contaminants in natural aquatic systems is of a primary importance for water resources management and treatment optimization. Among them, polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in the environment and have been identified in a number of soils, effluents, river sediments, as well as in the colloidal compartment of surface waters (Bouloubassi and Saliot, 1991; Countway et al., 2003). Although PAHs also occur naturally, high levels of those molecules in the environment are usually attributed to point and non-point pollution sources such as oil spills, automobile exhaust fumes, and coal refining processes (Achten and Hofmann, 2009; Short et al., 2007). According to eco- and human toxicity exposure tests (Hua et al., 2009; Jeffrey et al., 1977), mutagenic or carcinogenic effects of PAHs can be expected in the natural environment.

It has long been recognized that natural organic matter (NOM) has an indisputable role in the sorption and leaching of hazardous organic chemicals such as PAHs in the environment (Chin et al., 1997; Countway et al., 2003; Gauthier et al., 1987; Peuravuori, 2001). Various techniques have been used to examine the binding capacities of NOM towards PAHs such as adsorption, Nuclear Magnetic resonance (NMR), chromatography, and fluorescence methods (Ghosh et al., 2000; Hur et al., 2009; Nakashima et al., 2007; Pan et al., 2006). Most of these classical approaches require the determination of free pollutant concentrations, before and after their binding with dissolved natural organic matter. However, the necessary separation steps often lead to a poor quantitative estimate of binding capacity. On the other hand, a fluorescence quenching technique does not require such a separation step. Therefore, fluorescence spectroscopy offers unique advantages in the study of contaminant binding to NOM either dissolved, suspended or within sediment matter.

Humic substances represent a predominant component of natural organic matter. They are commonly described as amphiphilic heterogeneous compounds of about 1-2 nm in size (Sieliechi et al. 2008). The organization of humic substances, either polymeric or supramolecular assembly, remains a debated issue. Their molecular size has been shown to strongly influence the binding capacity and the environmental transport of both non-polar (Chin and Weber, 1989; Chiou et al., 1987) and polar organic contaminants such as pesticides (Piccolo et al., 1996, 1998). The polarity of humic material could also affect pollution transport. Previous reports have suggested either a partition of contaminants between water and micelle-like structures of humic substances and/or binding to well-defined sorption sites of the humic substances (Engebretson and Wandruska, 1994; Sierra et al., 2005). Therefore, a better understanding of the humic substance building blocks and structure can certainly be attained by relating their sorption properties with their chemical characteristics. One approach is to correlate the “binding” coefficients determined by quenching measurements with others hydrophobic experimentally determined parameters. Indeed, this kind of analysis based on pseudo-micellar behaviour (Engebretson and Wandruska, 1994; Sierra et al., 2005) revealed that an increase in pyrene fluorescence with temperature in natural organic matter (NOM) solution could be related to the decrease in dielectric constant of water, or to a reduction of pyrene binding to NOM. Besides, as water becomes a poorer solvent, humic polymers are thought to coil up and “squeeze” out the solvating water. Such dehydration phenomenon has been offered in explanation of the well-known cloud-point behaviour of non-ionic surfactants (Myers, 1992). The increased pyrene fluorescence intensity with an increased temperature in the presence of some humic acids may thus be ascribed to a pyrene incorporation into the micelle. Since the interaction may take place in the excited state of the fluorescence compound and be

collisional in nature, it could be referred to as “dynamic quenching”. The excitation energy is transferred to the quencher and usually nonradiatively dissipated. Alternatively, the fluorophore and the quencher may associate in the ground state, leading to species that lacks the original fluorescence characteristics. This is termed “static quenching”. Therefore, understanding that pyrene binding enthalpy is negative is not enough to explain the rise of pyrene fluorescence in DOM solutions with temperature.

Until now, the pyrene fluorescence spectrum has mainly been used to determine solubility values and binding constants of non-polar organic compounds to different sorbents. Thus, this hydrophobic probe turned out to be a very useful in situ tool for determining the polarity of natural organic matter fractions (Engebretson and Wandruska, 1994).

Recent research (Quagliotto et al., 2006) tends to build on such concepts and views natural organic matter (NOM) as “a competitive low-cost biosurfactant”. From process cost and environmental impact points of view, the bio-surfactant has the advantages of operating at much lower additive concentrations and should be available at significantly lower cost than the synthetic surfactants. However, the mechanistic basis for identifying the interactions between polycyclic aromatic hydrocarbons (PAHs) and natural organic matter (NOM) is not yet sufficiently clarified to predict sorption coefficients. One approach to circumvent the intrinsic complexity of humic substances consists in synthesizing functionally well-defined polymers integrating the main characteristics of amino-phenolic humic-like molecules (Jung et al., 2005). A comparison of the interaction of pyrene with (i) synthetic humic-like substances (SyHA) of different molecular weights made from the oxidation of catechol/glycine (CGHA) or catechol/triglycine (CTHA) oxidation, (ii) natural humic extracts from suspended matter centrifuged from Moselle

River water (France), was explored in the present paper. The amount of pollutant bound to each of the synthetic and natural humic acid fractions was correlated with their chemical characteristics.

This paper attempts to clarify the formation of micellar aggregates between pyrene and humic acids. The aim is to detect whether an interfacial modification could explain the pyrene entrapment in micellar-like domains, and finally whether the pyrene has an effect on the formation of humic substances micelles-like.

Experimental section

Humic substances

Synthetic humic-like substances (SyHA) were prepared with catechol and glycine or triglycine as reactants, thus representing two different nitrogen sources in distinct syntheses. Two molecular weight synthetic humic-like substance (SyHA) fractions (1-3.5 kDa and > 8 kDa, respectively CTHA1 and CTHA2) were obtained for the catechol/triglycine synthesis. A greater than 8 kDa catechol/glycine synthetic humic-like substance (SyHA) fraction was also collected (CGHA). Andreux et al., (1980) polymerization protocol was used. Catechol (6.6 g) was dissolved in 500mL of a solution of sodium phosphate buffer $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ at pH 8 to obtain a 0.03M catechol solution. This was then mixed with an equal volume of buffered glycine or triglycine 0.03M solution. The resulting mixture was then allowed to react in a 2 L flask in the dark at 25 °C and under pure oxygen constant pressure (0.9 bar) for 5 days. After dialysis, (Spectra/Por 7 membrane- 1, 3.5 and 8 kDa molecular weight cuts-off-Roth-Sochiel Lauterbourg, France), the synthetic materials in a sodium form were freeze-dried and stored at 4 °C in the dark. Monitoring of the synthesis has been described elsewhere (Jung et al., 2005).

Natural organic matter (NOM) designates natural humic acids extracted from suspended matter taken from the Moselle river at Messein (France). The extraction was performed according to the International Humic Substances Society (IHSS) standard procedure (Thurman and Malcolm, 1981). Sodium saturated humic substances having a greater than 8 kDa molecular weight were dialyzed to remove excess salt and then freeze-dried. All natural and synthetic humic substances were collected under their sodium form. Two natural humic extracts corresponding to two different field sampling campaigns were obtained. Natural humic acids (NHA1 and NHA2) extractions were performed during the summer and autumn seasons, respectively.

Preparation of Pyrene-Humic substances solutions

Pyrene (99+%) was purchased from Sigma-Aldrich Chemicals (Lyon, France) and carefully stored at 4 °C in the dark. Crystals were dissolved in ethanol (99.5+% spectrograde, Carlo Erba) to make a stock solution of 10^{-3} M.

Solutions of 10, 5 and 0.1 g.L⁻¹ of synthetic humic-like substance (SyHA) and natural humic acids (NHA) were first prepared in deionized ultra-pure Milli-Q water (Millipore). Dilutions of these solutions were made to obtain humic substances (HS) concentration ranging from 0.1 to 30 mg.L⁻¹ for fluorescence measurements and from 0.1 to 1 g.L⁻¹ for surface tension measurements. 20 mL aliquots of Synthetic humic-like substance (SyHA) and NHA solutions were then placed in glass beakers and mixed with the pyrene stock solution (10^{-3} M in ethanol) to obtain final concentrations of 10^{-6} M of pyrene. The volumetric content of ethanol introduced in the samples for fluorescence measurements did not exceed 0.1% and was thus considered negligible with respect to the solubility of pyrene in the samples. The pyrene-synthetic humic-like substance (SyHA) and pyrene-natural humic acids (NHA) solutions were then adjusted to pH 8

under stirring with dropwise addition of 0.1 M HCl. The pyrene-SyHA and pyrene-NHA solutions were allowed to equilibrate for one hour. Prior to fluorescence measurements, the samples were homogenized by stirring.

Surface tension measurements

Surface tension measurements were carried out with solutions prepared similarly as for the fluorescence measurements. The surface tensions (γ_s) were determined at pH 8 using the Wilhelmy plate technique both for humic materials alone and in presence of pyrene at 10^{-6} M. The measurements were performed with glass plates carefully cleaned with sulfochromic acid, rinsed with Milli-Q ultra-pure water, and then heated in a Bunsen burner flame to avoid organic contaminant traces. γ_s values were obtained at 25 \pm 0.1 $^{\circ}$ C, using a KSV Sigma-70 tensiometer model (Cahn, Texas, USA) and performed with a constant solution volume of 20 mL. The accuracy of measurements was \pm 0.1 $\text{mN}\cdot\text{m}^{-1}$. The surface tension of each solution was calculated from the average of two immersion/emersion cycles using the linear intercept at the origin of the receding branch. The average standard errors between measurements for synthetic humic-like substance (SyHA) fractions and natural extracts were 0.2 $\text{mN}\cdot\text{m}^{-1}$ and 0.35 $\text{mN}\cdot\text{m}^{-1}$, respectively. All experimental errors were found below 1 $\text{mN}\cdot\text{m}^{-1}$ (the maximum difference between two immersion/emersion cycles measurements and taking into account the experimental deviation). Critical micellar concentration (CMC) of humic substances have been determined from the break points of surface tension versus logarithm of humic acid concentration straight lines. The second horizontal line was plotted using the first γ stabilization value, that is to say when the difference between two γ mean measurements was at maximum three times the average standard error determined for the fractions.

Fluorescence measurements

Steady-state fluorescence measurements were performed on a Spex Fluorolog-3 spectrofluorometer (Jobin-Yvon, Stanmore, UK) using 0.5 mm slits for the excitation and emission monochromators. The instrument was equipped with a 450 W Xenon lamp (Jobin-Yvon). The sample temperature was maintained to 25.0 +/- 0.1 °C by a LTD6G temperature control unit (Grant, Sarose, UK). In order to compare the fluorescence intensities of all fluorescence spectra and to avoid time apparatus drift, the fluorescence was normalized with a 0.16 mg.L⁻¹ fluoresceine solution buffered at pH 10. Auger's correction (Auger et al., 1995) was used to correct the DOM absorption. Absorbance was measured by means of a Perkin Elmer (Boston, USA) Lambda-2 spectrophotometer. The synthetic humic-like substance (SyHA) and natural humic acids (NHA) intrinsic fluorescence were corrected by subtracting the emission intensities of SyHA and NHA solutions without pyrene.

Determination of binding constants by fluorescence

Fluorescence quenching identifies an interaction between the fluorophore and another species leading to a diminution or even an extinction of the fluorescence signal. This interaction may take place in the excited state of the fluorescence compound and may be collisional in nature, in which case it is referred to as “dynamic quenching”. The excitation energy transferred to the quencher, is usually dissipated non-radiatively. Alternatively, the fluorophore and the quencher may associate in the ground state, thus leading to species that lack the original fluorescence characteristics. In that case, it is termed “static quenching”.

Quenching interactions are described by the Stern-Volmer equation (Gauthier et al., 1986). The equation holds for both dynamic and static quenching, provided that only one type of quenching is operative and that the fluorophore is present as a single population. A linearisable Stern-Volmer plot (I_0/I vs $[Q]_0$) is then obtained, where I_0 and I are respectively the fluorescence intensity in the absence and in the presence of quencher, K_{SV} the Stern-Volmer quenching constant (slope), and $[Q]_0$ the quencher initial concentration.

The pyrene fluorescence spectrum exhibits five main emission vibronic bands called I_1 , I_2 , I_3 , I_4 and I_5 respectively at 372, 377, 382, 390 and 395 nm in ethanol after excitation at 332 nm (Kalyanasundaram and Thomas, 1977). For quenching measurements, the I_0/I_2 ratio was calculated, with I_2 and I_0 designating the pyrene fluorescence intensities at 377 nm in emission for a 332 nm excitation wavelength with and without humic substances, respectively.

Three replicates were run for each sample and for each quenching measurements to determine average binding constants.

Results and Discussion

Synthetic humic-like substance (SyHA) and natural humic acids (NHA) characterizations

The main characteristics of both synthetic and natural humic materials have been presented in a previous paper using a multi-analytical technique approach (Jung et al., 2005). A summary of those properties (elemental analysis, hydrophobic indices) is given in Table 1. The nitrogen contents for synthetic humic-like substance (SyHA) were found to be approximately three times higher in CTHA (1-3.5 kDa and > 8 kDa, respectively CTHA1 and CTHA2 obtained with catechol/triglycine) than in CGHA (> 8 kDa with catechol/glycine). The higher nitrogen content of NHA1 (natural humic acid summer extract) compared with NHA2 (autumn extract) may be explained by microbial decomposition by-products. H/C atomic ratio is a useful qualitative parameter when comparing the aromaticity and the hydrophobicity of humic substances (Chen et al., 1977; Thurman, 1985). The aromaticity increases with a decrease in H/C, and thus, CGHA is more aromatic than CTHA2 and eventually than CTHA1. Natural humic acids (NHA) were found more aliphatic than synthetic humic-like substance (SyHA) based on elemental analysis and on Fourier Transform InfraRed (FTIR) spectroscopy results (Jung et al., 2005). CTHA fractions are also presumed to possess more “labile” peptidic bonds and to present a lower aromaticity degree than that of catechol glycine humic acid. Finally, a great difference between natural and synthetic humic-like substance concerning both aromatic and peptidic structures is revealed by the elemental composition.

Surface tension measurements at the air/water interface

As illustrated in Figure 1, the surface tensions of all humic substances decrease with an increase in humic substances (HS) concentration. No significant differences between solutions of humic acid alone and humic acid and pyrene could be detected (data not shown).

Catechol triglycine low-molecular weight humic acid (CTHA1) and catechol-glycine humic acids (CGHA) solutions present a decrease of surface tension as a function of the logarithm of the concentration, that is reminiscent of those of natural soil and leaf humic acids extracts (Chin et al., 1996; Quagliotto et al., 2006; Tuckermann and Cammenga, 2004). On the other hand, the two natural humic acids extracts (NHA1 and NHA2) and catechol triglycine high-molecular weight humic acid (CTHA2) show hyperbolic curves that resemble some determined in the literature and reported on Figure 1 (Quagliotto et al., 2006). These different behaviours suggest that natural humic acids and CTHA higher molecular weight fraction (CTHA2) likely present a molecular crowding at the interface, whereas synthetic CGHA and CTHA1 seem to behave like less charged entities concerning the acidity view: they can deliver less protons in the solution than the other fractions (Table 1). The critical micellar concentration (CMC) values are reported in Table 2. The CMC of our natural extracts are twice lower than those determined in the literature for humic substances extracted from marine sediments (typically close to 1 g.L^{-1} according to Shinozuka and Lee, 1991) but close to those of compost humic acid-like (typically 403 mg.L^{-1} according to Quagliotto et al., 2006). The two natural extracts (summer, NHA1 and autumnal, NHA2) possess a similar critical micellar concentration (CMC) (with respect to experimental accuracy). Among synthetic fractions, CTHA2 is the only material that shows a true CMC. It can also be hypothesized that to allow the formation of a micellar phase, the humic substances

should present a good balance between functional groups (especially protein-like, when comparing results from CTHA to CGHA) and high molecular weight (comparing CTHA2 with CTHA1).

Stern-Volmer plots

The I_0/I_2 versus synthetic humic-like substance (SyHA) and natural humic acid (NHA) concentration plots are shown in Figure 2. For all humic fractions, the quenching plots present a linearisable evolution in the range of concentrations investigated. Moreover, the evolution of the quenched fluorescence in solution is very similar to other authors studies, especially for CTHA1 fraction (Peuravuori, 2001, presented on Figure 2). The slopes of Stern-Volmer plots decrease from synthetic catechol-glycine humic acid (CGHA), the two catechol-triglycine humic acid (CTHA2 and CTHA1) to natural NHA1 and NHA2.

It has been established (Gauthier et al., 1987; Lakowicz, 1999) that two distinct kinds of fluorophore can exist in solution, i.e., some molecules are bound to the humic-like material whereas others remain free in solution. The first group has a totally quenched fluorescence (this mechanism is usually called “static quenching”) whereas the second group shows no interaction with the humic matrix, but remains sensitive to other types of quenching (this mechanism is usually called “dynamic quenching”). Both quenchings (static and dynamic) can be observed by linearisable Stern-Volmer plots at concentrations generally below 20-30 mg.L⁻¹.

Since no significant change in interfacial tension was observed when adding the pyrene to the suspension of humic material, we can assume that the obtained values are mainly due to humic materials. Besides, all combined data suggest a close physical

association between the fluorophore and the quencher attributed to an association of the former into hydrophobic pockets.

The binding constants (K_{SV}) obtained for our systems (Table 2) are in good agreement with the range found in the literature for different polycyclic aromatic hydrocarbons (PAHs) complexed with natural organic matter (Borisover et al., 2006; Gauthier et al., 1986; Peuravuori, 2001). For both natural and synthetic humic-like substance, we can conclude that pyrene is incorporated into the hydrophobic sites of humic substances.

The much higher K_{SV} values for catechol-glycine humic acid (CGHA) may be explained by the differences in elemental analysis reported in Table 1. The H/C atomic ratio, which could provide an estimation of the aromaticity degree (Chen et al., 1977; Thurman, 1985), is substantially lower (greater aromaticity) for this humic substance. The greater aromaticity could also be responsible for an increase of van der Waals forces due to π bonding interactions between the aromatic structure of the pyrene and the aromatic moieties of the synthetic humic-like substance (SyHA) and natural humic acid (NHA) (Chin et al., 1997). Besides, the hydrophobic indices (HI in Table 1) calculated by reversed-phase high liquid chromatography in a water/acetonitrile elution gradient (Jung et al., 2005) for the different fractions show good correlation with the Stern-Volmer constants determined (Table 2). These results suggest that the more hydrophobic the humic substances at the local scale, the more effective the quenching fluorescence of pyrene. A previous work (Jung et al., 2005) has shown that catechol-glycine humic acid (CGHA) presents the most condensed aromatic structure. It can then be concluded that the more condensed the humic substance, the more efficiently quenched pyrene fluorescence. Moreover, the binding constants could be discussed with the total acidity determined in Table 1: the more the humic substances are acid, the less

pyrene could be associated with, whatever kind of acidity is concerned. Indeed, a previous study (Jung et al., 2005) showed that carboxylic groups are more abundant in the synthetic polymers whereas phenolic groups are ten times more numerous in natural substances. These results suggest that phenolic or carboxylic groups are repulsive for pyrene association with humic substances.

The very slight difference in the Stern-Volmer slopes relative to catechol triglycine higher molecular-weight humic acid (CTHA2) and catechol-glycine humic acid (CGHA) suggests that peptidic bonds do not significantly contribute during the first step of the interaction mechanism (static quenching). Other molecular parts of natural humic acid (summer extract, NHA1) not identified in this study, could be responsible for other complex photophysical electron charge transfer, which should then lead to higher I_0/I_2 values.

Moreover, our results showed that K_{SV} values obtained for synthetic humic acids are at least three times higher than those for natural ones. We can assume that synthetic humic-like substance (SyHA) hydrophobic domains are more tightly structured than those of natural humic acids (NHA). Pyrene also shows more significant diffusional freedom with NHA and a lesser tendency to be captured by humic acids.

Summarizing our results, there are two main domains for pyrene-humic acids (HA) associations with increasing HA concentrations:

(i) a low-concentration region in the 0-30 mg.L^{-1} range, where molecular photophysical “weak-adsorption” interactions between the fluoroprobe “pyrene” and the most hydrophobic HA sites take place. This region allows to determine the Stern-Volmer binding constants between pyrene and HA molecules;

(ii) a high-concentration region at least greater than 250 mg.L^{-1} corresponding to a “pseudo-micellar” organization phase at the air/water interface. This latter region reveals

that organic matter micellar-like behaviour is independent from pyrene entrapment capacity. In accordance with previous literature (Peuravuori, 2001; Borisover, 2005; Nakashima, 2007), these results confirm that a true entrapment in a micellar phase is not a prerequisite to the quenching process. Moreover, the presence of pyrene seems to have no effect on a humic micelle formation. Considering our aqueous pyrene concentration (0.2 mg.L^{-1}) and the mean binding constant of 10^4 determined in this study, we can expect the pyrene concentration in a micelle about 2000 mg.kg^{-1} . Such result is particularly meaningful for drinking water treatment, since pyrene could be entrapped in organic micelles contained in the raw surface water to be treated. Due to the coagulation process (such as charge destabilization), the pyrene could then be released at high coagulant concentration in the treated water.

Conclusion

Hydrophobic characteristics responsible for pyrene entrapment in humic structures are mainly due to first polarity (que veux tu dire par first polarity ??) and polarizability, both correlated with the aromaticity and the presence of aliphatic chains in humic acids, molecular weight, nitrogen nature and/or content and finally total acidity of the substances.

The comparison of natural and synthetic humic acid interfacial and Stern-Volmer analysis suggests that the hydrophobic interactions are responsible for the organization of the aggregates, regardless of HA origin. Therefore, even if the hydrophobic characteristics of humic acid are well correlated with the pyrene quenching capacity, the specific interactions with aromatic molecular sites depend on the whole tridimensional photophysical environment of humic acids in solution.

Surface tension measurements revealed an increase in surface activity as a function of concentration for each humic fraction independently of pyrene presence in solution. Those results confirm the possibility of specific intermolecular interactions occurring during pyrene entrapment within humic acids. From the comparison of SyHA and NHA, we showed that the presence of pyrene had no effect on humic micelle formation. The pyrene fluorescence quenching study pointed out the capacity of HA to incorporate large hydrophobic molecules into its micellar core, without change of its CMC value determined in the absence of other organic molecules.

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Appendix

γ_s : surface tension

[Q]₀: quencher initial concentration

CGHA: catechol/glycine humic acid

CMC: critical micellar concentration

CTHA: catechol/triglycine humic acid

CTHA1: CTHA having a 1-3.5 kDa mean molecular weight

CTHA2: CTHA having a > 8 kDa mean molecular weight

FTIR: Fourier Transform InfraRed

HI: hydrophobic indice

HS: humic substances

I: fluorescence intensity in the presence of quencher

I₀: fluorescence intensity in the absence of quencher

I₁, I₂, I₃, I₄, I₅: pyrene fluorescence intensity respectively at 372, 377, 382, 390 and 395 nm
(in ethanol after excitation at 332 nm)

IHSS: International Humic Substances Society

K_{SV}: Stern-Volmer quenching constant

NHA: natural humic acids

NHA1: NHA extracted during the summer season

NHA2: NHA extracted during the autumn season

NOM: natural organic matter

PAH: polycyclic aromatic hydrocarbon

SyHA: synthetic humic-like substance

References

Achten, C., Hofmann, T., 2009. Native polycyclic aromatic hydrocarbons (PAH) in coals - a hardly recognized source of environment contamination. *Sci. Total Environ.* 407, 2461-2473.

Agence de l'Eau Rhin-Meuse, <http://www.eau-rhin-meuse.fr/Berm/htm>

Andreux, F., Golebiowska, D., Metche, M., 1980. Oxydative polymerization of O-diphenols in presence or absence of amino-acids. Topics on (catechol-glycocolle) and (catecholdiglycylglycine). General Assembly of Polyphenols Group Report, Logrono, Spain, Bulletin 9, 178-188.

Auger, R.L., Jacobson, A.M., Domach, M.M., 1995. Aqueous Phase Fluorescence Quenching Technique for Measuring Naphtalene Partition Coefficients in Nonionic Surfactant Micelles. *Environ. Sci. Technol.* 29, 1273-1278.

Borisover, M., Laor, Y., Bukhanovsky, N., Saadi, I., 2006. Fluorescence-based evidence for adsorptive binding of pyrene to effluent dissolved organic matter. *Chemosphere* 65, 1925-1934.

- Bouloubassi, I., Saliot, A., 1991. Composition and sources of dissolved and particulate PAH in surface waters from the Rhone Delta (NW Mediterranean). *Mar. Pollut. Bull.* 22, 588-594.
- Chen, Y., Senesi, N., Schnitzer, M., 1977. Information provided on humic substances by E4/46 ratios. *Soil Sci. Soc. Am. J.* 41, 352-358.
- Chin, Y.P., Aiken, G.R., Danielsen, K.M., 1997. Binding of pyrene to aquatic and commercial humic substances: the role of molecular weight and aromaticity. *Environ. Sci. Technol.* 31, 1630-1635.
- Chin, Y.P., Buterbaugh, J.M., Gustafson, T.J., Traina, S.J., Danielsen, K.M., 1996. Response to Comment on "Solubility enhancement and fluorescence quenching of pyrene by humic substances". *Environ. Sci. Technol.* 30, 1409-1411.
- Chin, Y.P., Weber, W.J., Jr., 1989. Estimating the effects of dispersed organic polymers on the sorption of contaminants by natural solids I: A predictive thermodynamic humic acid-organic solute interaction model. *Environ. Sci. Technol.* 23, 978-985.
- Chiou, C.T., Kile, D.E., Brinton, T.I., Malcolm, R.L., Leenheer, J.A., Mac Carthy, P., 1987. A comparison of water solubility enhancements of organic solutes by aquatic humic materials and commercial humic acids. *Environ. Sci. Technol.* 21, 1231-1234.
- Countway, R.E., Dickhut, R.M., Canuel, E.A., 2003. Polycyclic aromatic hydrocarbon (PAH) distributions and associations with organic matter in surface waters of the York River, VA Estuary. *Org. Geochem.* 34, 209-224.
- Engbreton, R.R., von Wandruska, R., 1994. Microorganization in dissolved humic acids. *Environ. Sci. Technol.* 28, 1934-1941.
- Gauthier, T.D., Seitz, W.R., Grant, C.L., 1987. Effects of structural and compositional variations of dissolved humic materials on pyrene K sub(oc) values. *Environ. Sci. Technol.* 21, 243-248.
- Gauthier, T.D., Syhane, E.C., Guerin, W.R., Seitz, W.R., Grant, C.L., 1986. Fluorescence quenching method for determining equilibrium constants for polycyclic aromatic hydrocarbons binding to dissolved humic materials. *Environ. Sci. Technol.* 20, 1162-1166
- Ghosh, U., Gillette, J.S., Richard, G.L., Zare, R.N., 2000. Microscale Location Characterization, and Association of Polycyclic Aromatic Hydrocarbons on harbour Sediment Particles. *Environ. Sci. Technol.* 34, 1729-1736.
- Hua, G., Lyons, B., Killham, K., Singleton, I., 2009. Potential use of DNA adducts to detect mutagenic compounds in soil. *Environ. Pollut.* 157, 916-921.
- Hur, J., Lee, D.-H., Shin, H.-S., 2009. Comparison of the structural, spectroscopic and phenanthrene binding characteristics of humic acids from soils and lake sediments. *Org. Geochem.* 40, 1091-1099.
- Jeffrey, A.M., Weinstein, I.B., Jennette, K., Grzeskowiak, K., Nakanishi, K., Harvey, R.G., Autrup, H., Harris, C., 1977. Structures of benzo[a]pyrene nucleic acid adducts formed in human and bovine bronchial explants. *Nature (London)* 269, 348-350.
- Jung, A.-V., Frochot, C., Parant, S., Lartiges, B.S., Selve, C., Viriot, M.-L., Bersillon, J.-L., 2005. Synthesis of amino-phenolic humic-like substances and comparison with natural aquatic humic acids: A multi-analytical techniques approach. *Org. Geochem.* 36, 1252-1271.
- Kalyanasudaram, K., Thomas, J.K., 1977. Environmental effects on vibronic band intensities in pyrene monomer fluorescence and their application in studies of micellar systems. *J. Am. Chem. Soc.* 99, 2039-2044.
- Lakowicz, J.R., 1999. *Principles of Fluorescence Spectroscopy*, second ed. Kluwer Academic/Plenum, New York.
- Myers, D., 1992. *Surfactants Science and Technology*, second ed. Wiley-VCH, Weinheim.

- Nakashima, K., Maki, M., Ishikawa, F., Yoshikawa, T., Gong, Y.-K., Miyajima, T., 2007. Fluorescence studies on binding of pyrene and its derivatives to humic acid. *Spectrochim. Acta, Part A* 67, 930-935.
- Pan, B., Ying, B.S., Liu, W.X., Tao, S., Lin, X.M., Zhang, X.X.M., Zhang, Y.X., Xiao, Y., Dai, H.C., Yuan, H.S., 2006. Distribution of sorbed phenanthrene and pyrene in different humic fractions of soils and importance of humin. *Environ. Pollut.* 143, 24-33.
- Peuravuori, J., 2001. Partition coefficients of pyrene to lake aquatic humic matter determined by fluorescence quenching and solubility enhancement. *Anal. Chim. Acta* 429, 65-73.
- Piccolo, A., Celano, G., Conte, P.J., 1996. Adsorption of glyphosate by humic substances. *J. Agr. Food Chem.* 44, 2442-2246.
- Piccolo, A., Conte, P., Scheunert, I., Paci, M., 1998. Atrazine Interactions with Soil Humic Substances of Different Molecular Structure. *J. Environ. Qual.* 27, 1324-1333.
- Quagliotto, P., Montoneri, E., Tambone, F., Fabrizio, A., Gobetto, R., Viscardi, G., 2006. Chemicals from Wastes: Compost-Derived Humic Acid-like Matter as Surfactant. *Environ. Sci. Technol.* 40, 1686-1692.
- Shinozuka, N., Lee, C., 1991. Aggregate formation of humic acids from marine sediments. *Mar. Chem.* 33, 229-241.
- Short, J.W., Irvine, G.V., Mann, D.H., Maselko, J.M., Pella, J.J., Lindeberg, M.R., 2007. Slightly weathered Exxon Valdez oil persists in Gulf of Alaska beach sediments after 16 years. *Environ. Sci. Technol.* 41, 1245-1250.
- Sielechi, J.-M., Lartiges, B.S., Kayem, G.J., Hupont, S., Frochot, C., Thieme, J., Ghanbaja, J., d'Espinose de la Caillerie, J.B., Barres, O., Kamga, R., Levitz, P., Michot, L.J., 2008. Changes in humic acid conformation during coagulation with ferric chloride: Implications for drinking water treatment. *Water Res.* 42, 2111-2123.
- Sierra, M.M.D., Rauen, T.G., Tormen, L., Debacher, N.A., Soriano-Sierra, E.J., 2005. Evidence from surface tension and fluorescence data of a pyrene-assisted micelle-like assemblage of humic substances. *Water Res.* 39, 3811-3818.
- Thurman, E.M., Malcolm, R.L., 1981. Preparative isolation of aquatic humic substances. *J. Am. Chem. Soc.* 4, 463-466.
- Thurman, E.M., 1985. *Organic Geochemistry of Natural Waters*, Martinus Nijhoff/Junk Publishers, Dordrecht, The Netherlands.
- Tuckermann, R., Cammenga, H.K., 2004. The surface tension of aqueous solutions of some atmospheric water-soluble organic compounds. *Atmos. Environ.* 38, 6135-6138.

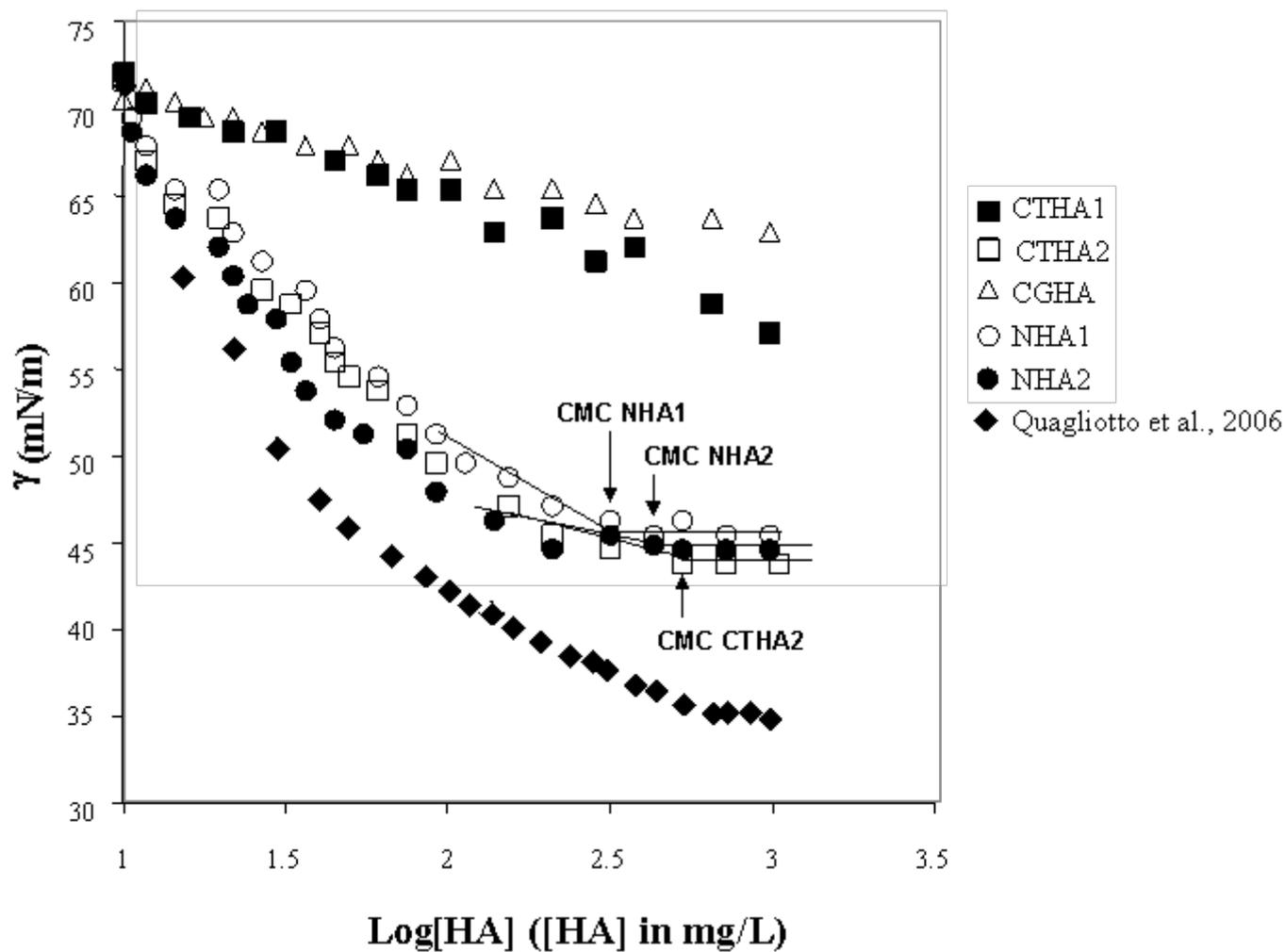


FIGURE 1. Surface tension versus humic acid (HA) concentration ($\text{mg}\cdot\text{L}^{-1}$). Experimental errors: not represented (smaller than marks).

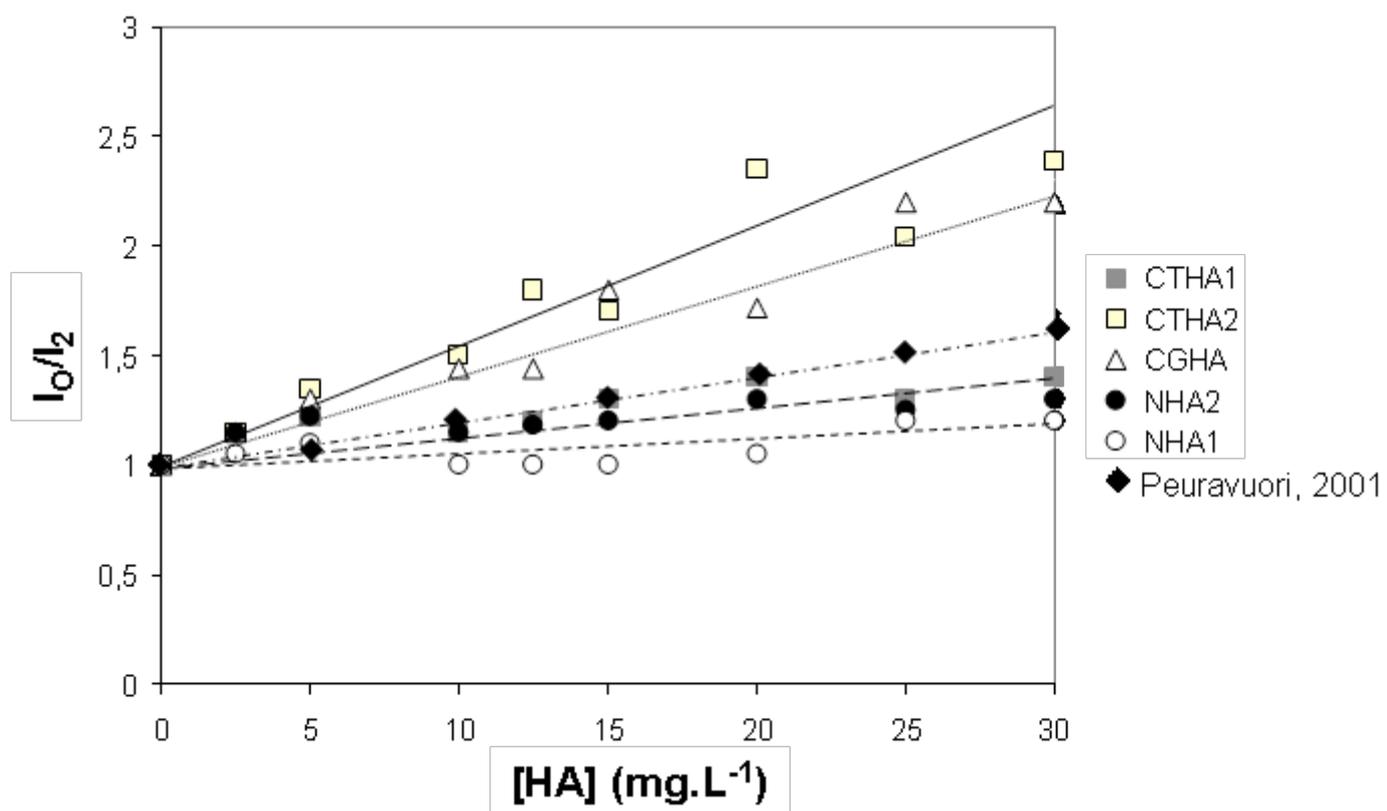


FIGURE 2. Synthetical and natural Stern-Volmer plots ($[HA]$ in mg.L^{-1}).
Experimental errors: not represented (smaller than marks).

Tables

TABLE 1. Humic substances under investigation (Jung et al., 2005).

TABLE 2. Stern-Volmer association constants and critical concentrations values for humic substances.

TABLE 1

| Humic acid fraction | O/C^a | H/O^a | (O+N)^a/C^a | H/C^a | Total Acidity (meq.g⁻¹) | HI^b |
|----------------------------|------------------------|------------------------|--|------------------------|---|-----------------------|
| CGHA | 0.73 | 0.032 | 1.49 | 6.72 | 3.7 | 84.6 |
| CTHA2 | 0.77 | 0.048 | 3.87 | 9.36 | 5.6 | 82.8 |
| CTHA1 | 1.40 | 0.048 | 5.61 | 16.56 | 5.4 | 76.6 |
| NHA1 | 0.40 | 0.224 | 2.45 | 22.44 | 13.1 | 51.1 |
| NHA2 | 0.56 | 0.128 | 2.08 | 19.32 | 18.0 | 52.2 |

^a Atomic ratio (O=100%-ΣC, H, N).

^b HI : Hydrophobic Indices determined by RP-HPLC (See Jung et al., 2005).

TABLE 2

| Humic acid fraction | K_{SV}^a (σ ^c = +/- 0.15 10 ⁴ mL.g ⁻¹) | CMC^b (σ ^c = +/- 50 mg.L ⁻¹) |
|----------------------------|--|---|
| CGHA | 5.50 10 ⁴ mL.g ⁻¹ | * |
| CTHA2 | 4.50 10 ⁴ mL.g ⁻¹ | 320 mg.L ⁻¹ |
| CTHA1 | 1.51 10 ⁴ mL.g ⁻¹ | * |
| NHA1 | 0.52 10 ⁴ mL.g ⁻¹ | 500 mg.L ⁻¹ |
| NHA2 | 0.31 10 ⁴ mL.g ⁻¹ | 450 mg.L ⁻¹ |

^a Stern-Volmer constants with 10⁻⁶ M pyrene, [HA] 1-30 mg.L⁻¹, pH 8.

^b Critical micellar concentration values determined as HA first concentration for γ stabilization value (* no stabilization value obtained).

^c Maxima standard deviations values for all fractions.