

Interactions of Three s-Triazines with Humic Acids of Different Structure

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The adsorption of three s-triazines (atrazine, terbutylazine, and simazine) on three different humic acids (HAs), before and after acidic hydrolysis, was studied at four diverse pH values from 3 to 4.5. The Freundlich sorption affinity (K_f) and intensity ($1/n$) of s-triazines were related to the chemical and structural composition of HA and used in a multivariate statistical analysis. At low pH, the sorption values for s-triazines were not directly justified by the content of carboxyl groups in original HA, while only an increase of phenolic carbons in hydrolyzed HA supported the increased s-triazine adsorption. The structural composition of both s-triazines and humic samples explained 86% of the significance in multivariate analyses, whereas the role of pH remained hidden in only 14% of the statistical significance. Adsorption of s-triazines was mainly related to carbon content, hydrophobicity, and aromaticity of HA, thereby implying a predominant binding role of weak dispersive forces. Steric properties explained the larger adsorption affinity of atrazine and terbutylazine with respect to simazine. The occurrence of charge-transfer interactions between atrazine and mainly hydrolyzed HA was further suggested by the increased number of free radicals detected in atrazine–HA complexes at different pH values. Charge-transfer interactions were postulated to occur when HA conformations became progressively controlled by aromatic components. This work indicates that humic matter rich in hydrophobic and aromatic constituents is more likely to adsorb s-triazines and reduce their environmental mobility.

KEYWORDS: Humic substances; adsorption isotherms; s-triazines; hydrophobic and charge-transfer mechanisms

INTRODUCTION

s-Triazines are selective persistent herbicides that are widely investigated due to their still large applications in forestry and pre- and postemergence in agricultural soils (1, 2). Even though these herbicides are now forbidden in some countries, the recalcitrance of s-triazines against chemical and biological degradation has led to their accumulation in the environment (3). As a result of their massive use, s-triazines and their metabolites have been detected at alarmingly high concentrations in soils, groundwaters, rivers, and lakes (4–6).

The transport, bioavailability, and fate of herbicides in soils are recognized to be controlled from the major part by soil sorption processes. A fundamental understanding of sorption

behavior is therefore important for accurate predictions of environmental load of released organic pollutants and the effective implementation of remedial strategies (7). For this reason, the mechanism of s-triazine sorption by soil organic matter, and in particular by humic substances, is a topic of great interest (8).

Despite the number of studies related to the interaction between s-triazines and humic substances (8–11 and references herein), the binding mechanism is still the subject of controversial discussions. (i) Ionic interactions, (ii) proton-transfer, (iii) electron-transfer, and (iv) hydrophobic interactions belong to the most discussed interactions attributed to the adsorption capacity of humic substances toward s-triazines. Summaries and contradictions related to these binding mechanisms are reported elsewhere (10–12). Furthermore, it has been suggested that the prevalent type of binding depends on chemical and conformational properties of humic substances because of the differences in their stereochemical nature and thus varying accessibility of specific functional groups on their surface (8, 12).

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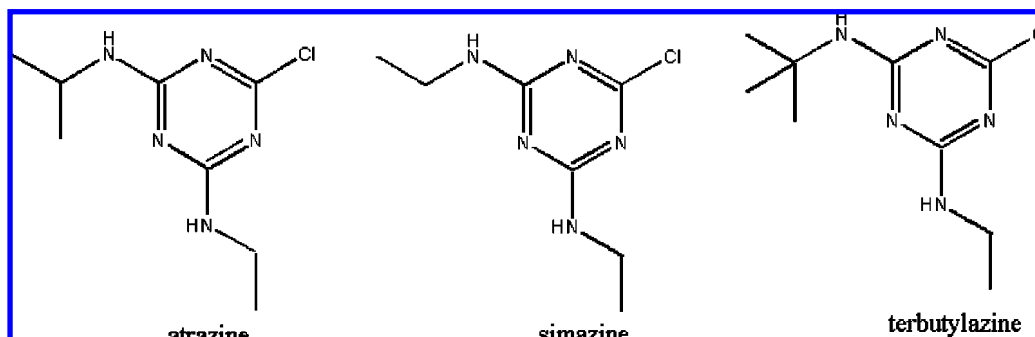


Figure 1. Molecular structures of s-triazines.

The objective of this work was to assess the molecular structure of humic acids (HAs) before and after strong acid hydrolysis and to investigate their adsorbing capacity toward selected s-triazines, namely, atrazine, simazine, and terbutylazine. A comparison of sorption affinity was made for HAs isolated from peat, lignite, and oxidized coal while varying the solution pH from 3.0 to 3.5, 4.0, and 4.5.

MATERIALS AND METHODS

Humic Substances. HAs were extracted from (i) P-HA, a peat soil (Dystric Histosol) near Lucca, Toscana, Italy; (ii) L-HA, a North Dakota Leonardite (Mammoth Int. Chem. Co.); and (iii) C-HA, an oxidized coal (Enricerche, SpA, Italy), by common procedures (13). The original material was shaken overnight in a 0.5 M NaOH solution under N₂ atmosphere. The HAs were precipitated from alkaline extracts by adding 6 M HCl until pH 1 and were extensively purified by three cycles of dissolution in 0.1 M NaOH and subsequent precipitation in 6 M HCl. The HAs were then treated with a 0.5% (v/v) HCl-HF solution for 36 h, dialyzed (Spectrapore 3, 3500 MW cutoff) against distilled water until chloride-free, and freeze-dried. Part of these HA samples underwent acidic hydrolysis with 6 M HCl at 200 °C overnight, and after they reached room temperature, the hydrolyzed HA residues (P-HA-H, L-HA-H, and C-HA-H) were dialyzed as done for the original HAs and freeze-dried. The elemental content of all HA samples was determined with a Fison EA 1108 Elemental Analyzer. Total acidity, carboxylic, and phenolic groups were determined according to the procedures described by Schnitzer (14). These analyses were conducted in triplicate, and standard deviations were calculated.

s-Triazine Herbicides. Three high-purity (99%) s-triazines with different functional groups, atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine), simazine [2-chloro-4,6-bis(ethylamino)-1,3,5-triazine], and terbutylazine (2-chloro-4-ethylamino-6-terbutylamino-1,3,5-triazine) were purchased from Dr. Ehrenstörfer Laboratories, Germany (Figure 1). Stock solutions of each herbicide were prepared by dissolving 20 mg of atrazine, 10 mg of simazine, and 10 mg of terbutylazine in 200 mL of methanol.

Adsorption Isotherms. Humic samples (25 mg) were added with solutions of the following ranges of concentrations: 0–25 ppm of atrazine, 0–10 ppm of simazine, and 0–15 ppm of terbutylazine. Different concentrations took into account s-triazine solubility in water. Aliquots of methanolic s-triazine stock solutions were diluted with 5 mL of 0.01 M CH₃COONa and adjusted to pH 3.0, 3.5, 4.0, and 4.5 with CH₃COOH. No dissolution of HA was observed at any pH. The mixtures were shaken at 25 °C in a screw-cap glass vial on a rotary shaker for 12 h. This time was previously verified to be necessary to attain sorption equilibrium. The suspensions were centrifuged, and the concentration of each s-triazine was left in the supernatant (where no HAs were present at any pH) and was determined by high-performance liquid chromatography (HPLC) analysis. All samples were made in triplicate at room temperature and in the dark to avoid photochemical reactions (15). Calculation of equilibrium sorption data was based on the Freundlich model:

$$C_s = K_f \times C_w^{1/n} \quad (1)$$

where C_s ($\mu\text{g/g}$) and C_w ($\mu\text{g/mL}$) represent the equilibrium solid- and liquid-phase concentration of the s-triazine, respectively. The logarithm

linearization of eq 1 allows measurement of Freundlich constant K_f (mL/g), which is a measure of sorption capacity, and $1/n$ (dimensionless), which corresponds to the line slope and is related to the number of adsorption sites of different surface energies (16).

HPLC Analysis. A Spectra System P 200 pump, equipped with 20 μL loop, 7725 Rheodyne Rotary Injector, a Hypersil-5 ODS precolumn and Hypersil-5 ODS column (250 mm \times 4.6 mm, i.d., 5 μL , HPLC Technology Ltd., United Kingdom), and a UV detector (Spectra System UV 2000) were operated at specific conditions for each triazine herbicide. Atrazine was determined by HPLC using an isocratic elution of 60% CH₃CN in H₂O at a flow rate of 2.0 mL min⁻¹ and UV detector set at 220 nm. Simazine was detected using an isocratic elution of 60% CH₃CN in H₂O at a flow rate of 1.7 mL min⁻¹ and UV detector set at 224 nm, while terbutylazine was eluted applying an isocratic flow of 65% CH₃CN in H₂O at 2.0 mL min⁻¹ and UV detector set at 223 nm. Two sets of calibration curves were used for each s-triazine, the first one involving a low concentration interval (0–5 ppm) and the second one covering a higher concentration range (1–25 ppm). The HPLC chromatograms were elaborated using a Labnet HPLC software.

NMR Spectra. Solid-state cross-polarization magic angle spinning (CPMAS) ¹³C NMR spectra of HAs were obtained with a Bruker AM 400 spectrometer at 100.6 MHz, using a rotating speed of 4000–4500 Hz. To analyze different structural components of humic samples, the peak intensities were measured at a contact time ranging from 1 to 3 ms. The samples underwent from 512 to 1024 scans (depending on the signal/noise ratio) with a relaxation delay of 2.2 s. An exponential multiplication corresponding to a line broadening of 8–16 Hz was applied. The ¹³C NMR spectra were integrated within the following chemical shift regions: 0–48 ppm, alkyl C; 48–105 ppm, O- and N-substituted C (aminoacids, carbohydrates, alcohols, etc.); 105–145 ppm, aromatic C; 145–165 ppm, phenolic C; and 165–190 ppm, carboxyl C.

Electron Spin Resonance (ESR). ESR spectra of the freeze-dried residues resulting from the adsorption of atrazine on C-HA and its hydrolyzed residue (C-HA-H) were recorded at room temperature with a Bruker AXS (Karlsruhe, Gerwas) ER-200D SRC ESR spectrophotometer operating at X-band (9.5 GHz), with a 100 kHz magnetic field modulation frequency, microwave power = 0.2 mW, at room temperature, and modulation amplitude of 0.5–10⁻⁴ Tesla. The g parameter was measured against 2,2-diphenyl-1-picrylhydrazyl (DPPH) standard ($g = 2.0036$).

Principal Component Analysis (PCA). Analytical adsorption data (Tables 3–5) were analyzed as one entire data set consisting of 18 samples (humic substances–triazine complexes) and eight variables (K_f and $1/n$ adsorption parameters at pH 3.0, 3.5, 4.0, and 4.5). The original data were recalculated into orthogonal linear combinations (principal components) of the standardized (autoscaled) variables using a correlation matrix. PCA was performed using the statistical software package XLSTAT 6.1 (Addinsoft SARL, France).

RESULTS AND DISCUSSION

Molecular Characteristics of HAs. Elemental analyses of humic materials before and after hydrolysis are reported in Table 1. The largest amount of carbon was found for oxidized coal (C-HA), followed by peat (P-HA) and lignite (L-HA). Hydroly-

Table 1. Elemental Content (g kg⁻¹) and Total, Carboxyl, and Phenolic Acidity (mequiv g⁻¹) in HAs before and after Hydrolysis^a

sample ^b	g kg ⁻¹					mequiv g ⁻¹			
	C	H	N	C/H	C/N	total acidity	carboxyl		phenolic acidity ^c
							acidity	phenolic acidity ^c	
P-HA	430	51	24	8.4	17.9	4.77 (0.63)	2.29 (0.01)	2.48	
P-HA-H	470	54	15	8.7	31.3	4.98 (0.10)	1.95 (0.04)	3.03	
L-HA	315	39	0.7	8.1	450	5.16 (0.36)	3.21 (0.13)	1.95	
L-HA-H	689	53	1.3	13	530	4.24 (0.27)	2.24 (0.02)	2.00	
C-HA	519	39	0.8	13	648	4.50 (0.44)	2.13 (0.14)	2.37	
C-HA-H	559	42	0.9	13	621	5.72 (0.62)	2.10 (0.02)	3.62	

^a Standard deviations are in parentheses. ^b P, peat; C, oxidized coal; L, lignite; and HA-H, hydrolyzed HA. ^c Obtained as differences between total and carboxyl acidity.

Table 2. Distribution of Signal Intensity in Different Regions (ppm) of ¹³C-CPMAS-NMR Spectra of Humic Fractions

sample ^a	relative intensity (% of total area)					aromaticity ^b	HI/HB ^c
	0–48	48–105	105–145	145–165	165–190		
P-HA	37.5	29.9	18.8	10.5	3.3	29.3	0.50
P-HA-H	35.3	26.5	22.9	12.6	2.6	35.5	0.41
L-HA	32.7	26.3	23.4	12.7	4.9	36.1	0.45
L-HA-H	28.9	25.7	26.2	16.0	3.3	42.2	0.40
C-HA	34.0	25.3	23.3	13.2	4.2	36.5	0.41
C-HA-H	30.2	24.2	27.1	15.0	3.4	42.1	0.38

^a Humic samples are as in **Table 1**. ^b Aromaticity = 100 × [(105 – 165)/(0 – 190)]. ^c HI/HB = [(48 – 105) + (165 – 190)]/[(0 – 48) + (105 – 165)].

Table 3. Freundlich Parameters, K_f (mL g⁻¹) and $1/n$, with Respective Determination Coefficients (r^2) for Atrazine Adsorption on HAs and Their Hydrolyzed (HA-H) Fractions at Different pH Values^a

pH	K_f	$1/n$	r^2	K_f	$1/n$	r^2
	P-HA			P-HA-H		
3.0	2.482 (0.005)	0.78 (0.04)	0.991	3.023 (0.011)	0.71 (0.05)	0.961
3.5	2.423 (0.007)	0.86 (0.03)	0.989	3.006 (0.009)	0.74 (0.02)	0.957
4.0	2.226 (0.004)	0.90(0.02)	0.964	2.911 (0.013)	0.81 (0.08)	0.991
4.5	2.162 (0.005)	0.90(0.01)	0.994	2.832 (0.008)	0.78 (0.06)	0.993
	L-HA			L-HA-H		
3.0	2.414 (0.010)	0.94 (0.02)	0.991	2.492 (0.003)	0.83 (0.03)	0.968
3.5	2.556 (0.012)	0.88 (0.03)	0.981	2.653 (0.016)	0.79 (0.07)	0.983
4.0	2.531 (0.003)	0.85 (0.05)	0.990	2.671 (0.013)	0.75 (0.10)	0.983
4.5	2.473 (0.014)	0.85 (0.01)	0.995	2.660 (0.009)	0.77 (0.03)	0.988
	C-HA			C-HA-H		
3.0	2.701 (0.008)	0.88 (0.06)	0.982	2.992 (0.011)	0.87 (0.05)	0.957
3.5	2.711 (0.006)	0.88 (0.07)	0.973	2.952 (0.006)	0.99 (0.07)	0.969
4.0	2.663 (0.010)	0.90 (0.05)	0.973	2.827 (0.003)	1.06 (0.06)	0.959
4.5	2.425 (0.018)	1.04 (0.03)	0.970	2.520 (0.009)	1.10 (0.03)	0.988

^a P, peat; C, oxidized coal; and L, lignite. Standard deviations are in parentheses.

sis resulted in a significant increase of carbon content in all humic materials, due to loss of proteinaceous materials (14). Conversely, the N content was decreased for P-HA, increased for L-HA-H, and did not change significantly for C-HA-H. Both hydrolyzed peat and lignite HA (P-HA-H and L-HA-H) exhibited larger C/H and C/N ratios than their corresponding untreated samples, while the ratios were substantially similar for C-HA-H.

The total acidities of the nonhydrolyzed HA were not statistically different, whereas those of hydrolyzed samples decreased in the order C-HA-H > P-HA-H > L-HA-H (**Table 1**). Acid hydrolysis led to an increased phenolic acidity in all humic samples and a decreased carboxylic acidity for L-HA-H and P-HA-H. The increased number of phenols may have originated from the hydrolysis of ester bonds, whereas carboxyl groups may have been lost through decarboxylation.

Table 4. Freundlich Parameters, K_f (mL g⁻¹) and $1/n$, with Respective Determination Coefficients (r^2) for Simazine Adsorption on HAs and Their Hydrolyzed (HA-H) Fractions at Different pH Values^a

pH	K_f	$1/n$	r^2	K_f	$1/n$	r^2
	P-HA			P-HA-H		
3.0	2.281 (0.002)	0.39 (0.04)	0.968	2.623 (0.011)	0.29 (0.05)	0.968
3.5	2.142 (0.009)	0.47 (0.03)	0.868	2.559 (0.006)	0.40 (0.02)	0.958
4.0	2.117 (0.007)	0.58 (0.02)	0.819	2.498 (0.010)	0.43 (0.05)	0.961
4.5	2.076 (0.004)	0.54 (0.03)	0.950	2.483 (0.008)	0.41 (0.06)	0.966
	L-HA			L-HA-H		
3.0	2.333 (0.017)	0.78 (0.03)	0.988	2.473 (0.005)	0.91 (0.06)	0.943
3.5	2.352 (0.013)	0.60 (0.05)	0.942	2.413 (0.011)	0.63 (0.02)	0.837
4.0	2.356 (0.010)	0.48 (0.11)	0.893	2.453 (0.015)	0.44 (0.13)	0.847
4.5	2.314 (0.015)	0.31 (0.07)	0.615	2.350 (0.009)	0.43 (0.06)	0.702
	C-HA			C-HA-H		
3.0	2.640 (0.010)	0.81 (0.09)	0.925	2.643 (0.015)	0.77 (0.05)	0.923
3.5	2.571 (0.014)	0.50 (0.06)	0.873	2.621 (0.005)	0.53 (0.07)	0.970
4.0	2.600 (0.008)	0.42 (0.08)	0.858	2.689 (0.011)	0.43 (0.02)	0.969
4.5	2.598 (0.016)	0.28 (0.18)	0.662	2.720 (0.013)	0.27 (0.06)	0.908

^a P, peat; C, oxidized coal; and L, lignite. Standard deviations are in parentheses.

Table 5. Freundlich Parameters, K_f (mL g⁻¹) and $1/n$, with Respective Determination Coefficients (r^2) for Terbutylazine Adsorption on HAs and Their Hydrolyzed (HA-H) Fractions at Different pH Values^a

pH	K_f	$1/n$	r^2	K_f	$1/n$	r^2
	P-HA			P-HA-H		
3.0	1.492 (0.010)	2.10 (0.04)	0.960	2.591 (0.007)	0.91 (0.03)	0.956
3.5	1.976 (0.021)	1.28 (0.06)	0.991	2.615 (0.011)	0.72 (0.02)	0.922
4.0	2.088 (0.017)	0.97 (0.05)	0.980	2.597 (0.013)	0.48 (0.02)	0.730
4.5	2.062 (0.006)	0.75 (0.02)	0.972	2.574 (0.006)	0.56 (0.01)	0.828
	L-HA			L-HA-H		
3.0	2.446 (0.007)	1.34 (0.03)	0.949	2.653 (0.003)	0.67 (0.06)	0.920
3.5	2.582 (0.010)	0.87 (0.02)	0.972	2.712 (0.009)	0.50 (0.01)	0.884
4.0	2.491 (0.005)	0.62 (0.14)	0.962	2.581 (0.010)	0.42 (0.11)	0.871
4.5	2.492 (0.013)	0.44 (0.02)	0.897	2.590 (0.004)	0.28 (0.01)	0.885
	C-HA			C-HA-H		
3.0	2.798 (0.005)	0.87 (0.03)	0.923	2.953 (0.011)	0.77 (0.05)	0.849
3.5	2.831 (0.010)	0.51 (0.06)	0.894	2.811 (0.015)	0.64 (0.01)	0.807
4.0	2.793 (0.004)	0.39 (0.09)	0.947	2.743 (0.001)	0.62 (0.05)	0.914
4.5	2.743 (0.012)	0.35 (0.08)	0.916	2.739 (0.012)	0.47 (0.02)	0.849

^a P, peat; C, oxidized coal; and L, lignite. Standard deviations are in parentheses.

Carbon distribution (**Table 2**) by signal integration in CPMAS-NMR spectra (unshown) showed that P-HA had a higher content of aliphatic (0–48 ppm) and substituted C (48–105 ppm) but a lower content of aromatic (105–145), phenolic (145–165 ppm), and carboxyl (165–190 ppm) carbons than L-HA and C-HA. Consequently, the aromaticity index was found to be greater for L-HA and C-HA than for P-HA, while the relative abundance of polar (hydrophilic) and apolar (hydrophobic) C (HI/HB ratio in **Table 2**) varied in the order P-HA > L-HA > C-HA. In general, acid hydrolysis led to reduction of alkyl-, O- and N-substituted-, and carboxyl-C but produced relatively more aromatic- and phenolic-C signals. The loss of carboxyl groups and enhancement of phenols confirms results of total acidities (**Table 1**).

Adsorption of s-Triazines on HAs. The equilibrium isotherms of s-triazines on original and hydrolyzed HAs, at different pH values, are reported in **Figure 2**. The maximum adsorption of s-triazines was in all cases observed at pH 3.0 and decreased with increasing solution pH. Because simazine ($pK_a = 1.65$), atrazine ($pK_a = 1.68$), and terbutylazine ($pK_a = 1.93$) are weak bases, increasing the pH resulted in a decrease of their cationic fraction in solution. Therefore, it is likely that at pH < 4.0, when s-triazines still maintain a degree of protonation, the adsorption on humic matter was enhanced due

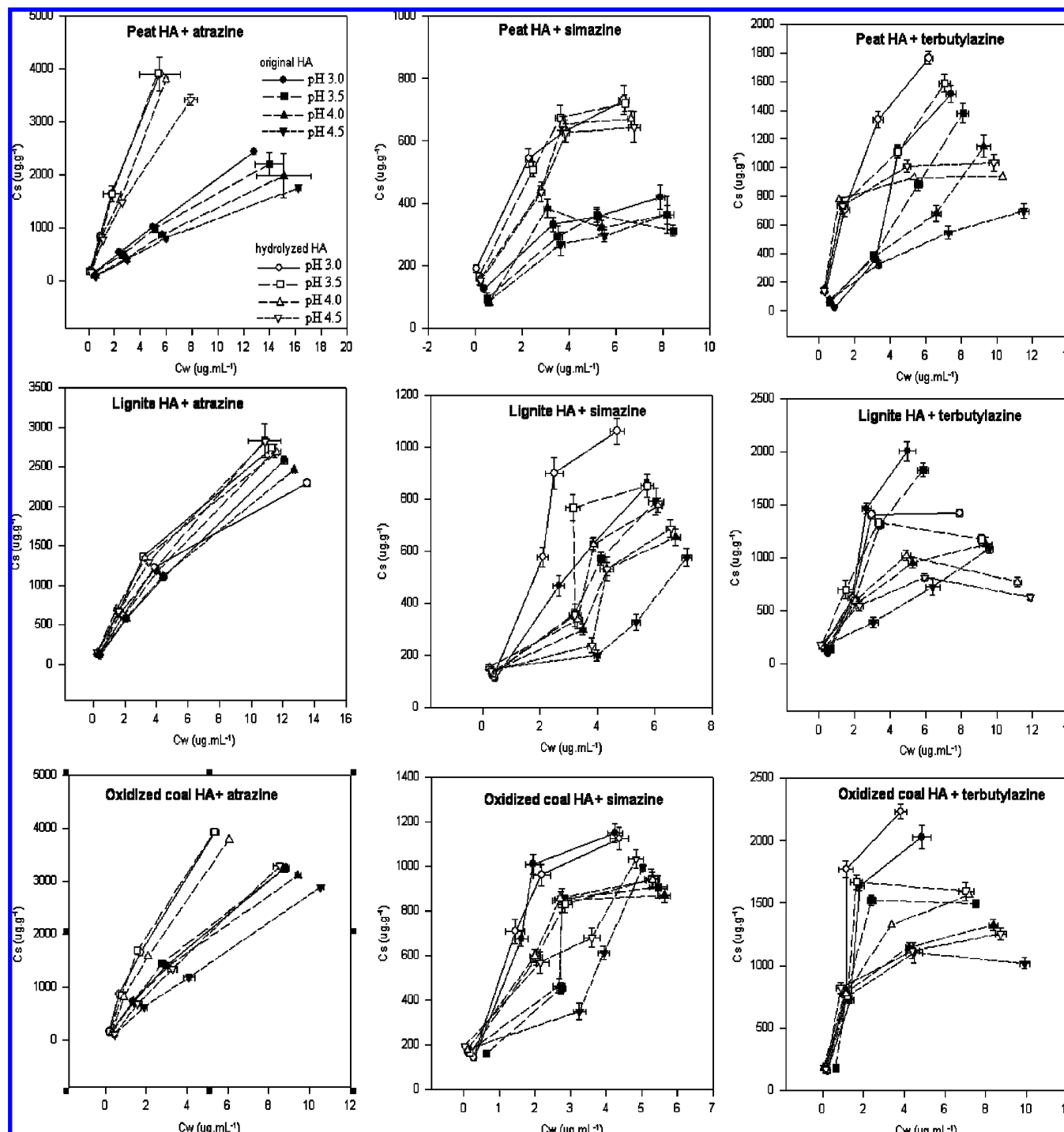


Figure 2. Adsorption isotherms for atrazine, simazine, and terbutylazine herbicides on HAs originated from peat soil, lignite, and oxidized coal and their hydrolyzed products at different pH values. Horizontal and vertical bars indicate standard deviations of the mean for C_s and C_w values, respectively.

to ion exchange interactions, while such mechanisms became progressively less effective at higher pH values (7).

However, the shapes of the Freundlich isotherms varied among triazines (Figure 2). The adsorption on original and hydrolyzed HA produced generally well distinct Atrazine isotherms, whereas such a distinction was not evident for the other two triazines. Simazine isotherms for P-HA differed substantially from that for P-HA-H, but no difference between original and hydrolyzed sample was noted for both L and C materials. Terbutylazine isotherms were similar for all humic samples and progressively passed from "S" to "L" types (17) with increasing pH, thereby indicating a progressive saturation or reduced affinity of adsorbing humic sites with pH.

The adsorption coefficients K_f and $1/n$ of the Freundlich model are summarized in Tables 3–5. The sorption affinity (K_f) of s-triazines for original humic materials at different pH values was generally in the order C-HA > L-HA > P-HA, although the K_f value for simazine was somewhat lower than for atrazine and terbutylazine. It is interesting to note that, while the P-HA K_f for terbutylazine was significantly lower than for atrazine and simazine at pH 3, the P-HA K_f values for the latter s-triazines became similar with increasing pH. This behavior may be explained with the steric hindrance of the terbutyl group in terbutylazine that prevented at pH 3 a closer proximity of the predominant cationic specie of terbutylazine to the P-HA surface. This despite ion-exchange interactions may have been

avored by both the large terbutylazine protonation ($pK_a = 1.93$) at pH 3 and the relatively high content of hydrophilic components of P-HA (see **Tables 1** and **2**). Conversely, when the adsorption pH was raised to 3.5 and above, the sorption affinity of terbutylazine on P-HA became similar to that of atrazine and simazine, possibly because of the decreasing importance of ion-exchange interactions and the increasing role of hydrophobic interactions for all s-triazines (18). Also, the $1/n$ factor, indicating sorption linearity, was much larger for terbutylazine adsorption on P-HA at pH 3 and steadily decreased with increasing pH to values similar to atrazine and simazine (**Tables 3–5**).

The sorption behavior of triazines was not different with more strongly adsorbing C-HA and L-HA materials. Except for a K_f decreasing tendency with pH observed for atrazine on C-HA, the triazines sorption affinity on C-HA and L-HA did not significantly vary with pH. Nevertheless, the $1/n$ factor decreased consistently and extensively with increasing pH for the adsorption of simazine and terbutylazine on C-HA and L-HA, whereas, in the case of atrazine, it remained substantially similar for L-HA and even somewhat increased for C-HA at pH 4.5. These results indicate the increasing role of hydrophobic interactions between s-triazines and humic materials rich in alkyl- and aromatic-C such as C-HA and L-HA (18).

Hydrolyzed HAs resulted in an increased s-triazines sorption capacity at all pH values, except for terbutylazine on C-HA-H at pH 4 and 4.5 (**Tables 3–5**). Because hydrolysis increased phenolic acidity, these functional phenolic groups in humic structures may account for the enhanced adsorption. Similarly, the increased herbicides adsorption may be correlated to the enhanced aromaticity of hydrolyzed samples. As by K_f values, the orders of adsorption were P-HA-H \approx C-HA-H $>$ L-HA-H, C-HA-H $>$ P-HA-H $>$ L-HA-H, and C-HA-H $>$ L-HA-H \approx P-HA-H for atrazine, simazine, and terbutylazine, respectively. However, as for original HAs, atrazine showed a greater sorption affinity toward hydrolyzed HA than simazine and terbutylazine.

A general reduction of $1/n$ values was noticed for s-triazines adsorption on hydrolyzed HAs, with the exception of simazine on L-HA-H and atrazine on C-HA-H (**Tables 3–5**). Particularly significant was the $1/n$ decrease for terbutylazine adsorption on P-HA-H and L-HA-H. Values of $1/n < 1$ indicate adsorption by heterogeneous matter where high energy sites are occupied first, followed by adsorption at lower energy sites, while $1/n = 1$ implies simple partitioning into amorphous adsorbent (19). Therefore, the decrease in $1/n$ can be taken as evidence of either losses of adsorbing sites or conformational changes leading to modification of adsorption surfaces in hydrolyzed samples.

To visualize the overall results of s-triazines adsorption on humic materials before and after hydrolysis (**Tables 3–5**), the data were analyzed by PCA. PCA is an unsupervised multivariate statistical method used for the recognition of similarities and/or dissimilarities among samples while reducing the high amount of original variables into just a few principal components retaining high percentage of the original information (20).

In this case, PCA reduced the original eighth dimensional space (i.e., eight original variables; for details, see the Materials and Methods) into just a two-dimensional plot, by extracting two principal components, PC1 and PC2, that covered 86% of total variance of original data (**Figure 3**). Negative scores on PC1 correspond to samples with higher $1/n$ values, while positive scores indicate samples having larger K_f coefficients. Samples with large $1/n$ and K_f parameters were also correlated along the positive side of PC2, in the first and second quadrant of **Figure 3**, respectively. As a result, PC2 differentiated humic

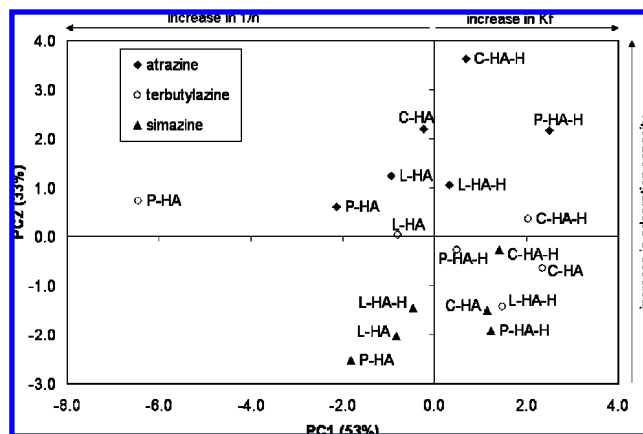


Figure 3. Score plot of s-triazines (atrazine, simazine, and terbutylazine) adsorption on HAs and their hydrolyzed (HA-H) fractions (P, peat; C, oxidized coal; and L, lignite). The two principal components, PC1 and PC2, were extracted from adsorption data shown in **Tables 3–5**.

materials according to their adsorption capacity (the larger the adsorption coefficients, the more efficient was the s-triazine adsorption), while PC1 indicates which of the two adsorption parameters (i.e., K_f and $1/n$) was more relevant. The PCA plot suggests that atrazine was the most easily adsorbed triazine, followed by terbutylazine and simazine. Furthermore, PCA analysis synthetically generalizes the adsorption behavior of humic samples: Atrazine was preferentially sorbed on C-HA, C-HA-H, and P-HA-H; terbutylazine showed the best adsorption affinity toward P-HA, C-HA-H, and L-HA, while C-HA-H represented the most efficient adsorbent for simazine.

The advantage of PCA is the possibility to combine both adsorption parameters, K_f and $1/n$, in one general comparison of adsorption efficacy of different humic materials. Conversely, the disadvantage of PCA is the lack of information on the influence of adsorption pH. The role of pH remained hidden in the 14% of nonextracted information from the original data set. While it is accepted that pH affects the mechanism and extent of adsorption interactions, the extracted 86% of variation by PCA indicated that it was not pH but rather the humic molecular structure to be the main driving force for the adsorption selectivity of s-triazines.

Role of Humic Structure on s-Triazines Adsorption. It has been previously shown that carboxylate groups of HA contribute to the adsorption of triazines by formation of either ionic or hydrogen bonds between humic COOH groups and basic N atoms in triazines (21). However, no significant correlation was found here between the number of COOH groups in humic samples and s-triazines adsorption. In fact, C-HA, with the largest adsorbing capacity of all original humic materials, contained an amount of carboxylate groups as low, if not lower, as other original samples. Because the significantly increased adsorbing capacity of hydrolyzed HA was accompanied by an enhanced phenolic acidity, the adsorption of s-triazines on humic matter seems to be rather correlated with humic phenolic groups. In particular, the most adsorptive C-HA-H and P-HA-H materials showed the largest phenolic acidity. However, the variation in s-triazines adsorption can not be explained only by the number of phenol groups, since the largest phenolic acidity (**Table 1**) was found for P-HA that resulted as the least adsorbing material for atrazine and simazine.

Other studies (8, 12, 18, 22) hypothesized hydrophobic interactions to play a key role in binding s-triazines to humic substances. In fact, these poorly soluble herbicides increase solubilization through partitioning into humic hydrophobic

Table 6. Semiquinone Content for HAs from Oxidized Coal before (C-HA) and after Hydrolysis (C-HA-H) and Their Adducts with Atrazine at Different pH Values

pH	atrazine (ppm)	C-HA		C-HA-H	
		semiquinones (10^{17} g^{-1})	line width (G) ^a	semiquinones (10^{17} g^{-1})	linewidth (G) ^a
3.0	0	27.5 ± 1.0	7.2 ± 0.2	38.8 ± 2.4	7.5 ± 0.2
	1	26.3 ± 1.2	7.2 ± 0.2	43.1 ± 2.2	7.3 ± 0.2
	5	25.7 ± 1.1	7.2 ± 0.2	47.1 ± 2.6	7.3 ± 0.2
	10	25.4 ± 1.2	7.2 ± 0.2	46.0 ± 2.3	7.3 ± 0.2
	25	30.9 ± 1.5	7.2 ± 0.2	45.2 ± 2.3	7.2 ± 0.2
3.5	0	30.3 ± 3.0	7.5 ± 0.2	41.8 ± 2.6	7.5 ± 0.2
	1	25.8 ± 2.2	7.3 ± 0.2	48.4 ± 2.8	7.3 ± 0.2
	5	27.5 ± 2.5	7.5 ± 0.2	47.0 ± 2.8	7.3 ± 0.2
	10	27.7 ± 2.3	7.6 ± 0.2	48.7 ± 3.0	7.5 ± 0.2
	25	26.0 ± 2.2	7.3 ± 0.2	49.8 ± 3.3	7.6 ± 0.2
4.0	0	27.0 ± 2.2	7.3 ± 0.2	40.9 ± 2.5	7.2 ± 0.2
	1	32.3 ± 2.9	7.5 ± 0.2	45.3 ± 2.7	7.7 ± 0.2
	5	31.3 ± 2.6	7.3 ± 0.2	49.0 ± 2.9	7.5 ± 0.2
	10	29.8 ± 2.6	7.9 ± 0.3	45.8 ± 2.6	7.3 ± 0.2
	25	25.0 ± 2.0	7.2 ± 0.2	39.5 ± 2.5	6.8 ± 0.2
4.5	0	24.9 ± 2.1	7.5 ± 0.2	40.0 ± 2.6	7.5 ± 0.2
	1	21.5 ± 1.8	7.5 ± 0.2	50.6 ± 3.6	7.5 ± 0.2
	5	23.0 ± 1.8	7.3 ± 0.2	48.9 ± 3.2	7.5 ± 0.2
	10	26.4 ± 2.1	7.5 ± 0.2	49.3 ± 3.3	7.5 ± 0.2
	25	24.3 ± 1.9	7.2 ± 0.2	49.3 ± 3.4	7.5 ± 0.2

^a 1 gauss (G) = 10^{-4} tesla (T).

domains (3), whereby they may be further stabilized by hydrogen bonding (23). In agreement with this, an increase of K_f values by a factor of 1.2–1.7 was observed here for the s-triazines adsorption on hydrolyzed P-HA-H, a material whose hydrophobic character (see HI/HB ratio in Table 2) was enhanced by acid hydrolysis. Nevertheless, the most absolute s-triazines adsorption was observed for C-HA and C-HA-H samples (Tables 3–5 and Figure 3). In fact, C-HA showed the largest hydrophobicity (lowest HI/HB ratio in Table 2) among the three original substrates. However, although the hydrophobicity of hydrolyzed C-HA-H was comparable to that of L-HA-H, it was still much lower than for P-HA-H (Table 2), thereby making it difficult to reconcile their different adsorption capacity only to hydrophobicity. Thus, it appears that the strong adsorption capacity of C-HA and L-HA, as well as that of their hydrolyzed derivatives, should also be explained by their concomitant large content of aromatic carbon (Table 2). This implies that, besides the dispersive forces due to general hydrophobicity, the adsorptions of s-triazines on humic samples at higher pH must be explained with π – π interactions between respective aromatic rings.

The electron-transfer interaction, sometimes referred to as charge-transfer bonding, is reported to occur between the electron-donor triazine rings and the electron-acceptor semiquinone and quinone structures of humic substances. While some findings indicated these interactions to be more important than ionic bonding (8), other results exclude this possibility (10, 24).

Here, ESR spectroscopy was used to compare the amount of semiquinone content in (C-HA)- and (C-HA-H)-atrazine complexes for the most adsorbing C-HA and C-HA-H humic materials. A significant enhancement of stable free radical content was observed for C-HA added with 25 ppm of atrazine at pH 3 (Table 6). A significant increase of stable free radicals with respect to control was also detected, regardless of pH, for all adsorption complexes formed between hydrolyzed C-HA-H and atrazine, except for 25 ppm of atrazine at pH 4. Such an increase in semiquinone radicals suggests the occurrence of an electron-transfer binding mechanism between the two reacting substrates (8, 10).

Our results suggest that the charge-transfer complexation between atrazine and C-HA is a function of humic conformational structure, as stabilized by pH. In fact, charge-transfer complexes with C-HA occurring at pH 3 only at the largest atrazine concentration (25 ppm) may be explained with the tight association of humic molecules by the abundant intermolecular hydrogen bonds existing at this pH and a consequent low availability of aromatic structures at humic surfaces. When pH was increased, intermolecular hydrogen bonds among humic molecules were reduced, thereby favoring dissociation of most acidic C-HA groups. The resulting negatively charged hydrated surfaces of humic matter should have reduced ionic-exchange interactions with atrazine. This may have limited its close approach to humic aromatic components and the importance of charge-transfer bonds. Concomitantly, less specific hydrophobic interactions of atrazine with the abundant alkyl components of humic matter may have been favored (25). A conformational change induced by pH and the consequent mutual rearrangement of hydrophobic and hydrophilic domains in HA are in line with previous results (10, 26, 27) and the concept of supramolecular structure that is currently advocated to explain the physical–chemical properties of humic matter (28).

Moreover, when atrazine interacted with hydrolyzed C-HA-H, a general increase in free radical content was observed at all pH values. This suggests that the conformational stability reached during hydrolysis, and characterized by enhanced carbon content, hydrophobicity, and aromaticity, may have reduced the importance of hydrophilic domains in atrazine adsorption and concomitantly favored a closer proximity between the atrazine and the more abundant aromatic components in the hydrolyzed sample.

This study shows that the adsorption mechanism of s-triazines on humic substances, rather than being controlled by solution pH, is more strongly influenced by the nature of s-triazines and the association of humic components. At low pH, while s-triazine adsorption is expected to occur by ion exchange, this mechanism could not be directly justified by the content of acidic carboxyl groups in humic samples. At higher pH, our findings suggest that the content of alkyl and aromatic humic components is mostly responsible for the adsorption of s-triazines. A contribution of charge-transfer interactions between the triazine ring and the semiquinone structure in humic substances is likely to occur when their conformational structures are prevalently controlled by aromatic components. On the basis of these results, it may be inferred that either natural or recycled (e.g., compost) humic matter particularly rich in aromatic and alkyl carbon is likely to enhance s-triazines adsorption on soil and limit both their vertical mobility in the profile and further risk of environmental contamination.

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