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Humic substances and napropamide interactions in aqueous solution: A fluorescence spectroscopy study

Ouarda Brahmia *

Laboratoire des Techniques Innovantes de Préservation de l'Environnement, Université des frères Mentouri (Constantine 1), Algérie.

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**Corresponding author. E-mail: ouarda.brahmia@gmail.com; Tel: (+213559666271)*

Abstract

The complexation of organic solutes with dissolved humic substances (HS) is an important process for the fate of these chemicals in natural aquatic systems. We used the Fluorescence Quenching technique to study the interactions between napropamide, a common herbicide, and ten dissolved humic or fulvic acids extracted from different soils and peats. The primary mechanism of napropamide quenching was determined to be static. The observed complexation constants ranged from 15.9 to 33.8 (L/g. Organic Carbon). Humic acids globally showed a better affinity for complexation with napropamide than fulvic acids with a particular mention for Bouzule and Scheyern humic acids. This may be explained in terms of hydrophobic interactions, in view of the globally hydrophobic nature of napropamide coupled with the fact that humic acids generally contain more hydrophobic regions than fulvic acids. Other possible types of interactions are also suggested. Napropamide binding to HS also depends on the HS elemental composition. It increases with the increase in the (N/C) mass ratio or the HS aromatic fraction but decreases with the increase in O/C mass ratio.

Keywords: Napropamide, Soil humic substances, Peat, Fluorescence quenching.

Introduction

Depending on the source where they come from, humic substances are divided into two groups, aquatic and terrestrial (soil). According to Stevenson et al. **[1]** these two groups differ slightly in their elemental composition, but their basic chemical structure is admitted to be similar. Soil organic matter (SOM) includes all organic compounds found in soils and it is classified into non-humic and humic substances. Humic substances in soils, or humus, are those organic materials extractable by strong bases and divided into humin, humic acid (HA) and fulvic acid (FA) according to their water solubility and resistance to precipitation to acids **[1,2]**. Humic substances are generally defined as a mixture of heterogeneous bulky organic substances present both in water and in soils. They exhibit complicated structures, characterized by the simultaneous presence of aromatic ring structures and the abundance of carboxylic and hydroxylic groups and other hydrophilic sites. Numerous studies showed that many properties of organic solutes are affected by complexation with soluble humic substances. Among these, we may mention an increase in water solubility of nonionic solutes, a decrease in the toxicity and bioavailability of organic solutes, a decrease in the adsorption of organic solutes to solid phases and a reduction in volatilization of volatile organic solutes **[3-5]**. The interactions between organic solutes and water-soluble humic substances (HS) can also alter the fate of these solutes in natural systems. It was established that the primary binding (association) of these solutes with dissolved humic substances in aqueous solution was caused by hydrophobic partitioning **[6-9]**. Many studies showed that quenching of the fluorescence of a solute molecule in the presence of humic acid results from the formation of solute-humic complexes, known as static quenching. However, despite considerable efforts from researchers, the exact mechanism and extent of complexation of organic solutes with dissolved humic substances are not well understood. To quantify the impact of dissolved humic substances on the organic solutes, it is necessary to study the strength of the

interactions occurring between them. Fluorescence Quenching (FQ) technique was largely used for this purpose. FQ can be described by Stern-Volmer equation **[10].**

$$F_0/F = 1 + K_{SV}[Q]\ldots \quad (1)$$

where F_0 and F are the fluorescence intensities in the absence and presence of the quencher Q, [Q] is the quencher concentration, K_{SV} is the Stern-Volmer constant. This latter can be calculated from the slope of the plot F_0/F vs the concentration of the humic acid (quencher). In the case of static quenching, which is due to the complex formation between the quencher and the fluorophore, K_{SV} is equal to the complexation stability constant K_b:

$$K_{SV} = K_b = [F\text{-}Q] / ([F].[Q])\ldots \quad (2)$$

where [F-Q] and [F] are the concentrations of the fluorophore-quencher complex and the free fluorophore respectively. This method has initially been developed for determining equilibrium constants for the association of Polycyclic Aromatic Hydrocarbons (PAHs) with dissolved humic and fulvic acids **[11,12]**. The association (binding) of PAHs with dissolved organic carbon was generally expressed as

$$F_0 / F = 1 + K_{OC}[DOC]\ldots. \quad (3)$$

where K_{OC} is the organic carbon partition coefficient. Napropamide is a non-ionic, moderately polar and hydrophobic herbicide. It is a commonly used herbicide. Its sorption capacity with dissolved humic substances is of overriding importance to its fate in natural systems. We may add that napropamide can give off strong fluorescence in proper conditions because of its fused aromatic rings. This makes it a particularly convenient model compound to study the interaction of Organics with dissolved HA and FA acids. Dissolved organic carbon (DOC) represents a large majority of the organic matter in most aquatic media. The ^{13}C NMR technique provides aromaticity of natural organic matter. However, this technique requires sophisticated instrumentation and substantial sample preparation. As a surrogate for aromaticity, many researchers used the specific UV absorbance at 254 (SUVA 254), which is the UV absorption at 254 nm normalised to the dissolved organic carbon concentration as a simple method for estimating the aromaticity of DOC in a given sample **[13-15]**. Weishaar et al. **[16]** showed a strong correlation between this parameter and DOC aromaticity. Generally, the influence of dissolved humic materials aromaticity on the extent of complexation of organic compounds with dissolved humic substances is poorly understood. Through the FQ technique, we aim to examine whether or not napropamide forms complexes with those tested isolated fractions (static quenching), to assess the aromaticity effect on the association napropamide-humic materials and to suggest possible modes of napropamide-humic material association.

2. Materials and methods

2.1 Chemicals and solutions preparation

Napropamide (N,N-diethyl-2-naphthalen-1-yloxypropanamide) was purchased from Sigma-Aldrich with the chemical structure presented in Figure 1.Water was purified using a Milli-Q (Millipore) device. The HS used within the current study were extracted by the GSF-Institute of Ecological Chemistry group (Munich), from four soils; Scheyern (Sch), Belle fontaine rendzine (BFR), Kaldenkirchen (Kal) and Bouzule (Bou), and two peats; W1 and W9B. These HS were described previously **[17]**. A single HA and three FAs named FA1, FA2 and FA3 were extracted from each of the four soils mentioned aboveagainst a single HA and a single FA from each of the two peats.We selected half the twenty isolated fractions and studied their interactions with the used herbicide. Theseare the following: *Sch HA, Kal HA, BFR HA, Bou HA, Peat W9B HA, Peat W1 HA, BFR FA2, Bou FA3, Peat W9B FA and Peat W1 FA.*

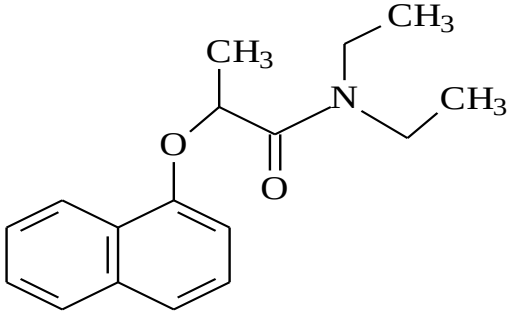


Figure 1: Napropamide Chemical Structure

2.2 Fluorescence experiments

For each humic substance, fluorescence measurements were performed in flasks on three samples: (a) napropamide alone, (b) HA or FA alone and (c) mixtures of napropamide/HA or FA. Before fulfilling fluorescence intensity measurements, HS-napropamide solutions were buffered at pH 6.5 with phosphate buffers (3.1×10^{-3} M) and left in the dark at room temperature for one day to allow equilibrium to be reached. All fluorescence measurements were performed on a Perkin-Elmer MPF-3L spectrofluorimeter equipped with an IP 28 photomultiplier. Napropamide exhibits an absorption maximum at 290 nm with a shoulder at 320 nm. Its emission spectrum shows a maximum at 340 nm. Based on this, for the lowest napropamide concentrations, ranging from 2.5×10^{-7} M to 5×10^{-6} M, the fluorescence intensity measurement was made with an excitation wavelength fixed at 290 nm and emission wavelength at 340 nm. For the highest concentrations, ranging from 8×10^{-6} M to 10^{-4} M, we changed the excitation wavelength to 320 nm while keeping the same emission wavelength. A correction factor is advocated to account for the apparent quenching, due to an attenuation of the excitation beam and/or absorption of emitted radiation by an excess concentration of the fluorophore or by the presence of any additional absorbing species in solution acting as an "inner filter". This correction was introduced as described by MacDonald et al **[18]**.The experiments were conducted to check whether the quenching of napropamide fluorescence by various dissolved humic substances and peats was static.

2.3 Analyses

DOC measurements were performed on Shimadzu TOC 5050 A analyzer. SUVA 254 values were determining by dividing the UV absorbance measured at 254 nm by the DOC concentration and were reported in the units of litter per milligram carbon per meter. UV-absorption measurements were recorded on a Cary 113 spectrophotometer (Varian) to determine the absorbance values at the excitation and emission wavelengths so as to correct the inner filter effects, as was previously described.

3. Results and discussion

3.1 Fluorescence spectra

Interactions of napropamide with HAs were studied by analysis of the napropamide fluorescence quenching induced by the humic fraction. The decrease of napropamide fluorescence intensity when a humic substance is added is illustrated in Figure 2, which shows the emission spectra of napropamide alone (Npr) and a mixture of "napropamide plus Kal HA". The fluorescence intensity decrease shows an association of almost 50 % of the product within the humic material. It is also to note that the quencher (humic acid) fluorescence intensity is negligible compared to that of the fluorophore (napropamide).

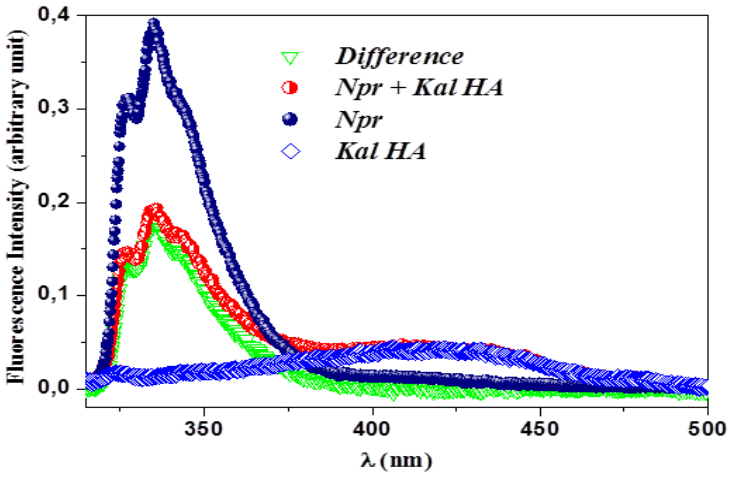


Figure 2: Fluorescence emission spectra of napropamide (Npr), "napropamide plus Kal HA" mixture, Kal HA and the difference between the latter two.
$[napropamide]_0 = 5\times10^{-6}$ M, [Kal HA] = 25 mg/L.

3.2 Complexation of napropamide by dissolved humic and fulvic acids

In a first set of experiments, we examined the interaction of napropamide with two HAs extracted from soils, i.e. Sch HA and Kal HA. We fixed the HAs concentration at 25 mg/L, then we varied the concentration of napropamide from $2.5.10^{-7}$ M to 5.10^{-6} M (figure 3 a) and from 10^{-5} M to 10^{-4} M (figure 3 b) in the case of Sch HA and from 2.5×10^{-5} M to10^{-4} M in the case of Kal HA (figure 4). The excitation wavelengths used were 320 nm or 290 nm as was described previously, while the emission wavelength was 340 nm for both. Napropamide was equilibrated overnight prior to the fluorescence intensity measurement. Consequently, any change in the fluorescence intensity was a reflection of the remaining unbound napropamide in solution. We assessed the fluorescence intensities of the compound alone (F_0) and in the presence of the dissolved humic acid (F). It appears from figures 3 and 4 that the decrease of napropamide fluorescence intensity is proportional to the initial napropamide concentration. This observation points to the establishment of an equilibrium state between free and bound napropamide. Hence, the observed quenching is most probably a static quenching, due to the formation of a complex between napropamide and the humic material as described by Gauthier et al **[19]**.

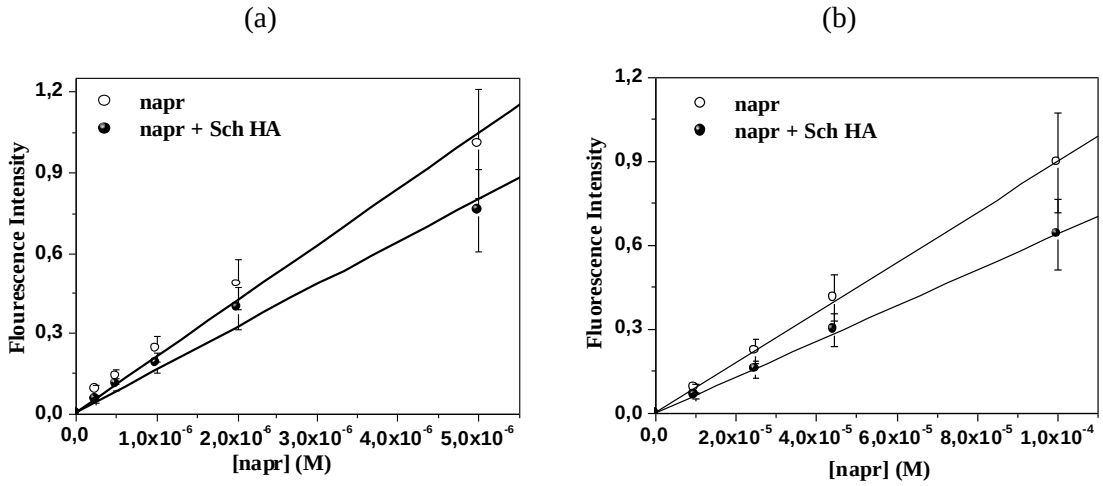


Figure 3: Napropamide fluorescence intensity in the presence (●) and in the absence (o) of the Sch HA (25 mg/L),
(a): $[napr]_0$= [2.5 .10^{-7} ; 5.10^{-6}] M, λ_{ex} = 290 nm, λ_{em} = 340 nm.
(b): $[napr]_0$ = [10^{-5} ; 10^{-4}] M, λ_{ex} = 320 nm, λ_{em} = 340 nm.

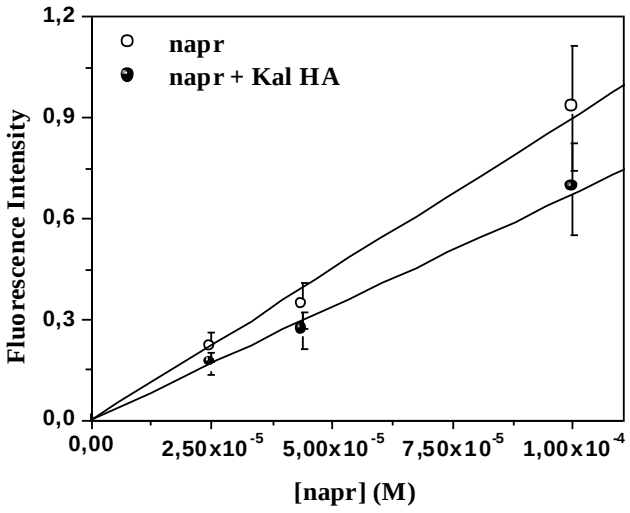
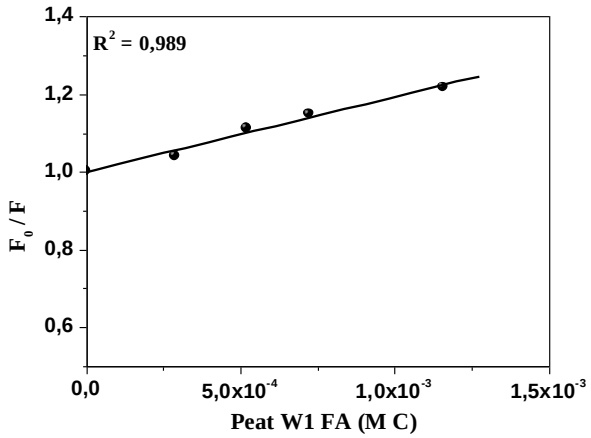


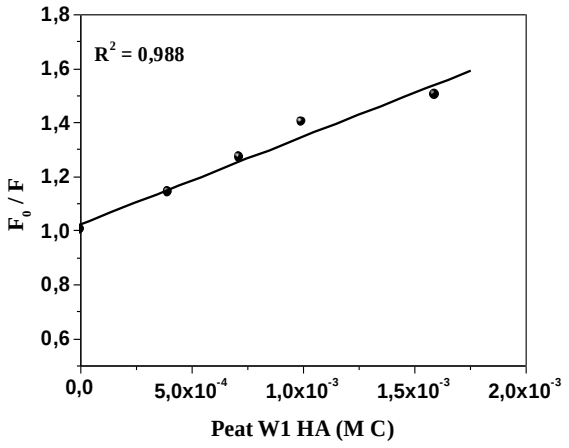
Figure 4: Napropamide fluorescence intensity in the presence (●) and in the absence (o) of Kal HA (25 mg/L), λ_{ex} = 320 nm, λ_{em} = 340 nm.

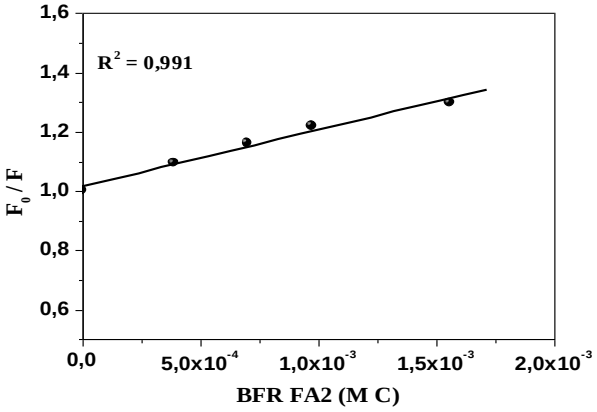
3.3Assessment of the complex formation constants

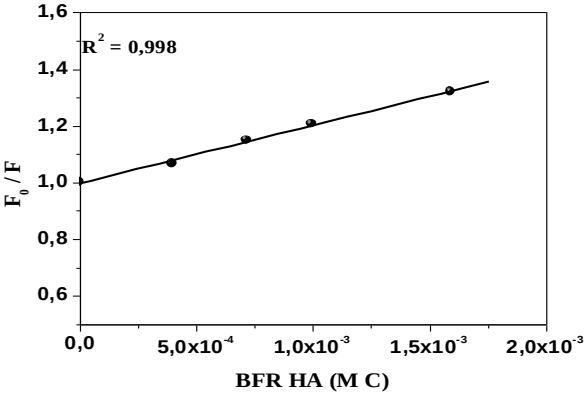
Experiments were conducted to test the applicability of the Stern-Volmer equation, aiming to provide us with a description of the association of napropamide with the HAs fractions and more exactly to enable us to check whether the fluorescence quenching of napropamide is due to static quenching (complex forming).

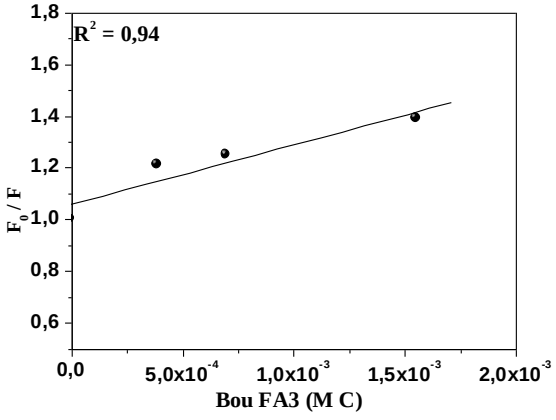
Hence, we fixed the fluorophore concentration at 5×10^{-6} M and varied the HA or FA concentration from 10 mg/L to 40 mg/L. The Intensity fluorescence corresponding to 5×10^{-6} M of napropamide alone was taken as F_0. As stated previously, HA or FA contributed with a small constant amount to the overall fluorescence of the solution. Therefore, the corrected fluorescence (F) of free napropamide was deduced by subtracting the HA or FA blank value from the measured value. Variations of the quenching ratio (F_0/F) vs the concentration of nine fractions of HA or FA, expressed as molarity of DOC (Dissolved Organic Carbon), are shown in figure 5. An excellent linear fit to Stern-Volmer equation was obtained for each of the dissolved humic or fulvic acids (R^2> 0.98, for eight of them, R^2 = 0.94 for the ninth). We may therefore conclude that the most probable primary mechanism of napropamide quenching is static.

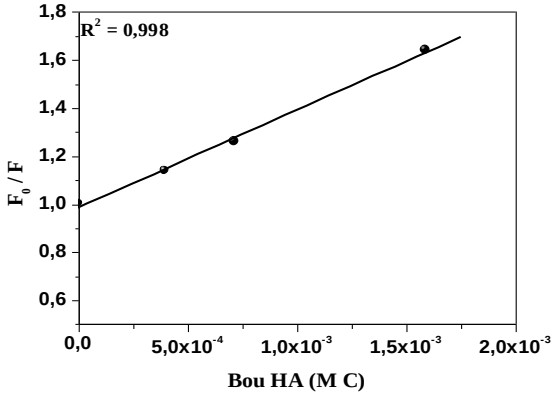












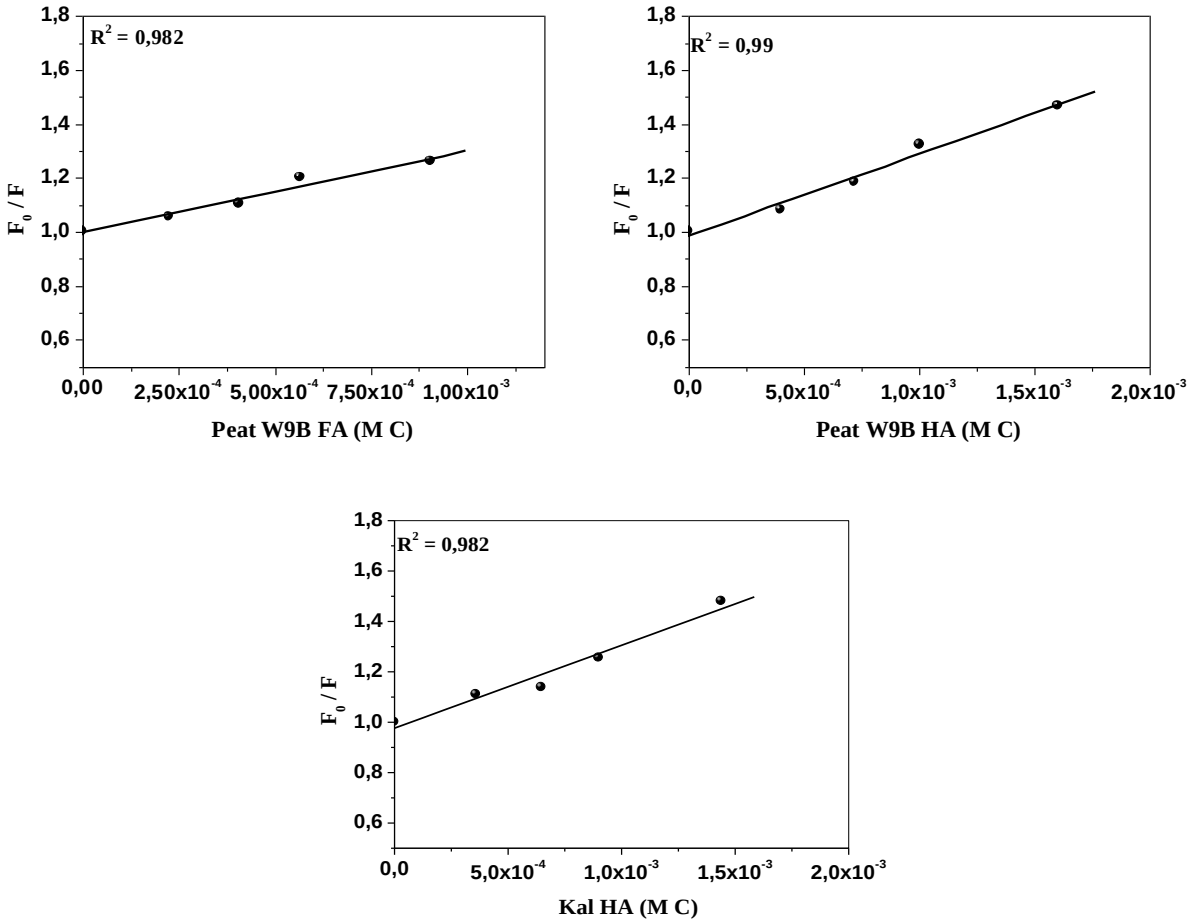


Figure 5: Stern-Volmer plots of Napropamide fluorescence quenched by dissolved humic and fulvic acids.

The slope of each of the nine straight-lines gives the value of the association (binding) constant for the corresponding fraction. Sch-HA stands as an exception because its binding constant was deduced from the slope of the linear plot of [F-Q] (the concentration of napropamide bound to Sch-HA) versus [F] (the concentration of free napropamide, figure 6), according to equation (2), [F-Q] = K [Q] [F]. As only a small fraction of Q complexes with napropamide, the concentration of Q at equilibrium, [Q], is taken to be constant, equal to $[Q]_0$.

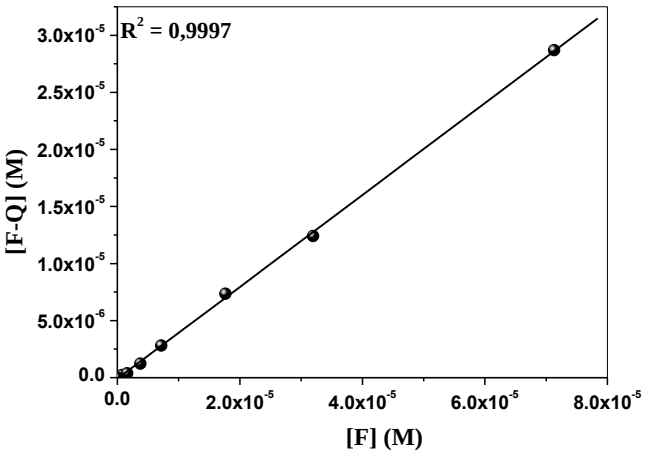


Figure 6: Quenched napropamide by Sch HA versus free Napropamide concentrations.

Table 1 summarizes the association constants, calculated on the basis on DOC (dissolved organic carbon). Our values range from 15.9 L/g C to 33.8 L/g C. They are close to those obtained on compounds displaying similar structures to napropamide, such as 1-naphthol **[19,20]**. The constant reported for the association of napropamide with IHSS-HA (humic acid of the International Humic Substance Society) is equal to 23.2 L/g C **[21]**, a value that falls at the heart of our constants range. The extent of quenching induced by HAs is higher than the one induced by FAs as evidenced by the higher K values, except in the case of Peat W9B, where humic and fulvic acids show the same binding constants with napropamide. This can be explained in terms of hydrophobic association as napropamide is hydrophobic, on one hand, and HA generally contains more hydrophobic regions than FA, on the other hand. Indeed, FAs contain more oxygenated functional groups and less aromatic rings than HAs, which makes them more polar and less hydrophobic than HAs. Our results agree well with similar fluorescence studies that have shown that humic acids may form stronger interactions with hydrophobic organic contaminants **[22, 23, 8]**. Lastly, we may note that Bou HA and Sch HA show the best affinity for complexation.

Table 1: Calculated binding (association) Constants for HAs and FAs used in the current study.

HA/FA fraction (quencher)	K_b (L/g C)	R^2
Peat W1 FA	16.1	0.989
Peat W1 HA	27	0.988
BFR FA2	15.9	0.991
BFR HA	17	0.998
Bou HA	33.8	0.998
Bou FA3	19.6	0.940
Peat W9B FA	25.2	0.982
Peat W9B HA	25.2	0.990
Kal HA	27.3	0.982
Sch HA	32.6	0.999

3.4. Constants association and some structural features of humic substances correlations

Many attempts were made to correlate the assessed binding constants with some structural features of the examined humic and fulvic acids. The results could provide us with meaningful information concerning napropamide interactions. Table 2 details the measured elemental mass content of each isolated fraction, namely the organic carbon (OC) content, ash content and the mass ratios (N/C, O/C and H/C). Table 3 illustrates the data of the ^{13}C NMR spectra and particularly the aromatic percentage in the region (110 -165 ppm) for all the isolated fractions. The data of table 2 and 3 were provided by (GSF-Munich). In table 4, we summarize the calculated SUVA 254 values for all the fractions.

Table 2: Elemental analysis for HAs and FAs used in the current study

	OC (%)	N/C	O/C	H/C	Ash (%)
Sch HA	49.41	0.082	0.565	1.204	4.0
Kal HA	43.27	0.078	0.633	1.367	14
Bou FA3	46.6	0.043	0.677	1.127	3.5
Bou HA	47.74	0.088	0.519	1.251	4.9
W1 FA	34.79	0.049	0.744	1.186	7.8
W1 HA	47.87	0.063	0.591	1.178	5.8
W9B FA	27.21	0.040	0.759	1.115	38
W9B HA	48.22	0.053	0.611	1.221	3.5
BFR FA2	47.13	0.058	0.664	1.204	4.1
BFR HA	47.87	0.099	0.555	1.281	7.2

3.4.1 Napropamide association constants and elemental content correlations

When we examine the impact of N/C and O/C mass ratios on napropamide association, we find that the binding constant K_b tends to increase when the N/C increases or when the O/C decreases (figure 7).

Table 3: Peak assignments for the ^{13}C NMR spectra for HAs and FAs used in the current study and their integrated area (%)

Assignments / Integration %						
	Alkyl-C 0-50 ppm	Methoxyl Alkyl-N 50-65 ppm	O-Alkyl-C 65-110 ppm	Aromatic and Phenolic 110-165ppm	Carboxylic carbon 165-185ppm	Carbonylic Carbon 185-230ppm
Sch HA	21.43	11.65	17.29	35.34	12.03	2.26
Bou FA3	18.61	7.43	15.19	33.37	21.84	4.77
Bou HA	19.61	9.19	14.40	32.89	17.38	6.98
Peat W1 FA	20.00	6.67	14.17	25.83	23.33	10.00
Peat W1 HA	19.66	6.84	17.95	31.62	19.66	4.27
Peat W9B HA	20.66	7.44	15.70	28.92	18.18	6.09
Peat W9B FA	16.67	7.94	13.49	24.61	23.81	11.49
BFR HA	28.40	10.80	18.40	26.4	14.40	1.60
BFR FA2	22.54	8.61	20.49	29.51	15.57	3.28
Kal HA	19.26	9.02	19.26	32.78	13.11	5.74

Table 4: The assessed SUVA 254 values for the used isolated fractions

Quencher	SUVA 254 (L.mg $C^{-1}.m^{-1}$)
Sch HA	5.24
Bou FA3	4.18
Bou HA	4.80
Peat W1 FA	5.11
Peat W1 HA	6.60
Peat W9B FA	5.4
Peat W9B HA	5.52
BFR FA2	4.00
BFR HA	3.91
Kal HA	5.52

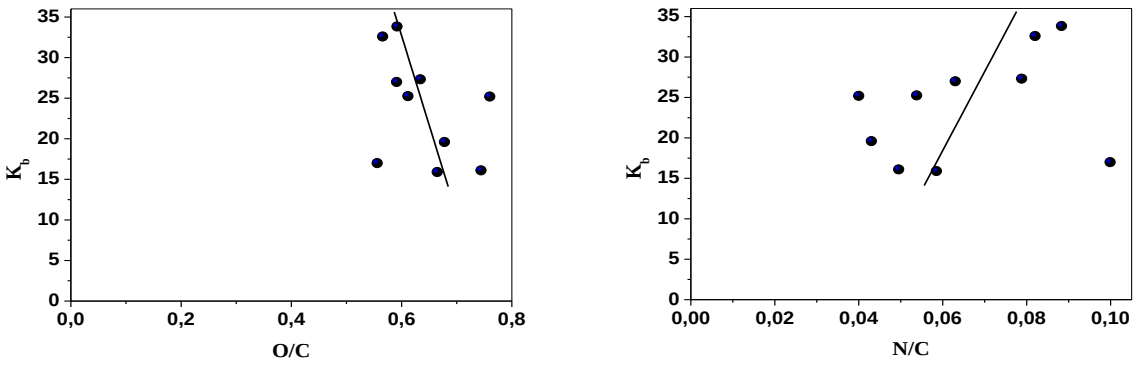


Figure 7: Binding constants variation versus O/C and N/C atomic ratios

When we consider the N/C and O/C mass ratios, it clearly appears from table 2 that nitrogen seems to favour the association of napropamide with humic substances while oxygen seems to be a disfavouring parameter. This suggests a positive role played by nitrogenated functions in strengthening the complexation between napropamide and the humic materials whereas the oxygenated functions do the inverse. Possible interactions between napropamide and the oxygenated or nitrogenated functions of the humic substances are polar intermolecular bonding, hydrogen bonding and electron-exchange processes. The latter have been suggested by several investigators **[1,24,25]** to justify the interaction of herbicides with soil organic matter. The strong H-

bonds that are expected to arise between water molecules (solvent molecules) and the oxygenated functions of the humic particle, should inhibit the fixation of napropamide in these hydrophilic regions and explain the adverse effect of oxygen on complexation. As Humic Acids are richer in nitrogen whereas Fulvic Acids are richer in oxygen, this may explain, at least partly, that HA display higher complexation constants with napropamide than FA.

3.4.2 Association Constant and aromaticity correlations

Does aromaticity promotes the association between napropamide and humic substances? To address this point, we show in figures 8 and 9 the plots of the binding constant versus the aromaticity percentage of the humic substances, expressed in terms of ^{13}C NMR in the region (110-165 ppm) and the SUVA 254, respectively. It seems quite clear from these graphs that the global trend is that the napropamide/HS binding constants increases with aromaticity. However, the determination coefficients (R^2) for the linear fits are moderate for both plots. This denotes that the binding constants are impacted by other HS structural parameters, such as the elemental content reported in the previous paragraph.

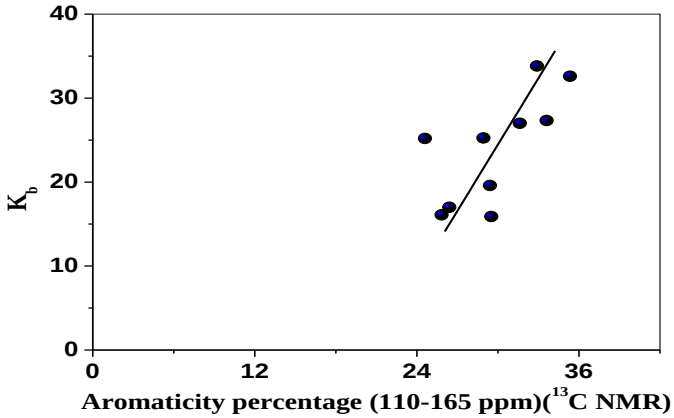


Fig. 8: Binding constants variation versus the aromaticity percentage (110-165 ppm) measured by ^{13}C – NMR.

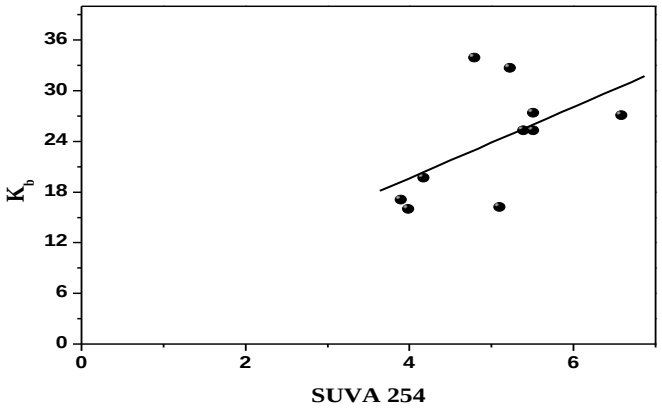


Figure 9: Binding constants variation versus the SUVA 254 values.

The efficiency of the aromatic fraction in promoting the complexation is most probably due to their ability to develop hydrophobic interactions with napropamide. Whatever the chemical nature of these interactions, the aromaticity is an important, but not the lone, factor that governs napropamide-humic materials interactions. The strong impact of aromaticity in the enhancement of the association between organics and dissolved humic substances was also reported by others similar studies **[26-28]**. It also appears from the SUVA 254 values that the complexation extent is generally more pronounced in the case of humic acids, in good agreement with their higher aromatic content.

Conclusion

Fluorescence quenching was successfully used to study aqueous interactions between Napropamide and fractions of humic and fulvic acids isolated from various soils and peats. This experimental procedure presents the advantage of being simple and fast. It also requires only small amounts of sample. The quenching was found to be static; i.e. there is formation of complexes of the fluorophore (napropamide) and the quencher (humic substance). Binding constants that were assessed from these experiments spread from 15.9 to 33.8 (L/g.C). Most values reported for "organic micropollutants/HS" systems lie in this interval.

We put forward some possible modes of "napropamide – HA/FA" association. Hydrophobic interactions seem to be the main one. Indeed, the aromatic fraction was found to be very efficient in promoting the complexation and thus can govern the napropamide - humic materials interactions. At the same time, oxygenated functions adversely affect the complexation extent, probably because of the presence of the strongly competitive water molecules. Thus, the napropamide complexation extent with HAs is higher than with FAs, HAs being richer in hydrophobic sites and FAs in oxygen. Hydrogen bonding and/or electron-exchange processes might contribute to napropamide-HS binding but have not been evidenced.

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