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Influence of Humic Acid on Adsorption and Desorption of Atrazine, Hydroxyatrazine, Deethylatrazine, and Deisopropylatrazine onto a Clay-Rich Soil Sample

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Adsorption and desorption properties of atrazine and some of its metabolites, hydroxyatrazine (AT-OH), deethylatrazine (DEA), and deisopropylatrazine (DIA), were studied with a clay-rich soil sample (clay content of 53%). A part of this soil was treated with humic acid (Soil-HA) to assess the influence of this important component of natural organic matter on adsorption and desorption processes. This study was performed using the batch approach with 1.0 g of soil, or Soil-HA, in 5.0 mL of 0.010 mol L^{-1} $CaCl_2$ solution containing the herbicide and the metabolites in a concentration range between 0.010 and 5.0 mg L^{-1}. After 24 h of contact time, the suspensions were centrifuged and the four compounds were quantified in the supernatant phases by high-performance liquid chromatography. The adsorption and desorption data of both Soil and Soil-HA were properly fitted by the linearized Freundlich equation. For the untreated soil, the adsorption affinity order evaluated as a function of the K_f values was AT-OH > AT > DIA > DEA, while desorption followed the order DEA > DIA ∼ AT > AT-OH. The presence of humic acid increased significantly the adsorption of all compounds, following the same affinity order observed for the untreated soil. Increase in adsorption was especially high for AT-OH and AT. On the other hand, the dealkylated metabolites, DEA and DIA, were more easily desorbed from the Soil-HA sample, suggesting that natural organic matter facilitates the leaching of these compounds. Desorption order in the presence of humic acid was DEA > DIA > AT > AT-OH.

KEYWORDS: Atrazine; atrazine metabolites; soil; humic acid; adsorption

INTRODUCTION

Atrazine (2-chloro-4-(ethylamino)-6-isopropylamine-s-triazine) is a selective herbicide widely used around the world in the pre- and postemergence, commonly in agricultural soils, and forestry applications. Because of the widespread use of atrazine to control annual grasses and broad-leaved weeds in crops such as sugar cane, maize, soybean, citrus fruits, as well as in railways, roadside verges, and golf courses, great attention has been given to its environmental impacts and monitoring (*1*−*8*). Atrazine and its metabolites have been detected in surface and groundwaters (*5*, *9*−*11*). Although the toxicological effects of atrazine and other triazines on humans are weaker than reported for chlorinated and organophosphorus pesticides, severe environmental problems can result from their persistence in soils and sediments, as well as their runoff to surface and groundwaters. A recent paper suggested that atrazine disrupts frog development at exposure levels lower than those found in natural waters (*12*).

The soil organic matter (SOM) has been considered the main soil component that controls the persistence, bioavailability, degradation, leachability, and volatility of herbicides (*8*, *13*−*15*). A complex mixture of carboxylic acids, amino acids, peptides, mono- and polysaccharides, and humic substances constitutes the soil organic matter, but the major constituents are the humic substances, which are comprised by humic and fulvic acids, and humin (*13*). The mechanisms involved in the association of atrazine with humic substances are proton transfer (*16*−*18*), hydrogen bonding, London−van der Waals forces, and formation of coordination complexes with a metal cation (*13*, *14*, *19*). Protonation is more pronounced for s-triazines with high pK_a values such as prometryne (pK_a 4.05) and prometone (pK_a 4.28) (*20*).

The adsorption of atrazine and some metabolites in soils, sediments, and clay minerals have been reported in the literature (*21*−*24*). Deethylatrazine (DEA) and deisopropylatrazine (DIA)

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Table 1. Solubility and pK_a of AT, AT-OH, DEA, and DIA[a]

	water solubility (μmol L^{-1})	pK_a	K_d (L kg^{-1})			
compound	ref *25*	ref *29*	ref *24*	ref *23*	ref *23*	ref *21*
AT	153	1.71	2.56	3.71	2.33	39.6
ATOH	35.5	5.15	132	11.85	8.0	135
DEA	1810	1.65	1.53	2.34	1.66	21.6
DIA	3740	1.58	3.04	4.18	3.24	135

[a] Some literature K_d values for partitioning of the chemicals with soils and sediments are also given.

are formed by biodegradation, but DEA is the metabolite of major concern, since it is considered as toxic as atrazine, while DIA is about 3 to 4 times less toxic (*2*). Furthermore, the water solubility of DEA and DIA are higher than atrazine (**Table 1**), facilitating the leaching of both compounds (*25*). Hydroxyatrazine (AT-OH) is formed by either chemical reactions in the soil or biodegradation, especially in the presence of high concentrations of fulvic acids and pH < 6 (*13*). The low water solubility of AT-OH and its high pK_a (5.15) favor the adsorption, even in soils with low content of organic matter. Besides, AT-OH and didealkylatrazine (DDA) are considered nonphytotoxic degradation products (*2*). DEA, DIA, and AT-OH are often detected in groundwaters (*5*, *9*, *26*), so that the understanding of their behavior in soils is of prime importance.

In a recent study, Krutz et al. (*23*) compared the adsorption and desorption of AT, AT-OH, DEA, and DIA in vegetated filter strip and cultivated soils, with both samples having very similar mineral composition but different contents of natural organic carbon. The purpose of the present paper is to study the adsorption of atrazine and its main metabolites in a rich-clay tropical soil sample, with low content of natural organic carbon, and to assess the influence of humic acid, in both adsorption and desorption processes, using the batch equilibration technique. Different from papers previously presented on this subject comparing different soils, a univariate approach was utilized, that is, the mineral composition was kept the same while varying only the organic carbon content from a single source of humic acid.

MATERIALS AND METHODS

Soil Sample. The soil sample was collected at the experimental farm of the Escola Superior de Agricultura Luiz de Queiroz da Universidade de São Paulo (ESALQ-USP) in the Piracicaba municipality, São Paulo state, Brazil, in an area with no history of application of herbicides. Fifteen surface samples were collected at depths between 0 and 20 cm from four different points and mixed to form a composed sample. The soil was air-dried and gently ground with a pestle and mortar to pass in a 1.0-mm sieve. The sieved sample was further dried in a vacuum oven at 35 °C until constant weight, a process that required approximately 48 h, and finally stored in a desiccator. Kaolinite is the dominant clay in this soil, with some contributions of sesquioxides (*27*). The soil properties are summarized in **Table 2**.

Table 2. Properties of the Studied Soil

CEC ($mmol_c$ dm^{-3})	pH[a]	sand (%)	silt (%)	clay (%)	total surface area (m^2 g^{-1})	total organic carbon (%)
185.2	5.2	29	18	53	49.3 ± 0.07	1.58 ± 0.04

[a] In 0.010 mol L^{-1} $CaCl_2$.

Humic Acid and Soil Preparation. A mass of 15 g of sodium salt humic acid from Aldrich was treated with 100 mL of 0.1 mol L^{-1} NaOH solution, previously degassed with N_2, and kept under N_2 bubbling for 30 min to minimize the humic acid oxidation. The suspension was centrifuged at 1000*g* for 30 min and the humic acid was precipitated by acidification with 6 mol L^{-1} HCl until pH 1. The resulting suspension was centrifuged and the humic acid was washed several times with deionized water until no Cl^- was detected in the washing water, which was verified by precipitation with $AgNO_3$ in acidic medium, that is, $[Cl^-] < 10^{-5}$ mol L^{-1}. Separation of the washing waters was made by centrifugation at 1000*g* for 15 min. The humic acid was resuspended with deionized water in a 250-mL volumetric flask, and the concentration was determined by the dry weight of 1.00-mL aliquots, taken just after the suspension was homogenized. The pH of the resulting suspension was 2.5 and the final concentration, in an ash free basis, was 28.0 g L^{-1}. The CHN composition was 49.7 ± 0.1% C; 4.3 ± 0.1% H; 0.65 ± 0.02% N; and 8.0 ± 0.5% ash.

The pH of the humic acid suspension was adjusted to 5.2 with 2.0 mol L^{-1} KOH solution. This pH was chosen to match the pH of a slurry containing 1.0 g of soil in 5.0 mL of 0.010 mol L^{-1} $CaCl_2$ solution. Thirty-five milliliters of the humic acid suspension (adjusted to pH 5.2) were added to 30 g of the sieved soil under constant agitation in a mortar. The slurry was stirred in a horizontal shaker for 6 h. After this time, 10 mL of deionized water were added to the mixture, which was then swirled in a horizontal shaker overnight. The aqueous phase was evaporated in an open flask and the resulting solid was dried in a vacuum oven at 35 °C for 48 h (until constant weight). The resulting modified soil was stored in a desiccator. The CHN composition was 3.3 ± 0.2% C; 1.03 ± 0.02% H; and 0.18 ± 0.01% N.

Apparatus and Reagents. A LC 9A Shimadzu high-performance liquid chromatograph (HPLC), equipped with an SPD 6 AV UV detector, and the LC Workstation Class-LC 10 software were used in all experiments for separation and quantification of atrazine and its metabolites. A SB C-18 Zorbax—HP column (3.5 μm, 150 × 4.6 mm) connected to a C-18 Phenomenex guard column was used. Sample injection was made with a rotary Rheodyne valve using a 20-μL sample loop.

A Metrohm 654 potentiometer (precision of 0.1 mV or 0.001 units of pH), coupled to a Ag/AgCl combination glass electrode, was utilized for all the pH measurements.

The analytical standards of AT, DEA, DIA, and AT-OH were supplied by Riedel-de Haën. Stock solutions (1000 mg L^{-1}) were prepared in methanol (HPLC grade). AT-OH was previously dissolved in 1 mL of 1.0 mol L^{-1} HCl solution and diluted with methanol. These standards, solids or solutions, were stored in a freezer at −18 °C.

Acetonitrile and methanol (HPLC grade) were purchased from J. T. Baker. Deionized water (Nanopure II Sybron Barnstead, Dubuque, IA) was used to prepare all solutions. Sodium salt of humic acid was purchased from Aldrich (catalog H1-675-2, lot 01816 HH). All other reagents used in this work were of analytical grade from Merck, Aldrich, or Sigma.

Adsorption and Desorption Experiments. A mass of 1.0 g of the dried soil, or humic acid treated soil (Soil-HA), was transferred to 12 glass centrifuge tubes with capacity of 10 mL. Suitable volumes of 0.010 mol L^{-1} $CaCl_2$ and of the stock solutions containing atrazine and metabolites (also in 0.010 mol L^{-1} $CaCl_2$) were added to the centrifuge tubes providing a total volume of 5.00 mL in each tube and the following total concentrations of atrazine and metabolites: 0.010, 0.025, 0.050, 0.10, 0.25, 0.50, 1.0, 2.5, and 5.0 mg L^{-1}. Since these concentrations were obtained by proper dilution of a stock solution containing the four compounds dissolved in pure methanol, the maximum methanol fraction in the adsorption tubes was 0.5% (v/v). A blank experiment in which no atrazine and metabolites were added to the soil suspension was also performed. The 1:5 soil-to-solution ratio follows the OECD guidelines (*28*). In parallel, an analytical curve was constructed using the standards of atrazine and metabolites at concentrations 0.01; 0.05; 0.25; 1.00; and 5.00 μg mL^{-1} in 0.010 mol L^{-1} $CaCl_2$. These standard solutions were prepared in glass centrifuge tubes similar to the ones used in the adsorption study. All centrifuge tubes (used for adsorption, blank, and analytical curve) were sealed, protected from light, and shaken in a horizontal shaker for 24 h. The suspensions were centrifuged at 1100*g* for 15 min and 3.50 mL of the supernatants were withdrawn with an automatic pipet and filtered in 0.45-μm

Durapore membrane Millex from Millipore. The filtered solutions were analyzed by HPLC.

The remaining materials inside the centrifuge tubes were used to evaluate desorption (*7*). First, 3.50 mL of 0.010 mol L^{-1} $CaCl_2$ were added to the tubes and the soil was resuspended. The tubes were sealed again, protected from light, and shaken for another 24 h.

The laboratory temperature during the contact time was kept at 25 ± 2 °C. The pHs of the suspensions after 24 h were between 5.1 and 5.2. All the experiments were carried out in duplicate.

HPLC Analyses. Acetonitrile and deionized water were previously filtered using a 0.45-μm PTFE Millipore filter. Two solutions constituted the mobile phase:

Solution A: 90% acetonitrile and 10% deionized water in 2.5 mmol L^{-1} ammonium acetate−acetic acid buffer with pH 4.5.

Solution B: 2.5 mmol L^{-1} ammonium acetate−acetic acid buffer with pH 4.5.

Helium was used as the degassing gas. The optimized pumping conditions under a flow rate of 1.2 mL min^{-1} were: 0−4 min, isocratic mode (22% A and 78% B); 4−9 min, linear gradient to 100% A; and 10−18 min, isocratic mode (22% A and 78% B). The UV detector monitored the absorbance at 220 nm. Before injection, standards and samples were buffered as the mobile phase. Samples with expected concentrations higher than the most concentrated standard (5.00 μg mL^{-1}) were diluted with 0.010 mol L^{-1} $CaCl_2$ solution.

The separation of the four compounds was easily achieved with retention times of 3.25, 4.05, 5.86, and 9.83 min for DIA, AT-OH, DEA, and AT, respectively. Detection limits for AT, AT-OH, DEA, and DIA were 0.8, 0.9, 0.7, and 0.3 μg L^{-1}, respectively. The quantification limits of AT, DEA, and DIA were 4 μg L^{-1}, which correspond to 18, 21, and 23 nmol L^{-1}, respectively. For AT-OH, the quantification limit was 6 μg L^{-1} (30 nmol L^{-1}). The higher quantification limit for AT-OH is due to its lower coefficient of molar absorptivity at 220 nm (*29*). Quantification was performed using calibration plots obtained under similar solution conditions used in the adsorption/desorption studies. The five calibration standards were maintained 24 h under shaking prior to constructing the analytical curve, but no significant peak area differences were observed in comparison to the ones obtained with freshly prepared standards. This fact indicates that there is no evidence of adsorption on the tube walls or compound degradation during the adsorption experiment time.

Solutions 0.10 or 1.00 μg mL^{-1} of AT, DEA, DIA, and AT-OH were analyzed in the absence and in the presence of blank extracts of the Soil-HA sample, revealing recoveries between 94 and 98%, which indicates that a cleanup step was not necessary.

Data Treatment. Adsorption data were treated by the linearized Freundlich equation:

$$\log\left(\frac{x}{m}\right) = \log K_f + \left(\frac{1}{n}\right)\log C \qquad (1)$$

where x/m is μmol of atrazine, or metabolite, adsorbed per kg of soil; C is the solution concentration of the herbicide or metabolite, and K_f and $1/n$ are empirical constants related to adsorption. The adsorption study was performed using a mixture of AT, DEA, DIA, and AT-OH, so that the K_f and $1/n$ parameters may be liable to competition effects among the chemicals for the adsorption sites. Wang et al. (*3*) reported that no competition between AT and AT-OH was observed onto a Laurentian soil (at an AT-OH to AT ratio of 0.5). On the other hand, Xing et al. (*30*) reported competition effects between prometone (PR) and AT for adsorption sites of a Cheshire soil at the PR to AT ratio of 5 or 10. This competition effect lowered the K_f and increased the $1/n$ parameters of atrazine adsorption.

RESULTS AND DISCUSSION

Contact Time. To define the contact time effect, 5.0 mL of mixtures of AT, AT-OH, DEA, and DIA at concentrations of 0.50 mg L^{-1} in medium of 0.010 mol L^{-1} $CaCl_2$ were shaken with 1.0 g of unmodified soil for periods of 2, 4, 8, 12, 16, 20, and 24 h. The solution concentrations decayed quickly during the first 2 h of contact time (**Figure 1**), reaching an apparent equilibrium after 24 h, so that this time was defined to perform the adsorption and desorption experiments. However, the true equilibrium can take much longer times to be reached (*31*). In previous papers, Pignatello and Xing (*31*) and Lesan and Bhandari (*32*) reported a rapid initial sorption of the organic solutes, which has been attributed to adsorption of the solute to mineral surfaces or partitioning into a "rubbery" fraction of the soil organic matter, while the slow sorption is believed to result from diffusion of solute into soil micropores or into highly cross-linked regions of the soil organic matter. Thus, the K_f and $1/n$ parameters reported in the present work are operationally defined for a contact time of 24 h, an approach that is valid for the comparison purposes, and has been used by other authors (*23*) as well.

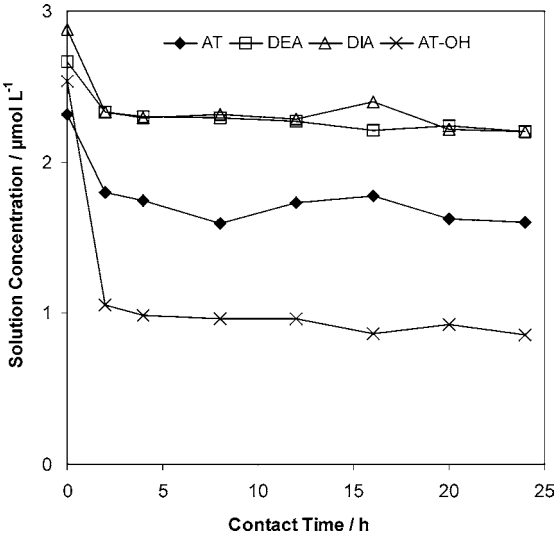

Figure 1. Effect of contact time on adsorption of atrazine and metabolites onto the unmodified soil sample. The initial concentration of all chemicals was 0.50 mg L^{-1} in medium of 0.010 mol L^{-1} $CaCl_2$.

Adsorption onto the Unmodified Soil Sample. The adsorption isotherms of AT and metabolites onto both soil samples were not linear, even in the low range of solution concentrations (**Figure 2**). As a consequence, the distribution coefficient (K_d), defined as $(X/m)/C$, is not constant, decreasing as the initial concentration of the chemicals increases. In the range of initial concentrations studied, the K_d values decreased from 9.0 to 1.5 L kg^{-1} for AT, from 23 to 5.4 L kg^{-1} for AT-OH, from 4.2 to 0.80 L kg^{-1} for DEA, and from 5.5 to 1.2 L kg^{-1} for DIA. These ranges are within the mean values reported in the literature for other soils (**Table 1**), with exception of those reported by Mersie and Seybold (*21*). However, nonlinearity occurs because the affinity for solute decreases progressively with the increase of solute concentration as adsorption sites become occupied. This behavior is characteristic of sorption processes arising from heterogeneous site-specific interactions (*30*) instead of the partition model, leading to values of $1/n < 1$. Nonlinearity is inconsistent with the partition model if all sorption in the soil is attributed only to the soil organic matter. Natural particles such as those composing soils, usually contain organic and mineral components, so that partitioning to organic matter may occur simultaneously to adsorption at water−organic or water−mineral interfaces (*30*), leading to nonlinear behavior as observed in the present work (**Figure 2**).

The linearized form of the Freundlich equation described very well the adsorption, since good correlation coefficients ($r^2 > 0.99$) were obtained for all compounds studied. The K_f and $1/n$ values obtained from the linearized Freundlich equation are

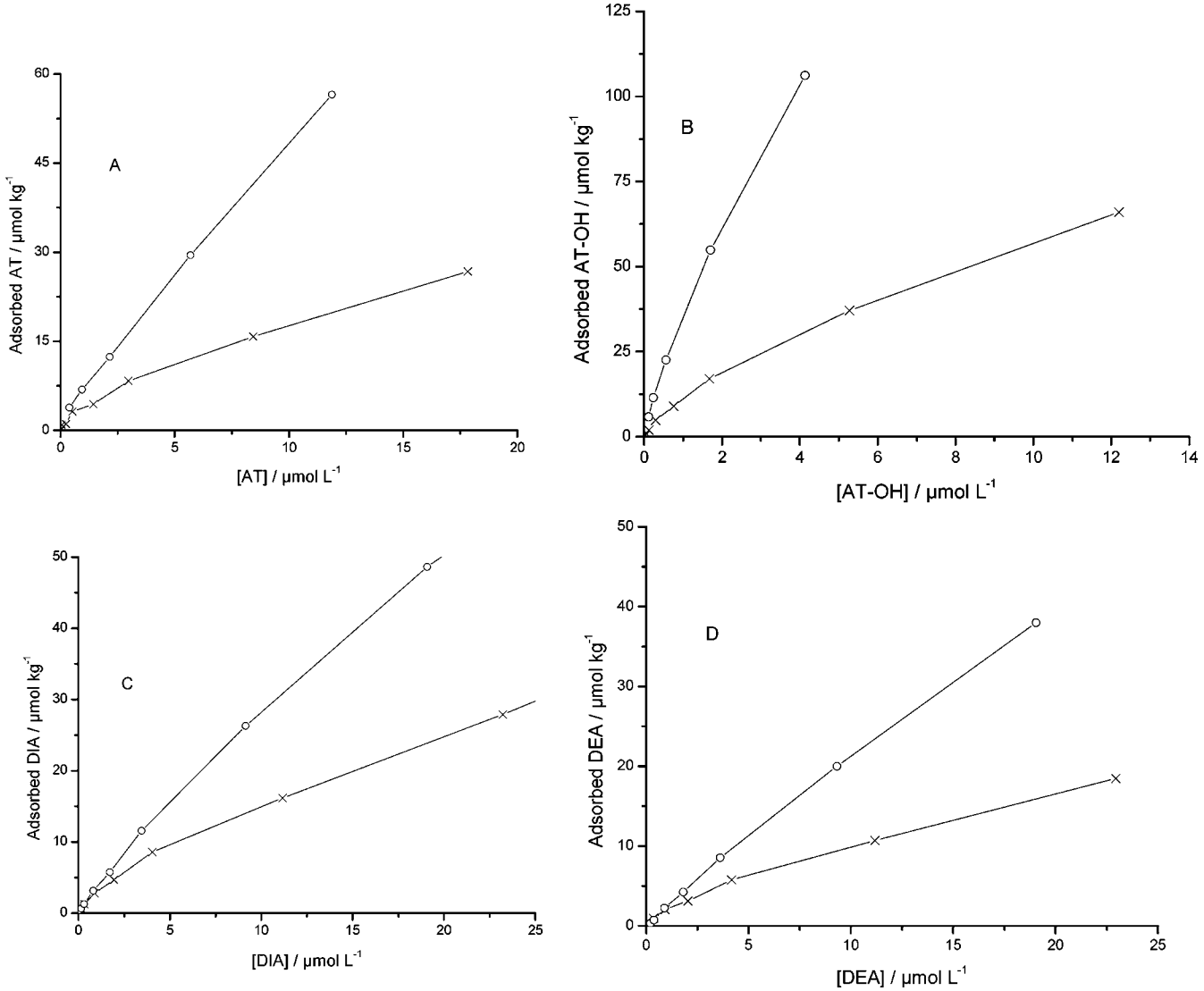

Figure 2. Adsorption isotherms of atrazine (**A**), hydroxyatrazine (**B**), deisopropylatrazine (**C**), and deethylatrazine (**D**) onto unmodified Soil (×) and Soil-HA (○) samples. Experiments were performed at 25 ± 2 °C in ionic medium of 0.010 mol L^{-1} $CaCl_2$.

Table 3. Adsorption and Desorption K_f ($\mu mol^{1-1/n}$ $L^{1/n}$ kg^{-1}) and 1/*n* Parameters Obtained from the Linearized Freundlich Equation for Unmodified Soil and Humic Acid Soil (Soil-HA)[a]

herbicide/ metabolite	param-eter	adsorption		desorption	
		soil	soil-HA	soil	soil-HA
AT	K_f	3.6 ± 0.2	6.9 ± 0.9	5.5 ± 0.2	8.2 ± 1.7
	1/*n*	0.73 ± 0.01	0.84 ± 0.03	0.74 ± 0.02	0. 8 ± 0.1
	r^2	0.994	0.993	0.995	0.988
AT-OH	K_f	11.1 ± 0.5	36.0 ± 1.9	14.2 ± 2.1	32.0 ± 1.5
	1/*n*	0.74 ± 0.01	0.79 ± 0.02	0.78 ± 0.11	0.89 ± 0.02
	r^2	0.997	0.999	0.998	0.999
DIA	K_f	2.6 ± 0.1	3.7 ± 0.2	4.6 ± 0.5	5.4 ± 0.4
	1/*n*	0.79 ± 0.02	0.88 ± 0.05	0.78 ± 0.02	0.90 ± 0.08
	r^2	0.995	0.999	0.998	0.998
DEA	K_f	1.75 ± 0.06	1.98 ± 0.09	3.2 ± 0.1	3.4 ± 0.3
	1/*n*	0.778 ± 0.008	1.06 ± 0.08	0.82 ± 0.01	1.12 ± 0.07
	r^2	0.993	0.996	0.993	0.990

[a] The results are average of two adsorption isotherms.

shown in **Table 3**. The adsorption coefficients, K_f, decreased in the order: AT-OH > AT > DIA > DEA, in agreement with the results previously reported by Mersie and Seybold (*21*) (**Table 1**), who studied the adsorption/desorption of atrazine and metabolites onto a wetland soil with organic carbon content of 9% (57% silt, 37% clay, 6% sand). Despite the similar order, the K_f values found by these authors are significantly larger than the K_f values found in the present work, in which a soil much richer in sand (29%) and clay (53%) but with lower C content (1.58%) was studied. The greater adsorption observed in the wetland soil studied by Mersie and Seybold (*21*) may be explained by the significantly higher content of organic matter in comparison to the agricultural soil studied in this paper.

At an initial concentration of 10 μg L^{-1} AT-OH, this metabolite is removed from solution to concentrations below the detection limit of the method (0.9 μg L^{-1}), with the removal decreasing to 52% for an initial concentration of 5.0 mg L^{-1}. For very low total concentrations (10 μg L^{-1}), the removal of AT is more significant (~60%) than DIA (52%), although for higher concentrations this order is reversed. Deethylatrazine is the less adsorbed species along all the concentration range studied, decreasing from 45 to 13% as the initial concentration increased from 10 μg L^{-1} to 5.0 mg L^{-1}. This adsorption process may be explained by the interaction of the compounds with the mineral phases of the soil, since several authors (*33*−*35*) have verified that clay minerals can also play an important role on adsorption of herbicides onto soils. The dominant clay in the studied soil is kaolinite, which is a 1:1 clay mineral that has

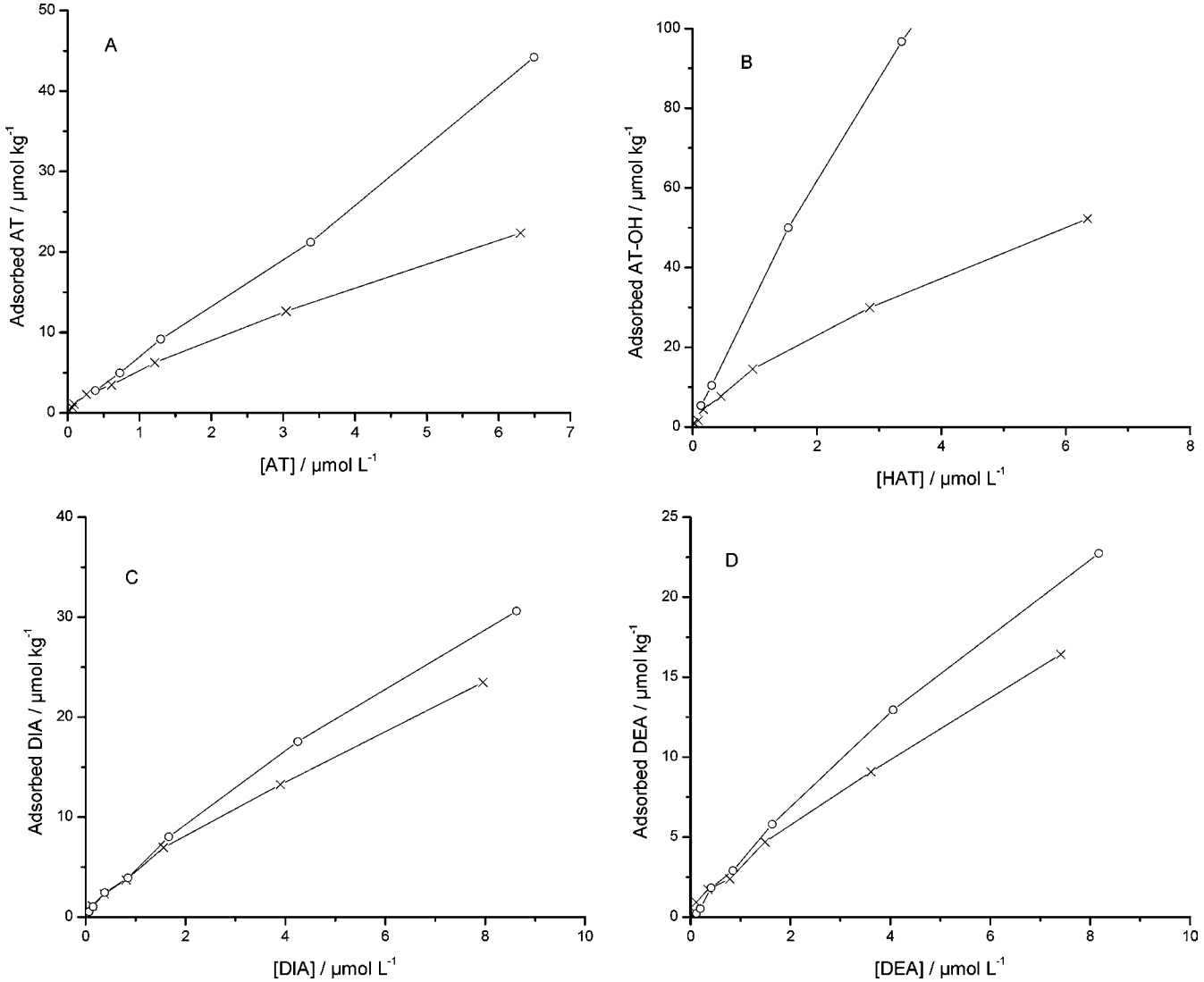

Figure 3. Desorption isotherms of atrazine (**A**), hydroxyatrazine (**B**), deisopropylatrazine (**C**), and deethylatrazine (**D**) from unmodified Soil (×) and Soil-HA (○) samples. Experiments were performed at 25 ± 2 °C in ionic medium of 0.010 mol L^{-1} $CaCl_2$.

lower adsorption capacity than 2:1 clays such as smectites (*36*). On the other hand, besides the presence of sesquioxides in this soil sample, Melo et al. (*37*) verified that kaolinite crystals in Brazilian soils contain high concentrations of Fe and are generally poorly crystalline. Much of this Fe is probably substituting for Al in the octahedral sheet, a composition that favors the formation of protonated AT and metabolites in the interlayer water, enhancing the adsorption by electrostatic interactions (*36*, *38*).

Adsorption onto the Humic Acid Modified Soil (Soil-HA). Since atrazine can bind to both the solid phase and the soluble fraction of humic acid (*13*, *16*−*20*, *39*), a first step in this study was to evaluate the fraction of humic acid that remained in solution under the experimental conditions used in the adsorption studies, that is, in the presence of 0.010 mol L^{-1} $CaCl_2$ and pH 5.2. This was performed by comparison of the absorbance (at 400 nm) of the solution obtained after centrifuging the soil suspension with solutions of known concentration of humic acid. This experiment revealed that about 99.4% of the humic acid initially added to the soil remained incorporated to the solid phase, either adsorbed to the soil or precipitated. Under the experimental conditions studied, the HA may precipitate by two processes: partial protonation of ionizable sites in pH 5.2 and formation of the insoluble Ca-humate in the presence of 0.010 mol L^{-1} $CaCl_2$.

The key role of natural organic matter on the adsorption of AT, DEA, DIA, and AT-OH by the soil is evidenced in **Figure 2**. An increase in the adsorption capacity in the presence of humic acid is confirmed when comparing the K_f values obtained for Soil-HA and Soil samples (**Table 3**). Increasing the organic carbon content from 1.58 to 3.3% increased the K_f value for AT-OH by a factor of 3.2 (**Table 1**). For AT, DIA, and DEA this factor was 1.9, 1.4, and 1.1, respectively. Adsorption of AT and metabolites onto the humic acid was not studied in the present work, but some authors have demonstrated that the adsorption capacities of the modified solid (soil or clay) are usually lower than the sum of adsorption capacities of the isolated untreated soil and the humic acid (*3*, *40*). This feature has been attributed to aggregation, "self-biding" and partial blockage of adsorption sites by the organic matter (*3*, *40*).

Results in **Table 3** show a small increase in the 1/*n* values for the Soil-HA sample, denoting an enhanced affinity between the chemicals and the solid phase (increase in the adsorption slope) in comparison to the untreated soil. However, for AT-OH and AT, the 1/*n* parameters obtained for the Soil-HA sample were not computed using the same concentration range as used for the Soil sample, since at low initial concentrations (up to

100 μg L^{-1}) the resulting solution concentrations were below the quantification limits, a fact that was not observed for the Soil sample. The linearized Freundlich equation does not compute these adsorption points, and this fact should be taken in account when comparing 1/*n* values of similar magnitudes for AT, AT-OH, DEA, and DIA (**Table 3**), which might imply an erroneous interpretation of similar affinities for all the compounds studied. For DEA and DIA, all points of the adsorption curve were used in the computation (initial concentrations from 0.010 to 5.0 mg L^{-1}), since because of the less intense adsorption with both Soil and Soil-HA, all solution concentrations of these metabolites were higher than the quantification limits.

The presence of functional carboxylic and phenolic groups in the humic acid molecules facilitates the adsorption process for AT as a neutral compound by physical forces such as van der Waals or hydrogen bonding. The pH is a key factor to control the cationic character of the AT, a weakly basic herbicide, as well as the ionization degree of the humic substances. Atrazine has a pK_a 1.71, so that a small adsorption of this herbicide is expected in agricultural soils, since only at low pH the conjugate acid concentration would reach significant values, favoring the adsorption (*20*). The pH of the studied soil was 5.2, which is not favorable to protonate AT, as well as DEA and DIA, for which the pK_a values are 1.65 and 1.58, respectively (*29*). On the other hand, the adsorption of AT-OH, with a pK_a of 5.15, was significantly higher than the other compounds (**Table 1** and **Figure 1**), in agreement with McBride (*20*), who states that the optimal adsorption of an organic base on negatively charged soil colloids occurs near the pH numerically equal to the pK_a of the organic conjugate acid. Wang et al. (*41*) report that AT-OH binds more strongly than AT to fulvic acid, with adsorption maximum occurring at pH near 3.8 and 1.6, respectively. These features suggest that the protonation in bulk solution plays a major role controlling the adsorption of the AT-OH acid conjugate onto organic matter and permanent-charged clays under typical environmental pH conditions, unlike AT, DEA, and DIA, which have pK_a smaller than typical agricultural soil pHs, although these compounds may be partially protonated in the interlayer waters between clay sheets (*36*, *38*). Martin-Neto et al. (*18*) demonstrated that AT-OH readily forms electron-transfer complexes with humic substances, emphasizing that these complexes are the probable explanation for the strong adsorption of this metabolite to soil natural organic matter.

Desorption Experiments. Desorption isotherms related the amount of the chemical retained by the soil to the solution concentration (**Figure 3**) after 24 h of contact time. The curves were properly fitted by the linearized Freundlich equation, with all $r^2 > 0.98$ (**Table 3**). Larger desorption K_f values denote greater proportion of the chemical retained in the solid phase, so that the following desorption order can be depicted for the Soil sample: DEA > DIA ~ AT > AT-OH. For the Soil-HA sample, the desorption K_f values indicate the following desorption order: DEA > DIA > AT > AT-OH, differing between DIA and AT, with a significant increase in the binding capacity of AT. For the points in which adsorption of AT and AT-OH was very intense (up to initial concentrations of 100 μg L^{-1}), the solution concentrations for these compounds were also below the detection limits after 24 h of desorption.

The results obtained suggest that the incorporation of humic acid enhanced significantly the soil affinity for AT and AT-OH, showing a trend of immobilization of these compounds in conditions of low coverage (initial concentrations between 0.010 and 0.10 mg L^{-1}). In summary, the presence of humic acid decreases desorption of AT and AT-OH, especially AT-OH, in the time scale of these experiments.

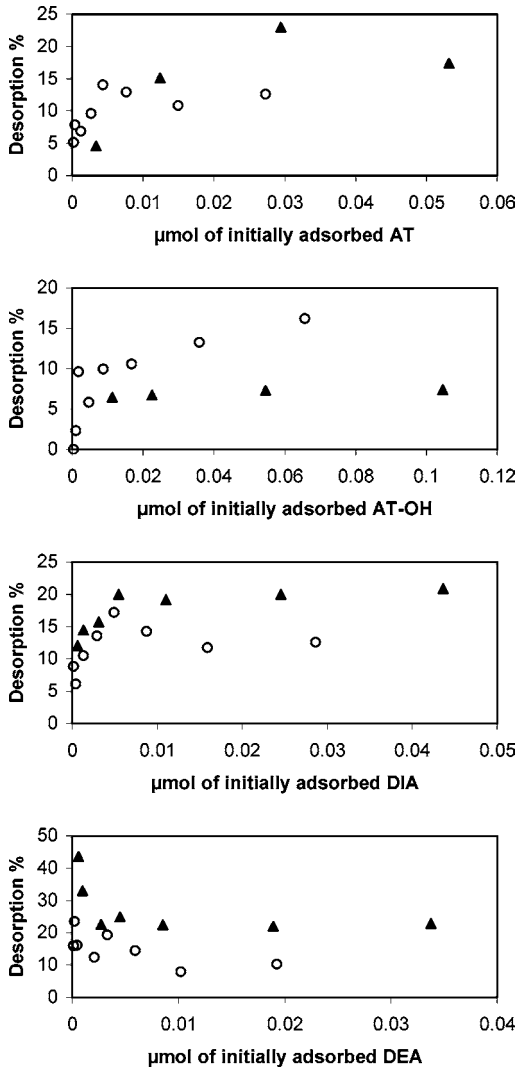

Figure 4. Desorption percentages as a function of the initially adsorbed amounts of AT, AT-OH, DIA, and DEA from Soil-HA (π) and unmodified Soil (○) samples.

For DEA and DIA, the desorption curves obtained for Soil and Soil-HA are quite coincident up to solution concentrations of about 2 μmol L^{-1}. No statistically significant variation of desorption K_f values were observed for desorption of DEA and DIA from both samples (**Table 3**), although at higher concentrations, the amounts of retained metabolites are higher in Soil-HA than in Soil (**Figure 2**). This is in agreement with Krutz et al. (*23*), who verified that the higher organic matter content of a vegetated filter strip soil compared to a cultivated soil may retard the transport of AT and AT-OH to surface and groundwaters but not the transport of DEA and DIA.

If desorption percentages are plotted as a function of the initially adsorbed amounts of compounds (**Figure 4**), one can observe that the desorption of AT-OH from the Soil-HA sample is systematically lower than from the Soil sample in all range of concentrations studied. Conversely, DEA and DIA have systematically larger desorption percentages from the Soil-HA sample, indicating that, despite the higher adsorption capacity of this material, these chemicals are more easily desorbed in comparison to the Soil sample. Desorption percentages of AT from Soil-HA are very low under conditions of low coverage but increase significantly as the amount of AT previously

adsorbed increases, becoming larger than observed for the Soil sample (**Figure 4**).

No sequential desorption steps were performed to verify if the chemicals can be exhaustively desorbed, but according to Moorman et al. (*7*) the release of the adsorbed AT occurs mainly in the first desorption step, although further small release of AT and metabolites also occurs during the successive desorption steps (*21*).

Environmental Implications. The AT-OH content in agricultural soils is frequently higher than the chloro-derivatives (*42*), a fact that can be explained by the more intense interaction between this compound and organic matter or mineral phases, as well as by its smaller water solubility, decreasing the desorption degree. Sorenson et al. (*43*) verified that AT-OH concentrations are higher in the top 10-cm soil layers, decreasing significantly in the layers at depths between 10 and 40 cm.

The results of our experiments indicate that DEA and DIA may be more easily leached from the topsoil than AT and AT-OH, even in the presence of high content of organic matter. The mobility of all compounds increases for the deeper layers of soils because of the smaller humus content. This fact, in addition to the greater chemical stability of DEA and DIA in comparison to AT, explains the frequent detection of these metabolites in groundwaters and surface waters (*5*, *10*, *11*). Attention should be given to monitoring these metabolites, mainly in tropical countries in which agricultural soils have low content of natural organic matter and favorable conditions for microbial degradation of AT.

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