

Adsorption of Glyphosate by Humic Substances[†]

A. Piccolo,* G. Celano,[‡] and P. Conte

Dipartimento di Scienze Chimico-Agrarie, Università di Napoli "Federico II", Via Università 100, 80055 Portici, Italy

Four humic substances were extracted from a peat (HA1) and a volcanic soil (HA2), an oxidized coal (HA3), and a lignite (HA4). The four humic materials presented distinct differences in their chemical and physical–chemical characteristics as assessed by chemical methods, ¹³C-NMR spectroscopy, and high-performance size exclusion chromatography. The interactions between these well-characterized and purified humic substances and the widely used glyphosate [*N*-(phosphonomethyl)glycine] herbicide were studied by means of adsorption isotherms. Adsorption of glyphosate was found to be surprisingly high and followed the order HA1 > HA2 > HA3 ≥ HA4. Humic extracts from soil adsorbed glyphosate even more than clay minerals, thereby indicating that the interactions with humic substances, in either a solid or dissolved form, are far more important than previously believed. Adsorption is explained by the multiple hydrogen bondings which can occur among the various acidic and oxygen-containing groups of both molecules. However, the order of adsorption did not simply follow the order of acidity but rather that of increasing aliphaticity and molecular size of humic substances. In fact, the least adsorbing humic materials from nonsoil sources showed a high content of aromatic structures and small molecular dimensions. These results reveal that the extent of glyphosate adsorption on humic substances varies considerably with their macromolecular structure and dimension and that is favored by a high degree of stereochemical flexibility combined with a large molecular size.

Keywords: *Adsorption; glyphosate; humic substances*

INTRODUCTION

Glyphosate, *N*-(phosphonomethyl)glycine, is the most extensively used herbicide in agriculture and other fields for the control of many annual and perennial weeds. One can find a sufficiently large literature on the chemistry of glyphosate, its mode of action, and its effects on plant metabolism (Malik et al., 1989), whereas relatively less information is available on the glyphosate behavior in soils (Torstensson et al., 1985). Glyphosate is reported to be rapidly inactivated in soil (Sprankle et al., 1975a). However, its inactivation appears not to be permanent since residual activity was found capable of injuring some plant species (Salazar and Appleby, 1982). Bowmer (1982) indicated that the amount of glyphosate left in solution in irrigation waters, after adsorption by deep silty sediments, was not insignificant. Glyphosate binding to soil was attributed to the phosphonic acid moiety reacting with polyvalent cations adsorbed on clay and, generally, on soil organic matter (Sprankle et al., 1975b; Hance, 1976). Other works have also related glyphosate adsorption to the clay content and the cation exchange capacity of the soils (Glass, 1987).

Detailed studies on the role of soil components on glyphosate adsorption were limited to cation-saturated clay minerals, and no investigations on the adsorbing capacity of humic substances have been previously reported in the literature, despite recognition that soil humic molecules adsorb most pesticides in soils (Weber and Miller, 1989). Furthermore, water-soluble humic substances are regarded as important carriers of organic

contaminants in the soil environment (Chiou et al., 1986). In fact, adsorption of xenobiotics to dissolved humic material may serve as a mechanism for enhanced mobility, even in homogeneous isotropic porous media (McCarthy and Zachara, 1989). Binding to humic substances has additional environmental implications, including effects on biodegradation, volatilization, hydrolysis, photolysis, and bioaccumulation of pollutants.

Recent spectroscopic investigations on the glyphosate–humic substances interactions have led Piccolo and Celano (1994) to demonstrate that glyphosate binds to humic substances through a hydrogen-bonding mechanism, and thus the herbicide may be adsorbed on or transported by humic substances. Moreover, glyphosate was found to be adsorbed on an iron–humic acid complex to a larger extent than that reported in the literature for clays and soils (Piccolo et al., 1995). This degree of adsorption was attributed to a ligand exchange mechanism whereby one or more hydroxyls of the iron hydration sphere can be exchanged by the glyphosate phosphono group.

The aim of this work was to investigate the interactions of glyphosate with humic substances. In particular, the extent of adsorption of the herbicide on purified humic substances extracted from different sources was assessed, and the effect of the humic substances' chemical properties on glyphosate adsorption was examined.

MATERIALS AND METHODS

Humic Substances. Humic substances were extracted from the following: (1) a volcanic soil (Typic Dystrandept) from the lake of Vico, near Rome; (2) a peat soil (Dystric Histosol) near Lucca; (3) an oxidized coal (Eniricerche, SpA); (4) a North Dakota Leonardite (Mammoth Chem. Co.). The extraction was carried out by shaking the original materials overnight in 0.5 N NaOH under a N₂ atmosphere by standard procedures (Stevenson, 1994). The humic acids (HAs) were precipitated

[†] Dipartimento di Scienze Chimico-Agrarie. Research Contribution 131.

[‡] Present address: Dipartimento di Produzione Vegetale, Università della Basilicata, Via Nazario Sauro, Potenza, Italy.

from the alkaline extracts by lowering the pH to 1 with 6 N HCl. The HAs were then purified by a double acid–base dissolution–precipitation procedure, a 0.5% (v/v) HCl–HF treatment for 36 h, and a dialysis in Spectrapore 3 tubes (3500 MW cutoff) against distilled water until chloride-free. The humic samples were then freeze-dried and stored in a desiccator. The four humic extracts (HA1, HA2, HA3, and HA4, respectively) were analyzed for their elemental content using a Perkin-Elmer 40C microanalyzer and for total, carboxylic, and phenolic acidities and ash content by standard methods (Stevenson, 1994).

Molecular Weight Distribution. Molecular weight distribution of humic materials was recorded with a high-performance size exclusion chromatography (HPSEC) system. The system consisted of a Perkin-Elmer LC-200 pump and a Perkin-Elmer LC-295 UV–visible detector, equipped with two columns, TSK G3000SW (600 mm) and TSK G4000SW (300 mm), in series, preceded by a TSK Guard-Column, and by a 0.2 μm inlet filter. Columns calibration was obtained by using globular proteins of known molecular weight (Pharmacia kit, 20–500 kDa). Elution was performed with 0.06 N NaNO_3 at pH 7 containing 0.3 $\text{g}\cdot\text{L}^{-1}$ NaN_3 . Freeze-dried humic materials were first dissolved into the NaNO_3 eluting solution at a concentration of 0.5 $\text{g}\cdot\text{L}^{-1}$, passed through a 0.2 μm filter, and injected into the HPSEC system through a 100 μL loop. Calculation of the weight-averaged (M_w), and number-averaged (M_n) molecular weights, and polydispersity (M_w/M_n) for the humic samples were done by the Perkin-Elmer-Nelson Turbochrom 4-SEC software, using the method described by Yau et al. (1979).

NMR Spectra. Quantitative ^{13}C NMR spectra were obtained by suspending 100 mg of each HA in 1 mL of 0.5 M NaOH for 1 day at room temperature under N_2 . The suspension was then filtered through acid-washed glass wool, which was then washed with 1 mL of D_2O (for a deuterium NMR lock signal). The filtrates were combined into a final volume of approximately 2 mL. Solution ^{13}C NMR spectra were recorded at 75.4 MHz on a Varian XL 300 spectrometer using conditions suitable to obtain quantitative intensity distributions. Briefly, these were by using inverse-gated decoupling, a 45° pulse, an acquisition time of 0.1 s, and a relaxation delay (decoupler off) of 1.9 s. The number of scans ranged from 80 000 to 100 000. The free induction decays were processed by applying from 20 to 50 Hz line broadening and baseline correction as allowed by the available Varian software. The chemical shift is expressed in ppm on a scale relative to external sodium 3-(methylsilyl)propionate (TSP) at 0 ppm. The spectra were divided into the following areas: 0–110 ppm, aliphatic C and substitute C (amino acids, carbohydrates, etc.); 110–160 ppm, aromatic and phenolic C; 160–190 ppm, carboxyl C. Areas were measured by an automatic integrator.

Glyphosate Adsorption on Humic Substances. Samples (50 mg) of each of the four HAs were shaken in a plastic screw-cap vials with 5 mL of increasing concentrations of glyphosate (25, 50, 75, 100, 200, 250 $\text{mg}\cdot\text{L}^{-1}$) in a 0.01 M solution of CaCl_2 for 24 h. A shaking time of 24 h was previously assessed to be sufficient to reach the adsorption thermodynamic equilibrium (Piccolo et al., 1995). At the end of shaking time, and for both series of adsorption, after centrifugation, the supernatant was removed and purified as follows, and its glyphosate content was determined. Glyphosate adsorption experiments were made in duplicate and at room temperature. The glyphosate-free acid (MW = 169.08 and 99.8% of purity), used in this study was kindly supplied by Monsanto, Italy. The pH of the contact solution was that of glyphosate-free acid in CaCl_2 (pH 3) and did not change appreciably after the shakings.

Purification of Glyphosate Extracts. To eliminate the cations and dissolved organic matter that may have passed into the supernatant after the shaking, and that interfere with the subsequent HPLC determination of glyphosate (Glass, 1984), the supernatant was further purified. A glass column of 20 cm length and 1 cm diameter, containing glass wool at the lower tip, was packed first with a layer of 0.8 g of Dowex 50 \times 4-400 cation exchange resin and then with a second layer of 0.3 g of XAD-2 resin (Serva, research grade). This packed column, with a void volume of 1 mL, was washed with 0.5 N

Table 1. Elemental, Ash, and Acid Functional Group Content of Humic Substances

sample	% C	% H	% N	% ash	acidities ($\text{mequiv}\cdot\text{g}^{-1}$)		
					total	COOH	PhOH
HA1	51.6	2.4	4.1	4.2	6.1	2.7	3.4
HA2	50.5	2.7	3.7	1.7	10.7	4.7	6.0
HA3	62.9	3.0	1.9	2.7	9.0	3.4	5.6
HA4	64.2	3.5	1.5	1.5	9.3	4.4	4.9

NaOH, eluted to neutrality with distilled water, washed with 0.5 N HCl, eluted to neutrality with distilled water, and finally eluted to a chloride-free point with Milli-Q grade purified water. A 1 mL aliquot of the supernatant solution containing glyphosate was then inserted into the column, under N_2 pressure (1.5 atm), only until disappearance into the resin layer. A 5 mL aliquot of Milli-Q grade purified water were then passed, under pressure, through the column, and the first 2.5 mL that eluted out of the column was collected. This purified fraction, well shaken, was used for the HPLC determination of glyphosate. The use of known concentrations of glyphosate through the described purification column ensured that the loss of herbicide was consistently less than 1%.

Glyphosate Determination. Glyphosate was determined by a HPLC apparatus (Perkin-Elmer, 400 series) employing a 100 mL loop, a UV detector at 190 nm, an anion exchange column (Partisil 10 SAX, 25 cm), and a Perkin-Elmer-Nelson, Model 2100, integrator equipment and software. The eluent was a 0.08 M potassium phosphate buffer at pH 2.1 and pumped at an isocratic flow rate of 1.6 $\text{mL}\cdot\text{min}^{-1}$ giving a retention time of 2.72 min for the glyphosate peak. This method was adapted from that reported by other authors (Lawrence and Leduc, 1978) and had a detection limit of 5 ppm.

RESULTS

Humic Substance Properties. All humic substances showed an ash content less than 5%. Table 1 also revealed that the elemental analyses for the humic material originating from soils (HA1 and HA2) had values similar to other soil humic acids previously reported (Schnitzer, 1978), whereas humic substances from oxidized coal and lignite (HA3 and HA4, respectively) showed higher C and H content but lower N content.

Total acidity, measured by wet chemistry methods, increased in the order HA1 < HA3 < HA4 < HA2. The same order was observed for the values found for the carboxyl groups, whereas the order of phenolic group content, obtained by difference between total acidity and carboxyl acidity, varied consistently (Table 1).

The liquid-state NMR spectra of the four humic extracts revealed significant structural differences as illustrated in Figure 1. The quantitative evaluation of the carbon distribution in the NMR spectra (Table 2) showed that the content of aliphatic C was in the order HA1 = HA2 > HA4 > HA3. The aromatic C content followed a reverse order, with the maximum amount of 72% being that of humic matter extracted from the oxidized coal (HA3). The content of carboxyl C reflected the order found by the wet chemistry method except for the value of humic substances from lignite (HA4) that appeared higher than the one reported in Table 1. Inconsistencies in carboxyl content determination between NMR spectroscopy and traditional methods were previously indicated (Piccolo et al., 1990).

The HPSEC chromatograms obtained for the four humic extracts are shown in Figure 2. The chromatograms revealed that both humic materials from soils (HA1, HA2) contained a high molecular weight fraction eluting at around the void volume, as is shown by a

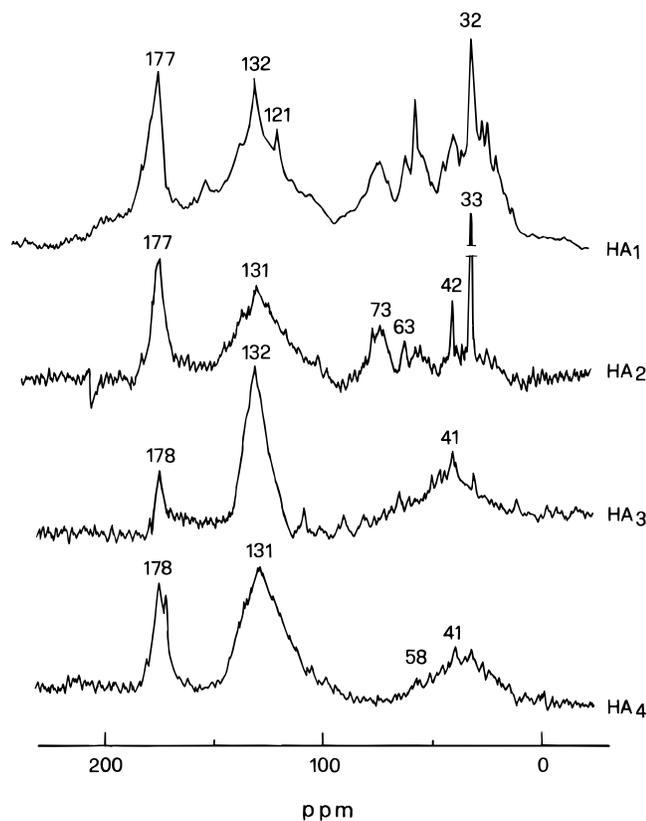


Figure 1. ^{13}C NMR spectra of humic substances

Table 2. Distribution of C Intensity in Different Regions (ppm) of ^{13}C NMR Spectra of Humic Substances

sample	relative intensity (% of total area)		
	0–110 (aliphatic C)	110–160 (aromatic C)	160–190 (carboxyl C)
HA1	52.8	40.7	6.5
HA2	53.6	37.0	8.4
HA3	18.0	72.0	10.0
HA4	29.8	55.7	14.5

noticeable peak for HA1 and by a less resolved but still visible absorbing zone for HA2. Lower molecular weight fractions were present in the humic material extracted from the more humified materials of oxidized coal (HA3) and lignite (HA4). In particular, the HA4 material showed a distinct peak at a higher retention time than for HA3, thereby indicating that the former humic acid was composed of material of lower molecular dimension than the latter. When the weight-averaged molecular weight, M_w , was calculated from the size exclusion chromatograms using the available software (Table 3), the order of molecular dimension of the four humic materials was confirmed except for HA3, which showed a slightly lower M_w than HA4. A similar order was found for the number-averaged molecular weight, M_n , with the humic material from the peat soil showing the largest dimension. The polydispersity, the M_w/M_n ratio, an index of the molecular size diversity present in each humic extract, was higher for the soil humic substances than for the material extracted from both oxidized coal and lignite (Table 3), as for the order HA1 > HA2 > HA3 = HA4.

Glyphosate Adsorption. The equilibrium adsorption isotherms of glyphosate on the four different humic substances are shown in Figure 3. HA1, from peat, revealed the highest rate of adsorption, reaching more than 7500 μg of glyphosate/g of adsorbent at the

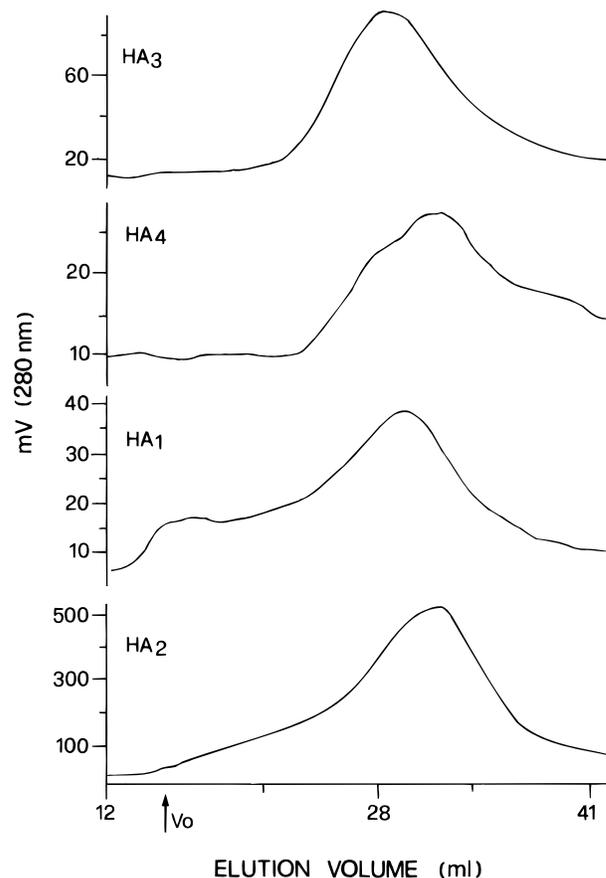


Figure 2. High-performance size exclusion chromatograms of humic substances.

Table 3. Weight-Average (M_w) and Number-Average (M_n) Molecular Weights and Polydispersity (M_w/M_n) of Humic Substances

sample	M_w	M_n	M_w/M_n
HA1	1.1×10^5	6.3×10^4	1.7
HA2	8.2×10^4	5.2×10^4	1.6
HA3	5.6×10^4	4.0×10^4	1.4
HA4	7.8×10^4	5.4×10^4	1.4

herbicide concentration of 250 $\mu\text{g}\cdot\text{mL}^{-1}$. The second highest adsorbing humic material was HA2, from volcanic soil, followed in order by HA3 and HA4. A comparison of the adsorbing capacity of the various HAs is reported in Table 4 for the initial glyphosate concentration of 100 $\mu\text{g}\cdot\text{mL}^{-1}$. At higher glyphosate concentrations, the adsorbing capacities of HA3 and HA4 tended to become similar (Figure 3).

The adsorption data over the range of concentrations studied here were used to fit both the Freundlich and Langmuir equations. For the Freundlich equation, $C_e = K_f C_w^{1/n}$, where C_e is the amount of glyphosate adsorbed ($\mu\text{g}\cdot\text{g}^{-1}$ of adsorbent), C_w is the equilibrium concentration of glyphosate ($\mu\text{g}\cdot\text{mL}^{-1}$), and K_f and n are constants that give estimates of the adsorptive capacity and intensity, respectively. For the Langmuir equation, $C_e = k_1 k_2 C_w / (1 + k_1 C_w)$, C_e and C_w have the same meaning as in the Freundlich equation, and k_1 and k_2 are indexes of binding strength and adsorption maximum, respectively. From the linearized forms of both the Freundlich and Langmuir equations (White and Zelazny, 1992), the above constants, K_f , n , and k_1 , k_2 , respectively, can be calculated as well as the relative corresponding determination coefficients (r^2). Their values for the adsorption of glyphosate on the four humic substances are shown in Table 5. The adsorption

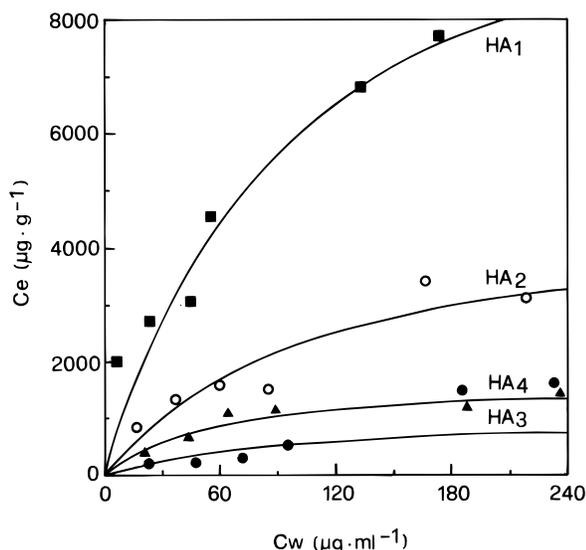


Figure 3. Adsorption isotherms for glyphosate on humic substances.

Table 4. Adsorption of Glyphosate^a by Humic Substances

sample	amount adsorbed ^b (mg·g ⁻¹)	sample	amount adsorbed ^b (mg·g ⁻¹)
HA1	4511 ± 14	HA3	1133 ± 5
HA2	1504 ± 4	HA4	514 ± 6

^a Initial concentration of glyphosate of 100 mg·mL⁻¹. ^b Means of two or more determinations and standard deviations.

Table 5. Freundlich and Langmuir Parameters with Respective Determination Coefficients (*r*²) for Glyphosate Adsorption on Humic Substances

sample	Freundlich			Langmuir		
	<i>K</i> _f	1/ <i>n</i>	<i>r</i> ²	<i>k</i> ₁	<i>k</i> ₂	<i>r</i> ²
HA1	454	0.55	0.95	0.010	11904	0.92
HA2	179	0.54	0.92	0.009	4807	0.78
HA3	98	0.51	0.84	0.016	1715	0.96
HA4	7	0.97	0.88	0.009	1108	0.73

constants for both Freundlich and Langmuir equations followed the same order observed for the adsorption isotherms (HA1 > HA2 > HA3 > HA4). The intensity of binding, the *n* constant in the Freundlich equation, showed that both humic substances from soils and from oxidized coal adsorbed glyphosate with equivalent strength. Moreover, such binding intensity was higher than that shown by the lignite humic matter. Conversely, the *k*₁ Langmuir values failed to show differences in binding strengths of glyphosate on the various humic materials.

DISCUSSION

The four humic substances used in this study presented significant differences in their chemical and physical-chemical characteristics (Tables 1–3). HA1 and HA2, both extracted from soils, contained more than 50% C in aliphatic, peptidic, and carbohydrate structures, whereas the older and more humified organic matter of lignite and oxidized coal produced humic substances with a much higher aromatic C. Such structural diversity is responsible for their different acidic reactivity, molecular dimension, and consequent glyphosate adsorption.

The amount of glyphosate adsorbed by humic substances was found to be surprisingly high. The Freun-

dlich adsorption constants, *K*_f, were significantly higher than those calculated for the glyphosate adsorption on three soils of different textural properties by Glass (1987) and on four different European soils by Piccolo et al. (1994). Furthermore, the amount of glyphosate adsorbed on humic material was of the same order of magnitude indicated by Glass (1987) for adsorption on clay minerals and their cation-saturated forms, montmorillonite and illite being stronger adsorbents than kaolinite. However, the *K*_f constants reported by Glass (1987) for the three clay minerals were significantly lower than those found for the soil humic materials, HA1 and HA2. The *K*_f of clay minerals were only comparable to the less adsorbing humic materials of this study, HA3 and HA4, which were extracted from nonsoil sources. These results indicate that humic substances isolated from soil are capable of a much higher glyphosate adsorption than both the soil itself and the mineral soil components.

In a previous study, the Freundlich *K*_f constant and the binding intensity, *n*, were sensibly higher than reported here when glyphosate was put in contact with a synthetic iron-humate complex that contained about 8% chelated iron (Piccolo et al., 1995). The particularly high adsorption of glyphosate on an iron-saturated montmorillonite had been previously reported (Glass, 1987). While the adsorption on soil had been related to the clay content and cation exchange capacity, the significant interaction with the cation-saturated clays (Glass, 1984) and with the iron-humate material (Piccolo et al., 1995) had been explained by the recognized capacity of glyphosate to strongly complex divalent and trivalent cations. McBride (1991) showed, by ESR spectroscopy, that a divalent cation such as copper was bound to glyphosate in 1:1 complexes by the amine N, and in 2:1 complexes by both carboxylate and phosphonate groups. In a study of glyphosate interactions with European soils containing different amounts of iron and aluminum oxides, Piccolo et al. (1994) indicated that the higher the iron and aluminum content, the larger the glyphosate adsorption was on soil.

In this study, however, the humic substances were thoroughly purified and contained less than 0.1% polyvalent cations. With such a low content of cations held by the humic substances, metal complexing cannot account for the significant adsorption of glyphosate.

Conversely, recent spectroscopic evidence (Piccolo and Celano, 1994) has proved that hydrogen bonding is a mechanism of interaction between glyphosate and purified humic substances. Hydrogen bonding can thus be advocated to explain the relevant glyphosate-humic material interactions reported in this study. In fact, the glyphosate molecule contains various electronegative atoms, which can act as both as hydrogen donor and hydrogen acceptor groups (Wauchope, 1976; Piccolo and Celano, 1993), and so does the humic matter due to the large content of oxygen-containing functions. However, other structural and stereochemical properties of humic substances must also play a role in glyphosate adsorption. In fact, had only hydrogen bonding been responsible for glyphosate adsorption, the order of humic-adsorbing capacity (HA1 > HA2 > HA3 ≥ HA4) should have been closely related to the content of acidic functions, which are likely to form hydrogen bonds with the corresponding glyphosate functions. That was not the case, since the order of acidities was HA2 > HA4 > HA3 > HA1 (Table 1). This order was generally confirmed by the NMR results (Table 2), whereby the

most glyphosate-adsorbing humic material, HA1, appeared as the least acidic compound.

A closer relation to the adsorbing capacity of humic substances was revealed by their content of aliphatic C and their molecular size. The two most adsorbing humic materials, HA1 and HA2, derived from soils, showed a lower content of aromatic C (Table 2) and a larger molecular dimension (Table 3) than the HA3 and HA4 which were the least adsorbing and most aromatic materials. In particular, HA1 revealed a molecular size close to double that of HA3 and HA4. These observations suggest that the larger the molecular size of the humic substances, the greater is the number of hydrogen bondings occurring between the herbicide and the humic molecule and, thus, glyphosate adsorption. Furthermore, a lesser degree of stereochemical rigidity, due to a lower content of aromatic rings, allows an easier penetration of the small herbicide molecule into the inner reactive sites of the humic macromolecule, thereby favoring glyphosate adsorption. The importance of the specific chemical structure and molecular dimension of humic substances in determining their capacity to interact with organic compounds in the environment had been already indicated (Piccolo, 1994). Also in the case of glyphosate, the occurrence of hydrogen bondings between the herbicide and humic substances is controlled not only by the content of hydrogen donor and acceptor groups but also by the molecular size and stereochemical flexibility of the humic material.

In summary, this work has shown that the adsorption of glyphosate on purified humic substances, far from being negligible, is comparable to if not higher than that observed for clay minerals and soils. However, the extent of adsorption may be variable since is dependent on the macromolecular structure and dimension of humic material. Results showed that humic substances from soils were capable of large glyphosate adsorption because of favorable molecular characteristics such as high molecular size and high stereochemical flexibility. These findings may well have implications in the prediction of residual glyphosate toxicities in soils with different organic matter properties. In fact, part of the glyphosate spread on soil may be only temporarily bound to humic material by hydrogen bondings and may be released after some time. Moreover, the herbicide mobility to deeper soil horizons may be controlled by the formation of relatively stable complexes between glyphosate and water-soluble humic substances.

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