

Changes in Dissolved Organic Carbon of Soil Amendments with Aging: Effect on Pesticide Adsorption Behavior

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The effect of aging in the soil of three organic amendments (OAs), one liquid (LF) and two solid ones (SF and AL), has been investigated and related to changes in soil adsorption of metalaxyl and tricyclazole. LF and AL have very high dissolved organic carbon (DOC) contents with low humification index values, whereas SF has a low DOC content but the highest amounts of highly humified material. All OAs increased the adsorption of tricyclazole, whereas adsorption of metalaxyl decreased in soils amended with LF and AL, due to competition with DOC for mineral adsorption sites. With aging, DOC from SF amended soils is not significantly affected and neither is adsorption behavior. On the contrary, the great reduction of DOC from LF and AL with aging has been shown to affect adsorption of metalaxyl and tricyclazole, and this effect is dependent on the pesticide, the nature of the DOC, and the type of soil, in particular its clay mineralogy.

KEYWORDS: Soils; organic amendments; aging; dissolved organic carbon; metalaxyl; tricyclazole

INTRODUCTION

The widespread occurrence of pesticides, especially in the groundwater, has stimulated research into the behavior of these agrochemicals in the environment. In particular, overuse of fungicides has been shown to result in pollution of water bodies through processes such as leaching and runoff (1–3). These losses are attenuated by the natural process of adsorption, because degradation, transport, and biological activity of pesticides are greatly influenced by adsorption on soil constituents, in particular soil organic matter (4, 5). Hence, organic amendments (OAs) can be used to reduce leaching of pesticides and its harmful effects, because this practice has been shown to modify soil surfaces, promoting adsorption (6–8) while providing an ecological and economical method of organic waste disposal.

The study of the influence of OA on pesticide behavior in soil is of great interest, especially when it is an issue very seldom considered when the decision is made to fertilize soil or dispose of organic wastes. Other aspects being considered are nutritional ones, risk of nitrate leaching, or accumulation of heavy metals in soils. The incorporation of OAs in soils introduces both solid organic matter and soluble organic carbon (DOC). In some cases DOC has been shown to reduce pesticide adsorption due to DOC–pesticide interactions and/or competition between DOC molecules and pesticide molecules for adsorption sites (9–14). This decrease in adsorption would enhance transport and

increase the risk of contamination of water bodies. Consequently, the effect of OA addition on pesticide behavior is not easy to predict, especially when liquid amendments are applied or when DOC concentrations resulting from the OA are high.

The nature of organic constituents of soils is likely to vary significantly among soils and even more among OAs of different origin, which consequently can affect adsorption of organic compounds. Chemical fractionation methods have been widely used in studies of the interaction of soil organic matter and pesticides, although such techniques may result in modification of the chemical nature of soil organic matter (15, 16). On the other hand, structural characterization of organic matter from soil or organic amendment origin has been accomplished by NMR (i.e., refs 17–19), IR (i.e., refs 20 and 21), and pyrolysis (i.e., refs 18 and 22) techniques. More recently, fluorescence spectroscopy has been used successfully to obtain information about the structure and humification of DOC (23–25), and, in previous work, we have related the information obtained by this technique with pesticide behavior in the soil (12, 26).

The aim of this paper is to evaluate the changes in DOC on organic amendment, immediately after the incorporation of the organic amendment and after incubation in the soil for 2 months, and to relate these changes with pesticide soil adsorption. This would help to find the key factors needed to design strategies to prevent contamination or to remediate it once produced and, in our case of study, serves as an example of reconciliation between agricultural development and environmental sustainability. Although it is realized that the DOC fraction of the OAs is a relatively small proportion of the total OA mass, it is to be

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Table 1. Physicochemical Properties of Soils (P1, P2, and P3) and Organic Amendments (SF, LF, and AL) and Humification Indexes (HIX = Fluorescence Intensity from 435 to 480 nm/Fluorescence Intensity from 300 to 345 nm, Equation 1) of Extracts from Soils and Organic Amendments

parameter	soils			organic amendments		
	P1	P2	P3	SF	LF	AL
TOC, ^a %	0.60	1.47	0.48	18.3	14.9	49
DOC, ^b mg/L	9.79	18.3	13.5	222.4	2843	3031
clay, %	16	53	68			
(I, K, M), ^c %	(59, 20, 21)	(36, 34, 30)	(56, 16, 28)			
silt, %	9	36	31			
sand, %	75	11	1			
dry matter, %				85	31	100
pH	7.1	7.6	8.3	9.4	5.1	5.4
HIX DOC	3.97	3.76	2.87	12.32	0.70	0.44
	(± 0.27) ^d	(± 0.43)	(± 0.32)	(± 4.42)	(± 0.05)	(± 0.10)

^aTotal organic carbon. ^bDissolved organic carbon. ^cIllite (I), kaolinite (K), and montmorillonite (M). ^dStandard deviation about the mean of three replicates.

expected that because of its mobility, the DOC fraction will have a significant effect on the fate of chemicals in the environment (27). The goals of the research reported here are to quantify and explain this effect on the fate of selected pesticides and to determine to what degree this effect is stable over time. For this purpose, three soils varying in their physicochemical properties, three different OAs, and two fungicides of different adsorption behaviors (metalaxyl and tricyclazole) were selected. Metalaxyl is a fungicide with residual and systemic activity, and it is used as a soil and foliar treatment. Muller and Buser (28) have detected amounts >100 ng/L of metalaxyl in agricultural drainage water, and Sukul and Spittler (29) have shown that it can leach to deeper soil horizons and reach groundwater in soils of low organic carbon content. It has been shown to adsorb on organic matter, although adsorption on soil mineral surfaces has also been reported, especially when low organic matter soils were considered (30–32). Tricyclazole is a systemic fungicide for the control of rice blast disease, and it adsorbs to soil to a much higher extent than metalaxyl (33). Triazole fungicides are widely used, and a possible cause for concern is their long persistence in soil (34).

MATERIALS AND METHODS

Pesticides, Soils, and OAs. Metalaxyl [methyl-*N*-(2-methoxyacetyl)-*N*-(2,6-xylyl)-DL-alaninate] (chemical purity > 97.7%) was supplied by Industrias químicas de Navarra, SA, Pamplona, Spain. This fungicide is a white powder with a melting point of 63.5–72.3 °C, a vapor pressure of 0.75 mPa (25 °C), a water solubility of 8.4 g/L (22 °C), and a molecular mass of 279.3 (35). Tricyclazole (5-methyl-1,2,4-triazolo[3,4-*b*][1,3]benzothiazole) (95.5% purity) was supplied by Eli Lilly (Indianapolis, IN). It is a crystalline solid with a melting point of 187–188 °C, a vapor pressure of 0.027 mPa (25 °C), a water solubility of 1.6 g/L (25 °C), and a molecular mass of 189.2 (35).

Three agricultural soil samples from southern Spain, which had not been treated with either metalaxyl or tricyclazole, were used in this study: P1, P2, and P3. All samples were collected from the 0–10 cm upper layer of the horizon, air-dried, sieved to pass a 2 mm mesh, and stored at room temperature. The physicochemical properties of the soil samples are given in **Table 1**. Soil pH was measured in a 1:2 (w/w) soil/deionized water mixture. Soil texture was determined according to the hydrometer method (36). The total organic carbon content (TOC) of the soils was determined according to the Walkley–Black method (37). Clay mineralogy (illite, montmorillonite, and kaolinite) was determined according to an X-ray diffraction procedure on oriented specimens (38). All measurements were done in triplicate.

Three organic amendments, solid fertiormont (SF), liquid fertiormont (LF), and alperujo (AL), were used in this study. SF and LF are commercial humic amendments derived by composting the solid and liquid waste, respectively, of the olive mill process, and AL is a residue

from the olive oil production industry. Some physicochemical properties of the OAs are also given in **Table 1**.

Amendment of the Soils and Incubation of Amended Soils. Soil samples (1 kg) were amended (10% w/w on a dry basis) with the three OAs (SF, LF, and AL), thoroughly mixed, air-dried, and sieved. Once amended, each soil was divided into two subsamples: 500 g was dried, sieved, and used for adsorption studies immediately after amendment (nonincubated soils), and 500 g of each amended soil was incubated at 22 °C for 2 months after the addition of the appropriate volume of water in order to obtain a moisture content equivalent to –0.33 kPa. These amended soils will be referred to as “incubated” soils or time 2 ($t = 2$) versus “nonincubated” soils or time 0 ($t = 0$) throughout this study. Moisture content was maintained constant throughout the incubation period by the weekly addition of distilled water as necessary. After 2 months of incubation, soils were dried, sieved, and used in adsorption studies in the same conditions as with “nonincubated” soils, as will be described later.

Characterization of DOC from OAs and Amended Soils: Spectroscopical Studies. DOC was extracted from OAs, soils, and amended soils (nonincubated and incubated) by treatment with a 0.01 M CaCl₂ solution, to minimize the extraction of clay and to mimic, to some degree, the ionic strength of the pore water (39). Triplicates of soils and amended soils were extracted in a ratio 1:2 (i.e., 5 g of sample and 10 mL of CaCl₂), and triplicates of OAs were extracted in a ratio 1:20, the proportion of amendment that exists during the extraction of amended soil (10% w/w). All extractions were performed on a dry mass basis. Suspensions were shaken for 15 min at room temperature, centrifuged for 15 min at 1000g, and then filtrated with 0.4 μm pore polycarbonate filter. The shaking time was kept as short as practically possible because it was attempted here to obtain material that could actually be in the dissolved state *in situ*. DOC of the extracts was measured at pH 2 with a Shimadzu TOC-5050A total carbon analyzer. The pH of the samples was adjusted by the addition of 1 μL of 2 N HCl/mL of sample and sparged with N₂ to avoid interference of carbonates in DOC determinations and to ensure that dissolved carbon determinations correspond to organic C.

To avoid concentration effects, the extracted DOC was diluted to have absorbance < 0.1 cm⁻¹ at 254 nm (40) and then acidified with 2 N HCl to obtain a pH of 2, because the pH of a solution can have a strong effect on fluorescence spectra (41, 42). All measurements were done at this pH. The fluorescence spectra, from 300 to 480 nm, were obtained in a Varian Cary Eclipse fluorescence spectrophotometer, using 1 cm cuvettes under excitation at 254 nm. The optical density of the samples at the excitation wavelength was determined with a Varian Cary 50 Bio UV–visible spectrophotometer, and the fluorescence emission spectra were corrected by multiplication with factor e^A , where A is the absorbance in cm⁻¹ at the excitation wavelength (23).

A humification index (HIX) was calculated from the fluorescence data as

$$\text{HIX} = \frac{\sum_{\text{WL}=435}^{480} I_{\text{WL}}}{\sum_{\text{WL}=300}^{345} I_{\text{WL}}} \quad (1)$$

where WL is the wavelength in nanometers and I_{WL} is the fluorescence intensity at this wavelength (12, 23).

DOC from the OAs (1:20 extraction on a dry mass basis) was further analyzed by Fourier transform infrared spectroscopy (FT-IR). Extracts were freeze-dried and analyzed as KBr disks using a Nicolet 5 PC spectrometer.

Pesticide Adsorption Studies. Adsorption isotherms of metalaxyl and tricyclazole on soils and nonincubated ($t = 0$) and incubated ($t = 2$) amended soils were measured using a batch equilibration method. Triplicates of 5 g (metalaxyl) or 2 g (tricyclazole) of each soil were treated with 10 mL of the corresponding fungicide solutions with a concentration (C_i) of 5, 10, 20, 50, or 100 μM made up in 0.01 M CaCl_2 . The suspensions were shaken at 20 ± 2 °C for 24 h and centrifuged at 1000g at the same temperature. Supernatants were filtered and equilibrium concentrations (C_e) determined by HPLC with a photodiode array detector. The following conditions were used: Nova-Pack C18 column, 150×3.9 mm; flow rate, 1 mL/min.; eluent system, 50:50 methanol/water (metalaxyl) and 20:80 acetonitrile/water (tricyclazole); injection volume, 25 μL ; detection wavelength, 230 nm for both fungicides. Quantification limits under these conditions were 0.5 and 0.2 μM for metalaxyl and tricyclazole, respectively.

Adsorption isotherms were obtained by plotting the amount of fungicide sorbed ($C_s = \mu\text{mol/kg}$) versus the equilibrium concentration ($C_e = \mu\text{M}$) and fitted to the Freundlich equation:

$$C_s = K_f C_e^{n_f} \quad (2)$$

Adsorption constants K_f and n_f , which indicate adsorption capacity evaluated at $C_e = 1$ μM and adsorption intensity, respectively, were calculated. Considering the range of initial solution concentrations used for both fungicides in adsorption studies, K_f values will indicate soil adsorption capacity at low concentrations, whereas n_f values give an indication of the evolution of adsorption when concentration in solution increases.

Statistical Treatment of the Data. Statistical analyses of experimental data were carried out with the program SPSS 11.0 for Windows. Significant statistical differences of all variables (DOC, TOC, HIX, and K_f) between the different treatments (freshly amended soils or $t = 0$ and aged amended soils or $t = 2$) were established by Student's t test at $P < 0.05$.

The amounts of DOC extracted from the freshly amended soils P1, P2, and P3 ($t = 0$), which is indicative of DOC adsorption to soils, were analyzed by ANOVA, considering DOC as the dependent variable. Significant statistical differences of DOC between the different amended soils were established by Tukey's test ($P < 0.05$).

RESULTS AND DISCUSSION

Characterization of DOC of the OA and Amended Soils.

The amount of DOC of the OAs is very high in the case of the liquid amendment LF and solid AL, the last with very high TOC (Table 1). The amendment of the soils with SF, LF, and AL resulted in an increase of TOC of the soils, especially AL amended soils (Table 2). No differences were observed in adsorption of DOC from SF among nonincubated soils (71, 75, and 75% of DOC sorbed on P1, P2, and P3, respectively), whereas DOC from LF and AL sorbed to a higher extent on soils P2 and P3, of higher clay content, when compared with the sandy soil P1, as indicated by the lower DOC of soils P2 and P3 amended with LF and AL when compared with P1 (Table 2, $t = 0$). This fact points to molecular differences between DOC from AL and LF and DOC from SF.

Table 2. Changes in pH, Total Organic Carbon (TOC), Dissolved Organic Carbon (DOC), and Humification Indexes (HIX) of Amended Soils with Incubation Time (t) [$t = 0$ (No Incubation) and $t = 2$ (Incubation for 2 Months at 40% w/w Moisture Content)]

organic amendment	soil	pH		TOC, %		DOC, mg/L		HIX	
		$t = 0$	$t = 2$	$t = 0$	$t = 2$	$t = 0$	$t = 2$	$t = 0$	$t = 2$
SF	P1	8.9	8.5	1.83	1.77	73.8 a ^a	88.0	8.41	9.79 ^b
	P2	7.4	8.3	1.76	1.72	73.4 a	58.0	7.61	9.72 [*]
	P3	8.6	8.6	1.33	1.14 [*]	68.3 a	76.0	7.06	10.6 [*]
LF	P1	6.5	7.9	1.70	0.97 [*]	1501 a	307 [*]	0.85	4.87 [*]
	P2	7.7	7.9	2.16	1.83 [*]	1198 b	220 [*]	0.98	4.51 [*]
	P3	8.0	8.1	1.65	1.31 [*]	988 c	189 [*]	0.94	4.66 [*]
AL	P1	6.8	8.7	3.29	2.43 [*]	1624 a	467 [*]	0.34	4.82 [*]
	P2	7.0	8.2	3.72	3.79	1343 b	515 [*]	0.37	4.60 [*]
	P3	7.8	8.5	3.22	3.24	1204 b	344 [*]	0.43	4.72 [*]

^a For each organic amendment, different letters (a–c) among soils indicate statistically significant differences ($P < 0.05$). ^b Values marked with an asterisk indicate that differences between $t = 2$ and $t = 0$ are statistically significant ($P < 0.05$).

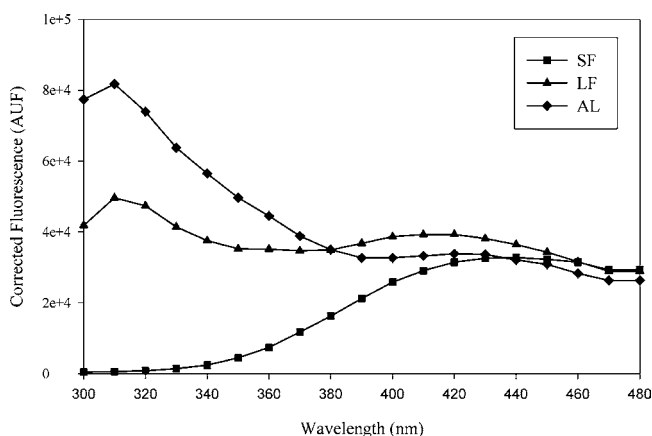


Figure 1. Emission fluorescence spectra of the DOC of the organic amendments SF, LF, and AL at pH 2 and an excitation wavelength of 254 nm.

Spectroscopic studies reveal that differences between amendments are not only in quantity but also in quality of DOC. The maximal fluorescence intensity of DOC from SF extract (Figure 1) appears at wavelengths >400 nm, indicating that their fluorescence is dominated by condensed molecules, presumably aromatic, which are typical for humic materials (23, 42–44). The LF and AL amendments have the maximal fluorescence intensity in the region near 300 nm (Figure 1). In this region, less condensed and nonhumified material tend to fluoresce. The intensity of this peak is much higher in DOC from AL than in DOC from LF. It should also be noticed that DOC from LF fluoresces to a higher extent at higher wavelengths (in the 400 nm region) than AL. The very low HIX values (Table 1) indicate that LF and AL contain great amounts of relatively nonhumified material. On the contrary, HIX values of DOC from SF show that this amendment contains the highest amounts of complex molecules (Table 1).

FT-IR spectra of DOC from OA extracts also show differences in the quality of DOC (Figure 2). The main feature observed in FT-IR spectra is the high intensity of the band near 1400 cm^{-1} in the SF extract when compared with the LF and AL extracts. This band corresponds to the carbonyl absorption band of the ionized carboxylic group (COO^-) (45). On the other hand, the band near 1700 corresponding to the carbonyl absorption band of the carboxylic group (COOH) is not present in the SF extract, whereas it is very clear in the LF and AL extracts, despite the proximity of the 1600 cm^{-1} band also

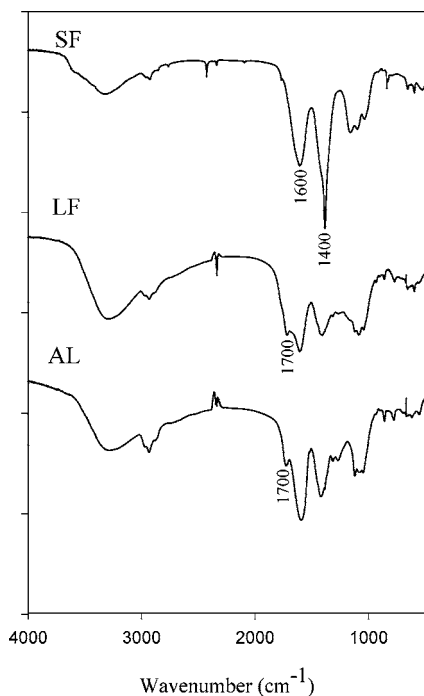


Figure 2. FT-IR spectra of the DOC of the organic amendments SF, LF, and AL.

associated with COO^- and water absorption. The higher amount of carboxylate in the SF extract is the reason for the high pH of SF when compared to LF and AL (**Table 1**).

The fluorescence spectra of extracts of the soils amended with SF and AL at time 0 (nonincubated soils) do not significantly alter spectrum characteristics of the respective OAs (**Figure 3**, solid symbols), with a maximum at high wavelengths (>400 nm) in the case of SF or at low wavelengths (near 300 nm) in AL-amended soils. In the case of LF-amended soils at time 0, the intensity of the peak of maximal fluorescence intensity, which appears near 300 nm in the LF extract (**Figure 1**), is reduced (**Figure 3**, solid squares), and this reduction is higher in soils P2 and P3, of higher clay content, in agreement with the higher adsorption of DOC measured in these soils (**Table 2**). In **Figure 3** it is also shown that the fluorescence intensity in the region near 300 nm in LF- and AL-amended soil spectra is higher in soil P1 than in soils P2 and P3, confirming that the DOC fraction that sorbs to higher extent on clay soils P2 and P3 is the fraction that fluoresces at lower wavelengths. This can be attributed to rapid adsorption to soil of the DOC fraction of LF fluorescing at lower wavelengths, whereas more complex (humified) organic material remains in solution. Preferential microbial utilization of DOC in these soils can also explain these results.

The HIX values of SF-amended soils at time 0 (**Table 2**) are higher than the corresponding unamended soils, although lower than the HIX of the SF extract (**Table 1**), which can be attributed to adsorption of the more condensed molecules in SF to soil. Plaza et al. (46) have observed that soil amendment with pig slurry, of smaller aromatic polycondensation and humification degree than soil humic acid, resulted in modification of soil humic acid and that this modification includes a reduction in aromaticity. In the case of LF- and AL-amended soils, HIX values slightly increase (LF-amended soils) or remain unaffected (AL-amended soils) (**Table 2**) when compared with the HIX values of LF and AL extracts (**Table 1**) and in every case are lower than the HIX values of the corresponding unamended soils (**Table 1**).

With residence time in the soil, DOC from SF-amended soils is not significantly affected ($P < 0.05$). Also, DOC from incubated soils ($t = 2$) amended with SF shows fluorescence spectra similar to those of the corresponding amended soils that were not incubated (**Figure 3**, solid and open circles). Consequently, HIX values show just a small increase with residence time (**Table 2**). In the case of LF- and AL-amended soils, DOC dramatically decreases in every soil after a 2 month incubation period (**Table 2**). The fluorescence spectra of DOC from LF- and AL-amended soils after incubation (**Figure 3**, open symbols) are very different from those at time 0: the peak in the 300 nm region, of very high intensity in AL nonincubated amended soils (**Figure 3**, $t = 0$) and the AL extract (**Figure 1**), disappears in LF- and AL-amended soils after incubation ($t = 2$). This results in a great increase in HIX values of DOC from LF- and AL-amended soils with incubation (**Table 2**). Fluorescence studies indicate that the great reduction in DOC in the LF- and AL-amended soils with incubation time is mainly due to a reduction in DOC molecules of low degree of complexation (probably smaller and more polar), which as discussed above has the lowest ability/tendency to fluoresce.

Three processes can be mainly responsible for this decrease in DOC: (1) microbial degradation (mineralization), (2) increase in DOC adsorption with residence time of OA in the soil, and/or (3) polymerization (humification) of the DOC, giving rise to more condensed molecules that fluoresce at higher wavelengths. If (1) is responsible for the great decrease in DOC in LF- and AL-amended soils with residence time, a similar decrease should be observed in TOC of incubated soils. An interesting feature is that TOC remained unaffected upon incubation in soils P2 and P3 amended with the solid amendment AL of very high DOC, despite the decrease in DOC (**Table 2**). These results suggest that the increase in adsorption of DOC with residence time can, in some cases, be also responsible for the decrease in DOC. Probably the longer time needed for soluble organic carbon from the solid amendment AL to pass into soil solution during the incubation period, when compared with the DOC from the liquid amendment LF, protects DOC from AL from microbial degradation, allowing an increase in adsorption with time to occur.

Adsorption of Metalaxyl and Tricyclazole in Nonincubated Amended Soils. Metalaxyl adsorption coefficients obtained from isotherms with unamended and amended soils (nonincubated, $t = 0$) are given in **Table 3**. Adsorption increases in the unamended soils in the order $\text{P3} > \text{P2} > \text{P1}$. Metalaxyl adsorption on soil P3, of very high clay content (**Table 1**), is much higher than on soils P1 and P2 (10 times higher). Soil P3, of very low TOC (**Table 1**), is a saline soil from the marshes of southern Spain affected by high salt concentrations, which gives rise to a surface alteration of its illitic clay that behaves like smectite (47, 48) and hence renders a very high adsorption capacity for water and polar organic compounds such as metalaxyl (32, 49).

When soils were amended with SF, adsorption increased in soils P1 and P2, which can be attributed to an increase in TOC (**Table 2**), and remained unaffected in soil P3 (**Table 3**), despite the increase in TOC from 0.48 to 1.33% and the high HIX of this OA (**Tables 1** and **2**). This fact corroborates the great affinity of metalaxyl for mineral surfaces (31, 32): the high mineral surface area available for adsorption in soil P3 is more important than the increased organic surfaces. Amendment with LF and AL ($t = 0$), of very high DOC contents (**Table 1**), increased metalaxyl adsorption in soil P1, whereas adsorption remained unaffected in $\text{P2} + \text{LF}$ and increased in $\text{P2} + \text{AL}$

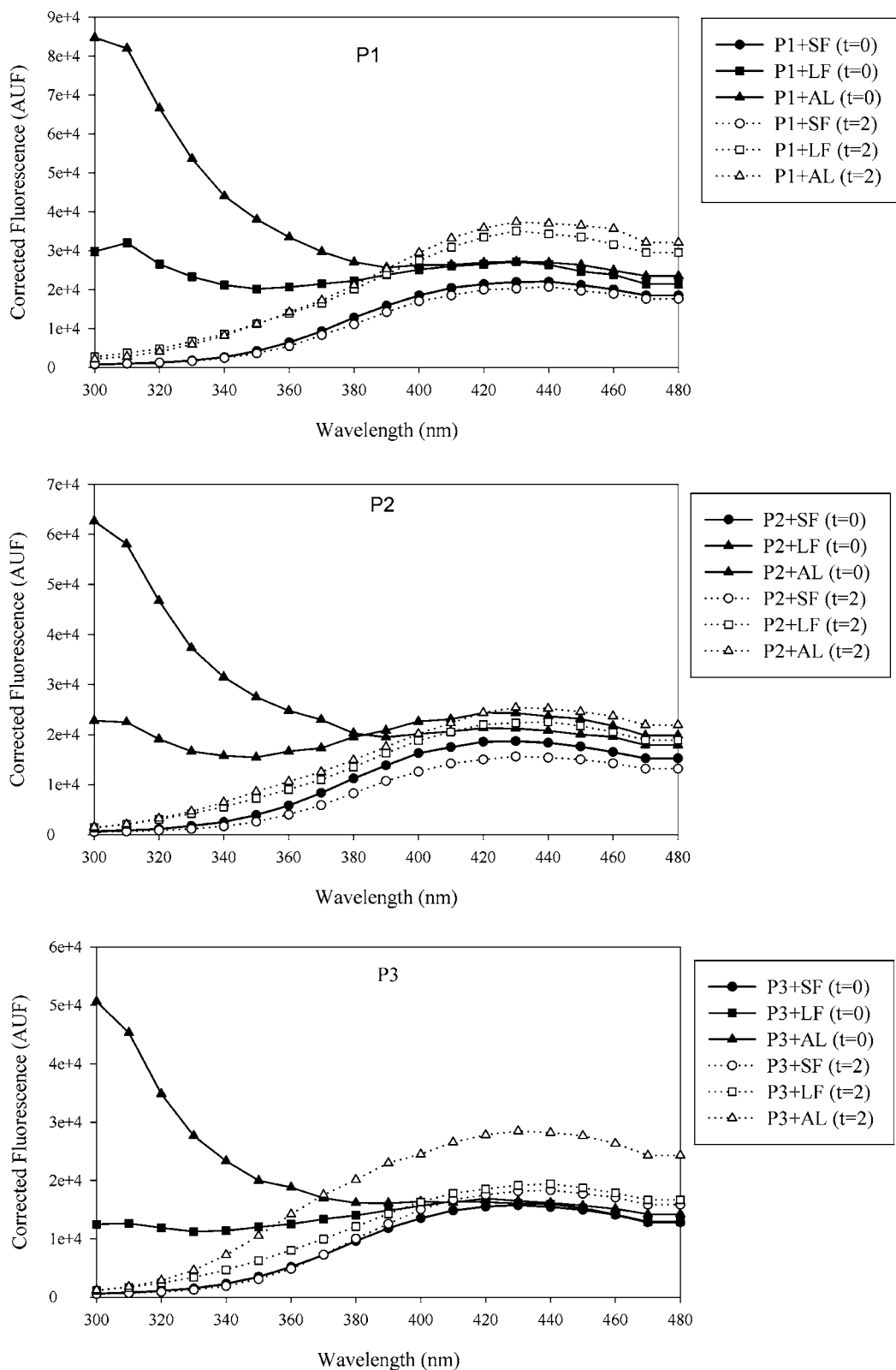


Figure 3. Emission fluorescence spectra of DOC from P1, P2, and P3 amended soils (nonincubated, $t = 0$, and incubated, $t = 2$) at pH 2 and under an excitation wavelength of 254 nm.

(Table 3). Adsorption on P3 decreased with both LF and AL. As shown in fluorescence studies, the LF and AL amendments are mainly constituted by molecules with low degrees of complexation (Table 1). The lack of increase in metalaxyl adsorption or the lower adsorption than unamended soil, despite the increase in TOC (Table 2), can be attributed to interactions between smaller DOC molecules from LF and AL and mineral

soil surfaces (12), competing with metalaxyl molecules for the same mineral sorption sites. This is corroborated by the higher adsorption of DOC from LF and AL on soils P2 and P3 when compared with soil P1 (Table 2).

Tricyclazole adsorption data obtained from adsorption isotherms are given in Table 4. Adsorption coefficients for every soil are higher than the corresponding data for metalaxyl, due

Table 3. Metalaxyl Sorption Coefficients in Unamended and Amended Nonincubated ($t = 0$) and Incubated ($t = 2$) Soils

soil	organic amendment	nonincubated soils ($t = 0$)			incubated soils ($t = 2$)		
		K_f	n_f	R^2	K_f	n_f	R^2
P1		0.29	1.2	0.98			
	SF	1.50	1.0	0.99	1.73	0.94	0.98
	LF	0.56	1.0	0.99	0.80 ^a	1.04	1.00
	AL	1.31	0.96	0.99	0.88 [*]	1.04	1.00
P2		0.47	1.2	0.99			
	SF	1.69	1.0	0.98	2.92 [*]	0.87	0.99
	LF	0.49	1.2	0.94	1.07 [*]	1.06	1.00
	AL	0.66	1.2	0.99	1.13 [*]	0.99	1.00
P3		5.48	1.1	1.00			
	SF	5.42	1.1	0.98	8.50 [*]	0.95	1.00
	LF	1.94	1.3	1.00	5.97 [*]	1.00	1.00
	AL	4.98	0.88	0.97	6.30	0.90	0.99

^a Values marked with an asterisk indicate that differences between K_f ($t = 0$) and K_f ($t = 2$) are statistically significant ($P < 0.05$).

Table 4. Tricyclazole Sorption Coefficients in Unamended and Amended Nonincubated ($t = 0$) and Incubated ($t = 2$) Soils

soil	organic amendment	nonincubated soils ($t = 0$)			incubated soils ($t = 2$)		
		K_f	n_f	R^2	K_f	n_f	R^2
P1		3.21	0.98	0.97			
	SF	16.2	0.77	1.00	24.9 ^a	0.68	1.00
	LF	9.08	0.91	1.00	5.94 [*]	0.88	0.97
	AL	11.3	0.90	0.99	15.5 [*]	0.82	1.00
P2		24.0	0.72	0.99			
	SF	34.3	0.71	1.00	40.4 [*]	0.64	1.00
	LF	35.7	0.62	0.99	12.3 [*]	0.78	1.00
	AL	23.0	0.78	1.00	19.1 [*]	0.86	0.99
P3		19.3	0.80	1.00			
	SF	32.5	0.76	1.00	39.8 [*]	0.71	0.99
	LF	15.4	0.95	0.99	26.5 [*]	0.75	1.00
	AL	17.2	0.93	0.99	32.4 [*]	0.76	1.00

^a Values marked with an asterisk indicate that differences between K_f ($t = 0$) and K_f ($t = 2$) are statistically significant ($P < 0.05$).

to its lower water solubility (50). In this case we do not find the very high adsorption on P3 observed for metalaxyl. On the contrary, higher adsorption was observed in soil P2, of higher TOC than P1 and P3. Soil P1, of very low TOC and clay content, shows a very low adsorption capacity for tricyclazole. The effect of OA addition to soil in adsorption behavior is completely different in the case of this fungicide. With the exceptions of P2 + AL, P3 + LF, and P3 + AL, OAs increased the adsorption of tricyclazole, and amendment with the highly humified SF rendered, in most cases, the highest adsorption coefficients when compared with the respective unamended soil. The increase in adsorption upon amendment is much higher in the low adsorptive soil P1. Adsorption of tricyclazole on P2 + AL, P3 + LF, and P3 + AL at concentrations $> 1 \mu\text{M}$ increases when compared with the respective unamended soil, as indicated by the higher n_f coefficients (Table 4). This increase in pesticide adsorption at high concentrations raises the possibility of the use of this OA in remediation in the case of pesticide point pollution or accidental spills. Organic matter is the most important soil component affecting the adsorption of tricyclazole (51), which can explain the increase in adsorption with OAs at high concentrations. However, the quality of organic matter appears to be very important because there is not a clear positive relationship of tricyclazole adsorption to soil TOC. Also, the lack of increase in adsorption at low concentrations (K_f) in the soils of

higher clay content (P2 and P3) and with the more polar (less humified) OAs (LF and AL) indicates that polar interactions, although not as relevant as in the case of metalaxyl, are also important in the case of this fungicide (52) and, hence, competition for mineral sorption sites can also take place (12, 26).

Adsorption of Metalaxyl and Tricyclazole in Incubated Amended Soils: Effect of OA Aging. Metalaxyl adsorption isotherms on amended soils incubated for 2 months were fitted to the Freundlich equation, and adsorption coefficients are given in Table 3, together with adsorption data at time 0 (nonincubated soils). When soils amended with SF were incubated for 2 months, metalaxyl adsorption on soil P1 remained unaffected, whereas it significantly increased ($P < 0.05$) on soils P2 and P3 (Table 3). No significant changes in the amounts of TOC or DOC were observed for P1 and P2 amended with SF (Table 2), whereas in the case of soil P3, with very high sorption capacity for metalaxyl, the significant decrease in TOC ($P < 0.05$) while DOC remains unaffected suggests mineralization of sorbed organic C in nonincubated soil blocking surfaces available for adsorption, resulting in further mineral surfaces available for metalaxyl adsorption. We have previously observed that H_2O_2 treatment for organic matter removal of the clay fraction of this soil increased adsorption of the polar herbicides thiazafuron and metamitron (49), which would corroborate our hypotheses.

Metalaxyl adsorption significantly increased ($P < 0.05$) with incubation ($t = 2$) in all soils amended with LF, and the highest increase ($\times 3$) was observed in the highly sorptive clay soil P3. All three soils amended with LF show higher and significant ($P < 0.05$) decreases in TOC than in DOC, especially soil P1 + LF (0.73% reduction in TOC versus 0.24% in DOC). This indicates that mineralization of the DOC in soil solution must be followed by desorption of sorbed organic carbon, which also further mineralizes, giving rise to a decrease in DOC of nearly 80% of the DOC present in nonincubated soils (from 1501 to 307 mg/L). The DOC remaining in solution has a higher degree of complexation, as indicated by fluorescence studies (Figure 3) and, hence, is more stable to mineralization and has a lower affinity for mineral surfaces than the smaller DOC molecules (12).

Adsorption of metalaxyl significantly decreases ($P < 0.05$) in P1 with aging ($t = 2$) of AL when compared with freshly amended P1. The higher reduction in TOC than in DOC again suggests desorption of sorbed organic carbon. However, this release of OC does not increase available surface for metalaxyl adsorption in this poorly sorptive soil. In the clay soils P2 and P3, TOC remained unaffected with aging, whereas DOC was highly reduced (80 and 70% in soils P2 and P3, respectively), suggesting an increase in adsorption of DOC from AL with time. This increase in adsorption of DOC significantly ($P < 0.05$) increases metalaxyl adsorption on P2 amended with AL due to generation of new organic surfaces, whereas no significant differences ($P < 0.05$) upon incubation of AL were observed in P3. These results reveal the heterogeneity of soil mineral surfaces with respect to adsorption of polar compounds. Soils P2 and P3 both have very high clay contents, although different clay mineralogies: P2 is rich in montmorillonite (34%), and P3 is rich in illite (56%). DOC sorption from AL to highly sorptive illite of P3 might block metalaxyl mineral sorption sites, which would be compensated by the generation of new organic surfaces upon DOC sorption, rendering adsorption capacities similar to those at $t = 0$ rather than at $t = 2$.

Tricyclazole adsorption significantly increases ($P < 0.05$) with aging ($t = 2$) in all three soils amended with SF (Table

4), despite there being no changes in the amount of TOC or DOC after 2 months of incubation in soils P1 and P2 and despite the decrease in TOC observed in soil P3 + SF with aging (Table 2). These changes can be attributed to changes in the quality of OM increasing the adsorption capacity for organic molecules. As shown in Table 2, the significant increase ($P < 0.05$) in HIX with incubation time observed for DOC from P3 + SF (Table 2) indicates that although the TOC is reduced, sorbed organic matter must be more humified and have a higher adsorption capacity for tricyclazole.

Changes in the adsorption of tricyclazole with incubation of LF and AL amendments, of very high DOC content, greatly differ from those observed for metalaxyl. When soils amended with LF were incubated for 2 months, adsorption significantly decreased ($P < 0.05$) in soils P1 and P2. It should be noted that in P2 adsorption is even lower than in unamended soil. This can be attributed to the decrease in TOC (Table 2), reducing hydrophobic interactions between tricyclazole and OM, which, on the contrary as for metalaxyl, are more important that the increase in available mineral adsorption sites when adsorbed organic matter desorbs into solution. On the contrary, as in soils P1 and P2, in the clay soil P3, K_f significantly increases from 15.4 to 26.5 (Table 4) despite the decrease in TOC (Table 2). This increase again shows the different adsorption behaviors of the two clay soils (P2 and P3) used in this study. The high adsorption capacity of the mineral surfaces of soil P3 and the importance of the "quality" of soil surfaces in adsorption is also revealed, and both seem to compensate the decrease in OM at least at low concentrations. In relation to this, Roldán et al. (52) reported previously that mineral surfaces can also account for tricyclazole adsorption. The lower n_f coefficient for tricyclazole in P3 + LF at $t = 2$ when compared to $t = 0$ (0.75 versus 0.95) suggests that the increase in adsorption upon amendment is more important at low solution concentrations.

With incubation time, adsorption of tricyclazole experiences a small but significant ($P < 0.05$) increase in P1 + AL and a small significant ($P < 0.05$) decrease in P2 + AL, whereas there is a large increase (significant at $P < 0.05$) in K_f for tricyclazole on P3 + AL (Table 4). As discussed above, TOC remains unaffected in soil P3, whereas DOC decreases with aging of AL in the soil, which has been attributed to an increase in adsorption of DOC from AL with incubation time. On the contrary, as with metalaxyl, in the case of the less polar tricyclazole, DOC molecules coating mineral surfaces produce an increase in hydrophobic surfaces that further sorb tricyclazole.

Our results indicate that changes in soil organic matter upon amendment depend on the nature of the organic amendment, the quality of the soil surfaces, and changes in organic matter with residence time in the soil. Changes in adsorption of two fungicides, differing mainly in the most important soil component affecting their adsorption (mineral surfaces, in the case of metalaxyl, and organic surfaces, in the case of tricyclazole), upon soil amendment, depend on soil characteristics, interactions between DOC and soil mineral surfaces (depending on the nature of DOM and on clay mineralogy), and changes in the quality and quantity of TOC and DOC upon immediate mixing as well as after incubation.

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