Influence of pine or oak wood on the degradation of alachlor and metalaxyl in soil

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Abstract

The objective of this work was to study the influence pine or oak wood added to soil as an amendment (5% w/w) had on the degradation rate of two pesticides, alachlor and metalaxyl, with different hydrophobic character. The formation of pesticide metabolites and the soil dehydrogenase activity in non-amended and amended soil samples were also monitored. The degradation of metalaxyl followed first-order kinetics, while the degradation of alachlor followed first-order or biphasic kinetics in the soil samples studied. The results indicated that the degradation rate was slower for metalaxyl than for alachlor, and for both pesticides followed the order: pine amended soil < oak amended soil < non-amended soil. The faster degradation rate in non-amended soil was attributed to the higher sorption of pesticides by wood amended soils.

The alachlor ethane sulfonic acid (ESA), and two metalaxyl metabolites (2-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid and N-(2,6-dimethyl-phenyl)-2-methoxy-acetamide) were detected during the incubation period. Soil dehydrogenase activity recorded close values in non-amended and amended soil treated with alachlor, but it was higher in wood amended soil treated with metalaxyl. Pine and oak wood increase the immobilization of the pesticides studied, but they also limit their bioavailability in soil by decreasing their degradation rate in amended soil.

Keywords: pesticide, metabolite, degradation, soil, wood residue, remediation.

Abbreviations: S, soil; SS, sterilised soil; S+P, soil amended with pine wood; S+O, soil amended with oak wood.
1. Introduction

Alachlor is a herbicide used in pre-emergence to control perennial grasses and many broad-leaved weeds in corn and other crops. Given its widespread use, alachlor has been found in soils and in both surface water and groundwater (Sánchez-Camazano et al., 2005; Vryzas et al., 2009). Soil organic matter (OM) is the major factor for alachlor adsorption, and it is sorbed to a lesser extent by clay colloids (Guo et al., 1993). Biodegradation is the single most significant mechanism for controlling the dissipation of alachlor in agricultural soils. Biological degradation by dechlorination in soil leads to the formation of the ethane sulfonic acid (ESA) metabolite (Stamper et al., 1998). Alachlor ESA is a more polar compound than alachlor and has been found in groundwater and in surface water more often and in higher concentrations than the parent herbicide.

Metalaxyl is a systemic acylanilide fungicide widely applied in different crops to eliminate different fungal species. Metalaxyl is highly soluble in water and has a low hidrophobicity, indicating low adsorption by soils and the possibility of leaching into groundwater. Metalaxyl has recently been found in groundwater at concentrations of up to 0.49 µg L$^{-1}$, which exceeds the 0.1 µg L$^{-1}$ EU limit (Hildebrandt et al., 2008). Several authors have indicated the importance of OM and clay content in the adsorption of metalaxyl by soils (Andrades et al., 2001). Its degradation in soil has been reported mainly as biodegradation (Sukul and Spiteller, 2000). Metalaxyl is degraded in soil by cleavage of the methyl ester group, forming the main acid metabolite (CGA-62826), although biodegradation of metalaxyl could occur by benzylic hydroxylation of the methyl chain or aromatic hydroxylation. A second metabolite (CGA 67868) is formed either directly from metalaxyl or from the metabolite CGA 62826 by N dealkylation. (Pesaro et al., 2004).
Recent work has shown that the water pollution caused by pesticides from point sources (spills, uncontrolled disposal, equipment washing water, etc.) can be more serious than that due to agricultural practice (De Wilde et al., 2007; Fait et al., 2007). The use of organic materials or wastes may prevent the mobility of pesticides from these point sources of contamination and enhance their biodegradation (Rodriguez-Cruz et al., 2007a). In recent years, different low-cost adsorbent systems (biobed, biomassbed, biofilter, etc.) have been developed to minimize point sources of pesticide pollution by retaining and degrading pesticides (Castillo et al., 2008; De Wilde et al., 2007; Fait et al., 2007). In particular, the use of wood residues as low-cost adsorbents has recently been developed as a new technology for the immobilization of heavy metals, dyes, pesticides, other organic compounds, etc., in soil (Gupta et al., 2009; Rodriguez-Cruz et al., 2007b; Shukla et al., 2002). In a recent study, Rodriguez-Cruz et al. (2007b) found that oak and pine wood can be effectively used as adsorbents of pesticides. The Freundlich constants (Kf) for alachlor and metalaxyl adsorption by oak and pine were related to the lignin content of these woods. However, the influence the addition of wood residues had on the degradation of pesticides in soils has been less studied (Grenni et al., 2009). The addition of an organic amendment to the soil affects the biodegradation of pesticides because the OM and nutrients added can strongly affect the structure and activity of bacterial and fungal populations as a result of the increased metabolism of the readily available nutrients (Briceño et al., 2007). Some organic amendments may stimulate biodegradation, but others can reduce it (Moorman et al., 2001; Rodriguez-Cruz and Lacorte, 2005).

The main objective of this work was to investigate the effects pine and oak wood added to soil as amendments had on the rates of degradation of alachlor and metalaxyl. These pesticides have different hydrophobic characters and water solubility and are
widely used in agriculture. Soil dehydrogenase activity was monitored in soil treated with alachlor or metalaxyl and amended with either pine or oak residues or non-amended in order to analyze the effect of the amendment and the pesticide on the microbial activity in the soil. The findings of this study provide information regarding the use of wood amendments for preventing soil and water contamination by pesticides with different characteristics.

2. Materials and methods

2.1. Soil and wood samples

Soil samples were collected from the surface layer (0-15 cm depth) of an agricultural field located in Aldearrubia (Salamanca, Spain). The soil was left overnight at room temperature to reduce moisture content and then sieved (< 2 mm). The soil was sandy-loam (11.8% clay, 13.6% silt and 74.5% sand), with 0.85% organic matter content, a pH of 6.3, and a cation exchange capacity of 4.8 cmol kg\(^{-1}\) (Rodríguez-Cruz et al., 2007a).

Pine and oak sawdust (< 1 mm) were obtained from a local industry in Salamanca (Spain). They had different lignin contents: 18.2% for oak and 24.4% for pine (Rodriguez-Cruz et al., 2007b).

The amended soils were prepared by uniformly mixing soil with oak or pine sawdust (5% w/w), similarly to other organic residues (Moorman et al., 2001). Sub-samples were analyzed to assess both the total organic carbon (TOC) and the soluble carbon contents, as described elsewhere (Rodríguez-Cruz et al., 2007b). The TOC content in the soil amended with pine (S+P) or oak (S+O) was 2.89% and 2.79%, respectively, and about fourfold greater than in non-amended soil (S) (0.72%).
Moreover, the soluble carbon content was higher in S+P (0.047%) and S+O (0.037%) than in S (0.008%). Sample pH varied between 5.9 (S+O) and 6.6 (S+P).

2.2. Chemicals

Alachlor (99.5% purity) was supplied by Chem Service (West Chester, USA). It is a herbicide with a water solubility of 240 µg mL\(^{-1}\) (pH 7, 20ºC) and a log \(K_{ow}\) of 2.63. The alachlor ESA was supplied by Monsanto Chemical Co. (St. Louis, MO, USA).

Metalaxyl (>98% purity) was supplied by Novartis Crop Protection AG (Basel, Switzerland). It is a fungicide with a water solubility of 8400 µg mL\(^{-1}\) (22ºC) and a log \(K_{ow}\) of 1.75 (Tomlin, 2003). The two metalaxyl metabolites studied (> 99% purity), 2-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid (CGA62826) and N-(2,6-dimethylphenyl)-2-methoxy-acetamide (CGA92370), were supplied by Syngenta Crop Protection AG (Münchwilen, Switzerland).

2.3. Degradation experiments with amended and non-amended soil

The pesticide degradation experiment was conducted in duplicate in accordance with SETAC guidelines (Lynch, 1995). The standard compound was added to soil (200 g) to obtain a final pesticide concentration of 1 mg kg\(^{-1}\). The final moisture content of the soils was adjusted to 60% of their maximum water holding capacity. Some soil samples were first sterilised by autoclaving at 120 ± 2 ºC for 20 min on two consecutive days (SS), and then treated with alachlor or metalaxyl, other soil samples (S) were treated only with alachlor or metalaxyl and others were treated with both pesticide and pine (S+P) or oak (S+O) sawdust. The soils were maintained in Erlenmeyer flasks plugged with sterilized cotton wrapped in gauze to allow air exchange. Soil moisture was kept constant throughout the experiments by periodic weighing and the replacement
of any losses with sterile water. Samples were incubated at 20 ± 0.5 °C in the dark. Solutions and instruments were sterilised and all steps were performed in a sterile cabinet.

2.4. Chemical analysis

Alachlor and metalaxyl and their metabolites were measured immediately after treatment and at different sampling times (0, 1, 2, 3, 6, 8, 10, 14, 20, 28, 51, 70, and 98 days). Two soil replicates (1 g) were taken from each microcosm and shaken with 5 mL of methanol for 24 h at 20°C for residue analysis. Samples were centrifuged and 4 mL of each supernatant were evaporated under an air stream using an Evaporator EVA-EC2-L (VLMGmbH, Bielefeld, Germany) and re-dissolved in 0.5 mL of methanol for analysis. The quantitative determination of alachlor, metalaxyl and their metabolites was performed by HPLC-DAD-MS (Waters Assoc., Milford MA, USA). A Waters Symmetry C18 (75 mm × 4.6 mm I.D., 3.5µm) column was used at ambient temperature. The mobile phase was 70:30 acetonitrile/water for metalaxyl and its metabolites and 80:20 acetonitrile/water for alachlor, and the flow rate of the mobile phase was 0.4 mL min⁻¹. The mobile phase was 90:10 acetonitrile/water for alachlor ESA with a flow rate of 0.5 mL min⁻¹. The sample injection volume was 10 µL. Detection by HPLC-DAD was at 196 nm for alachlor, 205 nm for alachlor ESA, and 194 nm for metalaxyl and its metabolites, and detection by HPLC-MS to confirm the identity of these compounds was carried out by monitoring the positive molecular ion (m/z) 238.2 for alachlor, 280.3 for metalaxyl, 266.2 for CGA62826 and 194.2 for CGA92370 and the negative molecular ion (m/z) 314 for alachlor ESA.

2.5. Soil dehydrogenase activity
Soil dehydrogenase activity considered as overall soil microbial activity was measured using the Tabatabai method (Tabatabai, 1994).

2.6. Statistical analysis of the data

The data obtained were subjected to analysis of variance. Standard deviation (SD) was used to indicate variability among replicates in the determination of pesticides or their metabolites and the least significant difference (LSD), at a confidence level of 95%, was determined to evaluate the effects of different soil treatments on dehydrogenase activity. The statistical software Statgraphics Plus version 5.1 (Statgraphics Plus Corp., Princeton, NJ, USA) was used.

3. Results and discussion

3.1. Degradation kinetics of alachlor and metalaxyl and metabolite formation

Figure 1 shows the degradation kinetics for alachlor and metalaxyl in non-amended soil (S), non-amended and sterilised soil (SS) and soil amended with pine (S+P) or oak (S+O). The data are plotted as residual concentrations of the pesticide (percentage of pesticide initially applied) against the time of incubation of each soil.

The degradation of alachlor followed first-order kinetics in the SS, S+O and S+P samples, but followed a biphasic pattern in the S sample with a rapid first phase and a slow second phase of degradation (Figure 1). The half-life ($t_{1/2}$) of alachlor in non-amended soil was calculated in the first phase because degradation was rapid (>90% in this first phase). The $t_{1/2}$ was 3.2 days and increased to a lesser extension in oak amended soil (16.7 days) and to a greater extension in pine amended soil (151 days) (Table 1). The degradation of alachlor in S+P was slower (<50% at the end of the
experiment) and the t_{1/2} value should be considered with caution given that only an r^2 of 0.53 was reached. A slight degradation was observed in the sterilised soil, which might be caused by chemical and abiotic factors other than photodegradation, as the soil samples were kept in the dark during the incubation period.

The higher t_{1/2} observed in the amended soils could be due to the higher TOC content of these soils compared to the non-amended soil. Given that alachlor is adsorbed mainly by the soil OM, its degradation is expected to depend on the OC content of the soil. Several authors have suggested that OM could play an important role in enhancing alachlor sorption by an amended soil (Dorado et al., 2005). Wood residues could enhance herbicide adsorption, decreasing the bioavailability and increasing the t_{1/2} of alachlor in amended soils. In addition, a higher adsorption of alachlor by soil amended with pine occurs due to the lignin content of the wood, which influences the sorption of pesticides by soils (Kf values were 22.4 and 41.4 for the adsorption of alachlor by oak and pine wood, respectively), as reported previously (Rodriguez-Cruz et al., 2007b). Other authors have also indicated that the addition of amendment (sewage sludge) to soil led to a decrease in alachlor degradation (Rodriguez-Cruz and Lacorte, 2005).

Concentrations of alachlor ESA were detected simultaneously to parent compound in non-sterile conditions. The evolution of this metabolite during the incubation period is included in Figure 2. The maximum amount detected was 36.7 µg kg^{-1} dry soil in S after 14 days. The amount then decreased, possibly due to the degradation of alachlor ESA and the low input of new metabolite in accordance with the residual parent compound (less than 20% of the original pesticide applied). In the S+O sample, a relative increase in alachlor ESA was recorded at 50 days of incubation after a rapid degradation of the parent compound (close to 30%) occurred between 28 and 70
days of incubation. This compound was not subsequently degraded at the end of the experiment (70 days). Lower amounts of this metabolite were found in the S+P sample according to a lower degradation of alachlor in this soil (Figure 1).

The degradation of metalaxyl in the non-amended and amended non-sterilised soil samples studied followed first-order kinetics (Table 1). The results indicated that no metalaxyl degradation occurred in the non-amended and sterilised soil (Figure 1). This suggests a microbial role in degrading this fungicide. The $t_{1/2}$ values indicated a slower degradation of metalaxyl in the S+P (144 days) or S+O (68.6 days) samples compared with the S (29.1 days) sample. As indicated for alachlor, an increase in metalaxyl adsorption by the amended soils occurs, with the fungicide being less available to degradation. The adsorption by pine amended soil could be higher than by oak amended soil according to the Kf values of metalaxyl by pine (8.28) and oak (4.95) wood (Rodriguez-Cruz et al., 2007b). Similarly, Fernandes et al. (2006) reported an increase in the $t_{1/2}$ of metalaxyl in a sandy soil with different organic amendments due to the increase in sorption, with the fungicide being protected from degradation.

The two metalaxyl metabolites studied (CGA62826 and CGA92370) were detected in the non-sterilised non-amended and amended soils (Figure 2). Concentrations of CGA62826 (acid metabolite) were higher than those of CGA92370. The maximum amounts of CGA62826 metabolite were 91.5 (S), 175 (S+O) and 240 (S+P) µg kg$^{-1}$ dry soil at 51, 70 and 98 days, respectively. For CGA92370 metabolite, the maximum amounts of 23.5 (S), 2.25 (S+O) and 2.61 (S+P) µg kg$^{-1}$ dry soil were recorded at 70, 51 and 6 days, respectively (Figure 2). The faster degradation of metalaxyl in the non-amended soil gave rise to a higher production of CGA92370 in comparison with the amended soils.
The dehydrogenase activity of non-amended and amended soils treated with alachlor or metalaxyl for different incubation times was monitored as an indicator of the overall microbial activity of the soils to determine the pesticide’s possible side effects on microbiological activity in the soil (Sukul, 2006) (Figure 3).

Soil dehydrogenase activity recorded a similar behaviour in non-amended and amended soil for soil samples treated with alachlor. The values were not significantly different (LSD=36.0, p<0.1). However, significant differences were noted for time (LSD=55.0, p<0.05). A maximum value of dehydrogenase activity was detected on day 14 for all soils and this was followed by a decrease from day 28 up to the end of the experiment on day 70. The increase was sharper in wood amended soils than in non-amended soil reaching values of 162, 227 and 277 µg TPF g\(^{-1}\) dry soil for S, S+P and S+O, respectively (Figure 3). The increase in the dehydrogenase activity observed at 14 days may correspond to a maximum activity of microorganisms to degrade alachlor in non-amended soil. A peak in the amount of its metabolite alachlor ESA was detected at this time (Figure 2), indicating that microbial growth was stimulated by the use of alachlor as a source of energy. Sukul (2006) reported that soil dehydrogenase activity is likely to be affected by pesticides. However the activity of microorganisms in amended soils might also be affected by the wood amendment as an additional carbon source. Some authors have noted the positive influence of organic amendments on the dehydrogenase activity of the microbial community (Moorman et al., 2001; Delgado-Moreno and Peña, 2007; Grenni et al., 2009).

The soil dehydrogenase activity in amended and non-amended soils treated with metalaxyl increased in the order S≤S+P<S+O (LSD=14.8, p<0.001). However, this activity was not significantly different over the entire incubation period for either non-
amended or amended soils (Figure 3). In a previous study, Pesaro et al. (2004) reported that changes in specific activities (e.g., pesticide degradation) are not necessarily reflected by bulk microbial activities such as dehydrogenase activity.

4. Conclusions

The use of wood residues to immobilize pesticides in soils affects the degradation kinetics of these compounds as shown in this study. The addition of a pine or oak wood amendment to the soil caused a significant reduction in the degradation rate of alachlor and metalaxyl. The specific activity of the microorganisms degrading the pesticides was negatively affected by the wood amendments due to the increased adsorption of pesticides by woods and the decreased bioavailability of the pesticide to be degraded. The results indicate that the use of wood amendments could be effective for limiting the leaching of pesticides in the soil, although the adsorption capacity of the amended soils should be taken into account, since it could decrease the degradation rate of these compounds. Further knowledge on the role wood residues play in influencing the degradation of pesticides in the soil will provide a better understanding of the bioavailability and potential toxicity of these contaminants and their metabolites.

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References


Table captions

Table 1. Kinetic equation, correlation coefficient and half-life values of alachlor and metalaxyl in non-amended (sterilised-SS and non-sterilised-S) and pine (S+P) or oak (S+O) amended soils.

Figure captions

Figure 1. Degradation kinetics of alachlor and metalaxyl in sterilised soil (SS), non-sterilised soil (S) and soil amended with pine (S+P) or oak (S+O) woods. Bars indicate the standard deviation of the replicates (n = 4).

Figure 2. Formation of alachlor ESA and metalaxyl metabolites CGA 62826 and CGA 92370 in non-sterilised soil (S) and soil amended with pine (S+P) or oak (S+O) woods. Bars indicate the standard deviation of the replicates (n = 4).

Figure 3. Dehydrogenase activity of non-amended (S) and amended soils (S+P and S+O) treated with alachlor and with metalaxyl at different incubation times. Bars indicate the standard error of the replicates (n = 2).
Table 1. Kinetic equation, correlation coefficient and half-life values of alachlor and metalaxyl in non-amended (sterilised-SS and non-sterilised-S) and pine (S+P) or oak (S+O) amended soils.

<table>
<thead>
<tr>
<th>Pesticide/Treatment</th>
<th>Kinetic equation</th>
<th>$r^2$</th>
<th>$t_{1/2}$ (d)</th>
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</thead>
<tbody>
<tr>
<td>Alachlor</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SS</td>
<td>$y = 98.935 e^{(-0.0035x)}$</td>
<td>0.78</td>
<td>198</td>
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<tr>
<td>S$^a$</td>
<td>$y = 101.65 e^{(-0.2187x)}$</td>
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<td>3.17</td>
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<td>S+P</td>
<td>$y = 76.54 e^{(-0.0046x)}$</td>
<td>0.53</td>
<td>151</td>
</tr>
<tr>
<td>S+O</td>
<td>$y = 90.738 e^{(-0.0414x)}$</td>
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</tr>
<tr>
<td>Metalaxyl</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>-</td>
<td>-</td>
<td>nd$^b$</td>
</tr>
<tr>
<td>S</td>
<td>$y = 108.75 e^{(-0.0238x)}$</td>
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<td>29.1</td>
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<td>S+P</td>
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<td>S+O</td>
<td>$y = 94.613 e^{(-0.0101x)}$</td>
<td>0.96</td>
<td>68.6</td>
</tr>
</tbody>
</table>

$^a$First-order equation calculated for the first degradation phase (0-6 days); $^b$nd, no degradation.
Figure 1
Figure 2.
Fig. 3.