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Binding of Atrazine to Humic Substances from Soil, Peat, and Coal Related to Their Structure†

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Partition coefficients for the binding affinities of atrazine to 16 different humic materials were determined by the ultrafiltration HPLC technique. Sources included humic acids (HA), fulvic acids (FA), and combined humic and fulvic fractions (HF) from soil, peat, and coal humic acid. Each of the humic materials was characterized by elemental composition, molecular weight, and composition of main structural fragments determined by ^{13}C solution-state NMR. The magnitude of K_{OC} values varied from 87 to 575 L/kg of C, demonstrating relatively low binding affinity of humic substances (HS) for atrazine. On the basis of the measured K_{OC} values, the humic materials can be arranged in the following order: coal HA ≅ gray wooded soil HA > chernozemic soil HA and HF > sod-podzolic soil HA ≅ peat HF > sod-podzolic soil FA ≫ peat dissolved organic matter. The magnitude of the K_{OC} values correlated strongly with the percentage of aromatic carbon in HS samples ($r = 0.91$). The hydrophobic binding was hypothesized as the key interaction underlying the binding of atrazine to HS.

Introduction

Atrazine (2-chloro-4-(ethylamino)-6-isopropylamino-*s*-triazine) is one of the most widely applied and persistent herbicide. Elevated concentrations have been found in groundwater, rivers, lakes, and soils (*1*). The fate of atrazine in the environment can be greatly affected by humic substances (HS) (*2*, *3*), which comprise from 50 to 80% of natural organic matter in water and soil ecosystems. So, it has been reported that the binding to HS affects degradation rate and toxicity of atrazine (*2*−*5*).

A partition coefficient (K_{OC}) can be used as a quantitative measure of the magnitude of binding affinity of HS for atrazine. In addition, it allows the calculation of the portion of herbicide bound to HS that is of importance for prediction of the atrazine fate in the soil and water environment. As a result, several investigations have been devoted to determination of K_{OC} for atrazine to HS (*6*−*13*). The reported K_{OC} values differ substantially in their magnitude (from 25 to 600 L/kg of C). This could be connected to the various reactivity of the humic materials having different structure; however, the nonoptimum analytical approach used for studying the atrazine−HS interaction can contribute as well. Given the high structural heterogeneity and irregularity of HS, another important factor could be the limited number of target humic materials (not exceeding three preparations) used for the previous studies. Until the present, a common opinion is missing concerning both the binding mechanism of atrazine to HS and the structural features responsible for the binding (*6*, *9*, *10*, *14*−*19*). Wang et al. (*16*) reported the preferential binding of atrazine to the higher molecular weight fraction of fulvic acids and proposed weak mechanisms of interaction, such as hydrogen bonding and hydrophobic bonding, to interpret their findings. Hydrogen bonding was also suggested as a governing mechanism by Sullivan and Felbeck (*15*) and Welhouse and Bleam (*17*), who suggested that particularly strong complexes could be expected between atrazine and acid functional groups of HS. Piccolo et al. (*18*) and Mueller-Wegener and co-workers (*19*, *20*) are in favor of a charge-transfer mechanism. However, the spectroscopic studies of Martin-Neto et al. (*21*) on this subject have indicated that a charge-transfer mechanism was not operative in atrazine−HS interaction. They gave evidence instead for a hydrogen bonding mechanism.

In this paper, we have determined the partition coefficients for a large set of humic materials of different origin (16 samples) including HS from different sources (soil, peat, and coal) as well as fractional composition (HA vs FA). We have used a broad range of molecular descriptors, including more specific ^{13}C NMR descriptors for deriving the structure−atrazine binding affinity relationships (*22*).

Our objectives were to (i) measure the K_{OC} of atrazine for a variety of humic sources and (ii) derive the correlation relationships between the atrazine binding affinities and the molecular descriptors of HS.

Experimental Section

Atrazine (99.97%) was purchased from Dr. Ehrenstorfer GmbH (Germany). Stock solution of 10 mg/L was prepared in distilled water (pH 5.5) and stored in the dark at 4 °C.

Humic materials (16 samples) used in this study were isolated from soil and peat and also included commercial humic acid from brown coal.

Soil humic acids (HA) were isolated from eight soils. These included five sod-podzolic soils near Moscow (related to the Spodosols) sampled from a forested site (HBW and HBW1), from cultivated soil (HBP1), and from a garden (HBG, HBG1); one gray wooded soil (related to the Alfisols) near Tula (HGW); and typical and meadow chernozems (related to the Mollisols) near Voronezh (HST and HS, respectively).

Soil fulvic acids (FA) were isolated from three sod-podzolic soils above near Moscow sampled from a forested site (FBW1), from cultivated soil (FBP1), and from a garden (FBG1). All the sampling sites are in Russia. The HA extraction was performed as described in ref *23*. In brief, a soil sample was extracted with 0.1 M NaOH. For calcerous soils (chernozems), the sample was first treated with 10% HCl. The alkali extract was treated with 0.3 M KCl to remove the organomineral colloidal particles. The HA and FA were obtained by standard fractionation technique using acidification of the extract to pH 1−2. The precipitated HA were desalted by dialysis. To isolate FA, the common XAD technique was used as described elsewhere for isolation of the aquatic HS (*24*). In brief, the acidic supernatant was passed through XAD-2 resin. The sorbed fraction of FA was recovered with 0.1 M NaOH and desalted with the use of a cation-exchanger resin.

Soil humic substances (nonfractionated, soil HF) were isolated by alkaline extraction from typical chernozem near Stavropol (SEL).

† This paper is dedicated to the 60th birthday of Professor Fritz Hartmann Frimmel.

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Peat humic substances (nonfractionated, peat HF) were isolated from the highland sphagnum peat (T4). Isolation procedure was as described elsewhere (*25*) and included preliminary treatment of a peat sample with an ethanol−benzene mixture (1:1 v/v) followed by an extraction with 0.1 M NaOH.

Water extract from woody-herbaceous peat (HTW) was prepared by adding distilled water to the peat sample (2:1, v/v). The suspension obtained was continuously stirred for 12 h. The water phase was filtered off through a 0.45-μm membrane filter followed up with desalination using a cation exchanger and concentrated using a rotor evaporator.

Peat humic acid (HTO) was a commercial preparation purchased from Biolar (Olaine, Latviya).

Coal humic acid (AGK) was a commercial preparation kindly provided by Biotechnology Ltd. (Moscow, Russia).

Structural Characterization of HS. *Elemental analyses* (C, H, and N) were conducted on a C, H, N analyzer (Carlo Erba Strumentazione 1106). Ash content was determined gravimetrically. The contents of all the elements were calculated on an ash-free basis. Oxygen contents were calculated as the difference between a weight of the ash-free HS sample analyzed and the sum of the C, H, N contents found. Moisture was not accounted for.

SEC analysis was performed at the facilities of the Division of Water Chemistry, Engler-Bunte Institute, Technical University of Karlsruhe, Germany, according to ref *26*. Toyopearl HW-50S gel was used for column packing. Polydextrans were used as markers for molecular weight calculations. HS solutions were prepared at a concentration of 1−2 mg of C/L by equilibrating with the SEC mobile phase (0.028 M phosphate buffer, pH 6.8) prior to the analysis.

Quantitative ^{13}C solution-state NMR spectra were recorded on a Varian VXR-400 spectrometer operating at 100 MHz. A weight of HS sample of 100 mg was dissolved in 3 mL of 0.1 M NaOD and transferred into a 10-mm NMR tube. All the spectra were recorded at 4-s delay time using inverse gate decoupling. These conditions were shown to provide quantitative determination of carbon distribution of the main structural fragments of HS (*27*). To quantify the observed spectra, the assignments were made after Kovalevskii et al. (*27*) and were as follows (in ppm): 5−108, aliphatic non- and O-substituted C atoms ($\sum C_{Alk}$); 108−165, aromatic non- and O-substituted C atoms ($\sum C_{Ar}$); 165−187, C atoms of carboxylic and esteric groups (C_{COO}); 187−220, and C atoms of quinonic and ketonic groups ($C_{C=O}$). To derive the molecular descriptors, the percentage of carbon in the given structural fragments was used.

Stock solutions of HS were prepared by dissolving a weight of 150 mg of air-dried sample in 10 mL of 0.1 M NaOH under continuous stirring. pH of the solution was adjusted to 5.5 using 0.1 M HCl and NaOH. Then, the solution was brought to a final volume of 50 mL using distilled water.

Binding Experiments. For measuring partition coefficients of atrazine for HS, a series of five atrazine−HS solutions were prepared. The concentration of atrazine in all the solutions was 2 mg/L, and the concentration of HS was adjusted in the range of 0.5−1.5 g of C/L by dilution to the stock solution of HS (3 g of C/L). The solutions were thoroughly mixed for 24 h at room temperature. The duration of the equilibration period was selected based on the findings reported in ref *28*. All the binding experiments were conducted in three replicates.

The species of atrazine freely dissolved in water and bound to HS were separated using ultrafiltration technique. The experimental design described in ref *29* used an Amicon ultrafiltration cell of a maximum volume of 10 mL and a filter membrane YM-2 of molecular weight cutoff of 1000 Da. When not in use, the membrane was stored in distilled water at 4 °C. The prepared HS−atrazine solutions were transferred into the ultrafiltration cell and filtered with continuous stirring at 4.6 atm. The concentration of organic carbon in the ultrafiltrate did not exceed 1 mg/L accounting for less than 0.1% of the initial concentration of HS used. We thus disregarded the portion of the HS-bound atrazine that leaked through the membrane.

The determination of atrazine in the ultrafiltrate was conducted using the HPLC technique as described elsewhere (*16*). The HPLC system used was Gold Model 126 equipped with UV detector. The Ultrasphere Beckman column 4.6 mm × 15 cm was used for separation. A mixture of acetonitrile and water (50:50, v/v) containing 3.18×10^{-3} M HCl (pH 2.5) was used as the mobile phase. The absorbance of eluate was detected at 220 nm.

Calculation of Partition Coefficients of Atrazine for HS (K_{OC}). The binding of atrazine (A) to HS can be quantitatively described by the following equilibrium constant:

$$K = \frac{[\text{A-HS}]}{[\text{A}][\text{HS}]} \tag{1}$$

where [A] and [A − HS] are the concentrations of atrazine nonbound (passing through the ultrafilter) and HS-bound (retained by the filter), respectively, and [HS] is the equilibrium concentration of HS.

As the total concentration of HS (C_{HS}) was always much larger than that of atrazine (C_A), [HS] can be put equal to C_{HS}. Because of the unknown stoichiometry of the interaction of atrazine with HS, the total concentration of HS is expressed, as a rule, on the mass basis with dimensional units of kilograms of C per liter (*3*). In this case, eq 1 can be transformed into the following expression for partition coefficient (K_{OC}) of atrazine to HS:

$$K_{OC} = \frac{1 - \alpha}{\alpha C_{HS}} \tag{2}$$

where $\alpha = [\text{A}]/C_A$ and $1 - \alpha = [\text{A} - \text{HS}]/C_A$ are the portion of atrazine nonbound and HS-bound, respectively. Therefore, the K_{OC} of atrazine can be determined as the slope of a linear plot, which can be described by the following experimental relationship:

$$\frac{C_A}{[\text{A}]} = K_{OC} C_{HS} + 1 \tag{3}$$

As the concentration of HS is usually expressed in kilograms of C per liter, the dimensional units of the above K_{OC} value was liters per kilogram of C. The above equation is similar to the Stern−Volmer equation widely used for determination of K_{OC} of polycyclic aromatic hydrocarbons (PAH) for HS (*30*).

Results and Discussion

Partition Coefficients. Typical plots of the relationship of $C_A/[\text{A}]$ versus C_{HS} are given in Figure 1 for the HS samples of different origin. They are calculated from the HPLC ultrafiltration data on the partitioning of atrazine between the species freely dissolved in the water and bound to HS. Apparently, fitting the experimental plots to the linear relationships yields high correlation coefficients (r^2 values varied from 0.95 to 0.99), which allows the obtained plots to be used for determination of the K_{OC} values of atrazine for HS. The corresponding data for all the humic materials are summarized in Table 1.

The obtained K_{OC} values for the humic materials of different origin lay in the range of 87−575 L/kg of C. The maximum K_{OC} value of 575 L/kg of C was observed for coal HA (AGK) and HA of gray wooded soil (HGW), whereas the minimum value of 87 L/kg of C was obtained from the water

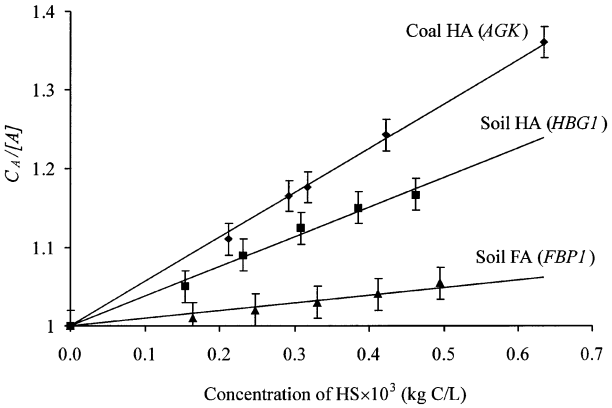

FIGURE 1. Determination of the atrazine partition coefficient K_{OC} for HS of different origin from the ultrafiltration HPLC experiments. Bars correspond to confidential intervals at $n = 3$ and $P = 95\%$.

TABLE 1. Partition Coefficients of Atrazine (K_{OC})[a] for Humic Substances Used in This Study

sample	source of HS	K_{OC}[b]
	Peat Humic Substances	
T4	sphagnum peat	377 ± 23
HTW	water extract of peat	87 ± 5
	Soil Humic Acids	
HBW	sod-podzolic soil, forest	380 ± 20
HBG	sod-podzolic soil, garden	400 ± 24
HBW1	sod-podzolic soil, forest	281 ± 17
HBP1	sod-podzolic soil, tillage	181 ± 30
HBG1	sod-podzolic soil, garden	380 ± 23
HGW	gray wooded soil, forest	575 ± 34
HS	meadow chernozemic soil	501 ± 31
HST	typical chernozemic soil	404 ± 23
	Soil Fulvic Acids	
FBW1	sod-podzolic soil, forest	192 ± 12
FBP1	sod-podzolic soil, tillage	110 ± 10
FBG1	sod-podzolic soil, garden	275 ± 17
	soil humic substances	
SEL	typical chernozem	444 ± 25
	Commercial Preparations	
HTO	peat humic acid	300 ± 20
AGK	coal humic acid	575 ± 35

[a] L/kg of C. [b] ± corresponds to a confidence interval at $n = 3$ and $P = 95\%$.

extract from peat (HTW). The obtained values agree well with the reported K_{OC} values for atrazine binding to dissolved humic materials determined by different analytical techniques ranging from 41 to 600 L/kg of C (*8*, *9*, *11*−*13*). According to the obtained data, the maximum binding capacity of HS for atrazine can be estimated to be as large as 0.9 mg of atrazine/g of C or 12 μmol of atrazine/g of HS, which is in the range of the previously reported values varying from 2.2 (*16*) to 21.3 (*31*) μmol of atrazine/g of HS. A comparison of the atrazine K_{OC} values with those for PAH obtained for the same humic materials (*22*) shows that the atrazine K_{OC} are 2−3 orders of magnitude lower than those for PAH.

On the basis of the measured K_{OC} values, the target humic materials can be arranged in the following order of affinity for atrazine: coal HA ≅ gray wooded soil HA > chernozemic soil HA and HF > sod-podzolic soil HA ≅ peat HF > sod-podzolic soil FA ≫ peat DOM. The soil HA samples studied had the higher binding affinity for atrazine than FA extracted from the same soil. It is of importance to note that the binding affinity of both HA and FA extracted from the garden sod-podzolic soil was higher than that of the HA and FA extracted from the virgin (a forested site) and cultivated sod-podzolic soils. Among the soil humic materials studied, the HA and FA isolated from the cultivated sod-podzolic soil had the lowest binding affinity for atrazine but was much higher than that of the water extract of peat.

The obtained results show clearly that the binding affinity for atrazine of the target humic materials varies greatly with respect to their source and fractional composition. Our next goal was to establish the structure−activity relationships for atrazine binding to HS.

Relationship between Structure and Binding Affinity of HS for Atrazine. To relate atrazine binding to HS structure, we used the same approach described in detail in our previous paper on binding HS to PAH (*22*). It is based on a numerical description of the structure of HS by a combination of the molecular descriptors of elemental, structural fragments, and molecular weight composition. The atomic ratios (H/C and O/C) were used as the descriptors of elemental composition, the percentages of carbon in the main structural groups ($C_{C=O}$, C_{COO}, $\sum C_{Ar}$, and $\sum C_{Alk}$) were used as the descriptors of structural fragments composition, and the weight-averaged molecular weight (M_w) was used as a descriptor of the molecular weight composition. The corresponding data are summarized in Table 2.

Among the molecular descriptors used, the strongest correlation with atrazine K_{OC} values was observed for $\sum C_{Ar}$ and $\sum C_{Ar}/\sum C_{Alk}$. The corresponding correlation coefficients (r) were 0.91 (Figure 2) and 0.87, respectively, demonstrating the statistical significance of the obtained relationships at $P > 99\%$ ($n = 16$). These results are consistent with the reported studies (*32*) correlating K_{OC} values with the percentage of aromatic carbon in the humic material. At the same time, there were no statistically relevant correlations observed with the amount of carbonylic ($C_{C=O}$) and carboxylic carbon (C_{COO}), with atomic ratios, and with M_w.

For understanding the mechanism underlying the binding of atrazine to HS, of particular importance is that a similar relationship between K_{OC} values and aromaticity of HS was observed for binding of PAH to HS (*22*). The more prevalent the hydrophobic aromatic core is as compared to the hydrophilic (mostly polysaccharidic) aliphatic periphery, the greater the hydrophobicity of the HS. Thus, the obtained results could have been interpreted as a key role of hydrophobic interactions in binding of atrazine to HS as was previously reported (*7*, *10*, *11*, *16*). The charge-transfer interaction might also contribute in binding of atrazine to HS as was hypothesized by the different investigators (*6*, *18*−*20*). A lack of correlation of the obtained K_{OC} values with the percentage of carbonylic and carboxylic carbon does not corroborate the reported findings, suggesting a dominant role of hydrogen bonding or proton transfer in atrazine binding to HS (*15*, *17*, *21*). The above mechanisms can prevail at pH < 4 when atrazine becomes protonated (*28*). However, such conditions are not environmentally relevant, which makes the mechanisms of ion exchange and proton transfer hardly feasible as an explanation of the binding of atrazine to HS in soil and water ecosystems.

Environmental Implication. The K_{OC} values reported in this paper are of major importance for prediction of atrazine behavior in different aquatic and soil media. As indicated by the K_{OC} values obtained, the toxicity of atrazine applied to agricultural crops will depend not only on the content of humic matter in the soil but also on its quality as well. Thus, the same application rate of atrazine is expected to result in the lowest herbicidal impact on the mollisol (chernozem) containing the largest amount of HS of highly aromatic character and result in the greatest herbicidal impact on the humic depleted, tilled sod-podzolic soils that contain HS of predominantly aliphatic character. Our experimental estimates of the toxicity of atrazine introduced into soils of

TABLE 2. Molecular Parameters of Humic Substances Used in This Study

sample	source of HS	H/C[a]	O/C[a]	M_w[b]	$C_{C=O}$[c]	C_{COO}[c]	$\sum C_{Ar}$[c]	$\sum C_{Alk}$[c]
			Peat Humic Substances					
T4	sphagnum peat	1.07	0.54	22	1	16	43	40
HTW	water extract of peat	1.49	0.61	10	4	13	16	73
			Soil Humic Acids					
HBW	sod-podzolic soil, forest	0.99	0.58	19	1	17	45	38
HBG	sod-podzolic soil, garden	1.11	0.48	22	4	20	33	40
HBW1	sod-podzolic soil, forest	1.02	0.51	19	3	21	26	50
HBP1	sod-podzolic soil, tillage	1.36	0.66	20	1	15	46	38
HBG1	sod-podzolic soil, garden	1.21	0.62	19	2	19	42	37
HGW	gray wooded soil, forest	1.04	0.63	21	1	19	47	33
HS	meadow chernozemic soil	0.86	0.51	17	2	14	57	28
HST	typical chernozemic soil	0.77	0.52	15	3	15	54	28
			Soil Fulvic Acids					
FBW1	sod-podzolic soil, forest	0.99	0.60	8	4	20	34	43
FBP1	sod-podzolic soil, tillage	1.15	0.75	9	2	25	26	47
FBG1	sod-podzolic soil, garden	1.04	0.63	9	3	18	41	37
			Soil Humic Substances					
SEL	typical chernozemic soil	1.03	0.58	19	1	18	45	36
			Commercial Preparations					
HTO	peat humic acid	1.05	0.57	18	3	13	44	40
AGK	coal humic acid	0.97	0.76	15	1	17	58	25

[a] H/C and O/C ratios are calculated on ash- and water-free basis. [b] M_w values are determined by SEC (calibration by polydextrans) and given in kDa. [c] Content of carbon in the structural fragments is determined by ^{13}C NMR spectroscopy as the integral intensity (%) of the following spectral regions (ppm): 220−187 ($C_{C=O}$), 187−165 (C_{COO}), 165−108 (C_{Ar}), and 108−5 (C_{Alk}).

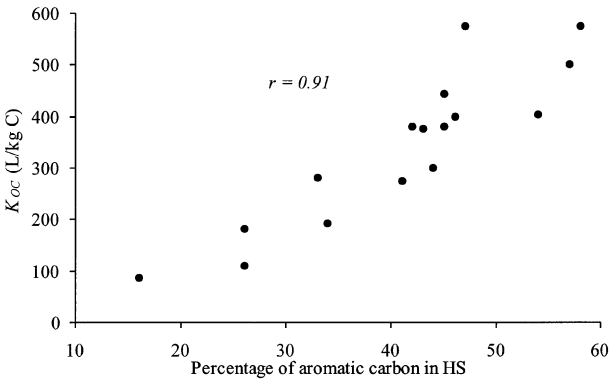

FIGURE 2. Correlation between the K_{OC} values of atrazine and aromaticity ($\sum C_{Ar}$) of HS used in this study.

different type and use showed that, as expected from the content and aromaticity of HA in soils, the lowest toxicity of atrazine at the same application rate was observed for mollisols (typical and meadow chernozems) (*33*).

On the other hand, the structure−activity relationships for atrazine binding to HS point out that aromatic-rich humic materials are the most efficient binding agents for atrazine. Hence, the coal humates can be effectively used to minimize the residual toxicity of the atrazine-treated agricultural fields. The advantage of humate application is their combined action as detoxifying agents and organic fertilizers. The quantification of detoxifying properties of humic materials in relation to atrazine and other herbicides will facilitate an effective use of humates for detoxification of bound residues of herbicides.

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