1

2

3

4   **Influence of pine or oak wood on the degradation of alachlor and**

5   **metalaxyl in soil**

6

7   **M. Sonia Rodríguez-Cruz*, Jesús M. Marín-Benito, José M. Ordax, Hanane**

8   **Azejjel, María J. Sánchez-Martín**

9

10   Institute of Natural Resources and Agrobiology of Salamanca, IRNASA-CSIC, P.O.

11   Box 257, 37071 Salamanca, Spain

12

13   *Corresponding author: Telephone: (+34) 923219606; Fax: (+34) 923219609. E-mail

14   address: msonia.rodriguez@irnasa.csic.es (M.S. Rodríguez-Cruz).

15   **Abstract**

16   The objective of this work was to study the influence pine or oak wood added to

17   soil as an amendment (5% w/w) had on the degradation rate of two pesticides, alachlor

18   and metalaxyl, with different hydrophobic character. The formation of pesticide

19   metabolites and the soil dehydrogenase activity in non-amended and amended soil

20   samples were also monitored. The degradation of metalaxyl followed first-order

21   kinetics, while the degradation of alachlor followed first-order or biphasic kinetics in

22   the soil samples studied. The results indicated that the degradation rate was slower for

23   metalaxyl than for alachlor, and for both pesticides followed the order: pine amended

24   soil < oak amended soil < non-amended soil. The faster degradation rate in non-

25   amended soil was attributed to the higher sorption of pesticides by wood amended soils.

26   The alachlor ethane sulfonic acid (ESA), and two metalaxyl metabolites (2-[(2,6-

27   dimethylphenyl)-methoxyacetylamino]-propionic acid and N-(2,6-dimethyl-phenyl)-2-

28   methoxy-acetamide) were detected during the incubation period. Soil dehydrogenase

29   activity recorded close values in non-amended and amended soil treated with alachlor,

30   but it was higher in wood amended soil treated with metalaxyl. Pine and oak wood

31   increase the immobilization of the pesticides studied, but they also limit their

32   bioavailability in soil by decreasing their degradation rate in amended soil.

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

34

35   **Abbreviations:** S, soil; SS, sterilised soil; S+P, soil amended with pine wood; S+O, soil

36   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).

62   Recent work has shown that the water pollution caused by pesticides from point

63   sources (spills, uncontrolled disposal, equipment washing water, etc.) can be more

64   serious than that due to agricultural practice (De Wilde *et al.*, 2007; Fait *et al.*, 2007).

65   The use of organic materials or wastes may prevent the mobility of pesticides from

66   these point sources of contamination and enhance their biodegradation (Rodriguez-Cruz

67   *et al.*, 2007a). In recent years, different low-cost adsorbent systems (biobed,

68   biomassbed, biofilter, etc.) have been developed to minimize point sources of pesticide

69   pollution by retaining and degrading pesticides (Castillo *et al.*, 2008; De Wilde *et al.*,

70   2007; Fait *et al.*, 2007). In particular, the use of wood residues as low-cost adsorbents

71   has recently been developed as a new technology for the immobilization of heavy

72   metals, dyes, pesticides, other organic compounds, etc., in soil (Gupta *et al.*, 2009;

73   Rodríguez-Cruz *et al.*, 2007b; Shukla *et al.*, 2002). In a recent study, Rodríguez-Cruz *et

74   al.* (2007b) found that oak and pine wood can be effectively used as adsorbents of

75   pesticides. The Freundlich constants (Kf) for alachlor and metalaxyl adsorption by oak

76   and pine were related to the lignin content of these woods. However, the influence the

77   addition of wood residues had on the degradation of pesticides in soils has been less

78   studied (Grenni *et al.*, 2009). The addition of an organic amendment to the soil affects

79   the biodegradation of pesticides because the OM and nutrients added can strongly affect

80   the structure and activity of bacterial and fungal populations as a result of the increased

81   metabolism of the readily available nutrients (Briceño *et al.*, 2007). Some organic

82   amendments may stimulate biodegradation, but others can reduce it (Moorman *et al.*,

83   2001; Rodriguez-Cruz and Lacorte, 2005).

84   The main objective of this work was to investigate the effects pine and oak wood

85   added to soil as amendments had on the rates of degradation of alachlor and metalaxyl.

86   These pesticides have different hydrophobic characters and water solubility and are

87 widely used in agriculture. Soil dehydrogenase activity was monitored in soil treated

88 with alachlor or metalaxyl and amended with either pine or oak residues or non-

89 amended in order to analyze the effect of the amendment and the pesticide on the

90 microbial activity in the soil. The findings of this study provide information regarding

91 the use of wood amendments for preventing soil and water contamination by pesticides

92 with different characteristics.

93

94 **2. Materials and methods**

95

96 *2.1. Soil and wood samples*

97   Soil samples were collected from the surface layer (0-15 cm depth) of an

98 agricultural field located in Aldearrubia (Salamanca, Spain). The soil was left overnight

99 at room temperature to reduce moisture content and then sieved (< 2 mm). The soil was

100 sandy-loam (11.8% clay, 13.6% silt and 74.5% sand), with 0.85% organic matter

101 content, a pH of 6.3, and a cation exchange capacity of 4.8 cmol kg^{-1} (Rodríguez-Cruz

102 *et al.*, 2007a).

103   Pine and oak sawdust (< 1 mm) were obtained from a local industry in

104 Salamanca (Spain). They had different lignin contents: 18.2% for oak and 24.4% for

105 pine (Rodríguez -Cruz *et al.*, 2007b).

106   The amended soils were prepared by uniformly mixing soil with oak or pine

107 sawdust (5% w/w), similarly to other organic residues (Moorman *et al.*, 2001). Sub-

108 samples were analyzed to assess both the total organic carbon (TOC) and the soluble

109 carbon contents, as described elsewhere (Rodríguez-Cruz *et al.*, 2007b). The TOC

110 content in the soil amended with pine (S+P) or oak (S+O) was 2.89% and 2.79%,

111 respectively, and about fourfold greater than in non-amended soil (S) (0.72%).

112   Moreover, the soluble carbon content was higher in S+P (0.047%) and S+O (0.037%)

113   than in S (0.008%). Sample pH varied between 5.9 (S+O) and 6.6 (S+P).

114

115   *2.2. Chemicals*

116   Alachlor (99.5% purity) was supplied by Chem Service (West Chester, USA). It

117   is a herbicide with a water solubility of 240 µg mL^{-1} (pH 7, 20ºC) and a log K_{ow} of 2.63.

118   The alachlor ESA was supplied by Monsanto Chemical Co. (St. Louis, MO, USA).

119   Metalaxyl (>98% purity) was supplied by Novartis Crop Protection AG (Basel,

120   Switzerland). It is a fungicide with a water solubility of 8400 µg mL^{-1} (22°C) and a log

121   K_{ow} of 1.75 (Tomlin, 2003). The two metalaxyl metabolites studied (> 99% purity), 2-

122   [(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid (CGA62826) and N-(2,6-

123   dimethylphenyl)-2-methoxy-acetamide (CGA92370), were supplied by Syngenta Crop

124   Protection AG (Münchwilen, Switzerland).

125

126   *2.3. Degradation experiments with amended and non-amended soil*

127   The pesticide degradation experiment was conducted in duplicate in accordance

128   with SETAC guidelines (Lynch, 1995). The standard compound was added to soil (200

129   g) to obtain a final pesticide concentration of 1 mg kg^{-1}. The final moisture content of

130   the soils was adjusted to 60% of their maximum water holding capacity. Some soil

131   samples were first sterilised by autoclaving at 120 ± 2 °C for 20 min on two consecutive

132   days (SS), and then treated with alachlor or metalaxyl, other soil samples (S) were

133   treated only with alachlor or metalaxyl and others were treated with both pesticide and

134   pine (S+P) or oak (S+O) sawdust. The soils were maintained in Erlenmeyer flasks

135   plugged with sterilized cotton wrapped in gauze to allow air exchange. Soil moisture

136   was kept constant throughout the experiments by periodic weighing and the replacement

137 of any losses with sterile water. Samples were incubated at 20 ± 0.5 °C in the dark.

138 Solutions and instruments were sterilised and all steps were performed in a sterile

139 cabinet.

140

141 *2.4. Chemical analysis*

142   Alachlor and metalaxyl and their metabolites were measured immediately after

143 treatment and at different sampling times (0, 1, 2, 3, 6, 8, 10, 14, 20, 28, 51, 70, and 98

144 days). Two soil replicates (1 g) were taken from each microcosm and shaken with 5 mL

145 of methanol for 24 h at 20°C for residue analysis. Samples were centrifuged and 4 mL

146 of each supernatant were evaporated under an air stream using an Evaporator EVA-

147 EC2-L (VLMGmbH, Bielefeld, Germany) and re-dissolved in 0.5 mL of methanol for

148 analysis. The quantitative determination of alachlor, metalaxyl and their metabolites

149 was performed by HPLC-DAD-MS (Waters Assoc., Milford MA, USA). A Waters

150 Symmetry C18 (75 mm x 4.6 mm I.D., 3.5μm) column was used at ambient

151 temperature. The mobile phase was 70:30 acetonitrile/water for metalaxyl and its

152 metabolites and 80:20 acetonitrile/water for alachlor, and the flow rate of the mobile

153 phase was 0.4 mL min^{-1}. The mobile phase was 90:10 acetonitrile/water for alachlor

154 ESA with a flow rate of 0.5 mL min^{-1}. The sample injection volume was 10 μL.

155 Detection by HPLC-DAD was at 196 nm for alachlor, 205 nm for alachlor ESA, and

156 194 nm for metalaxyl and its metabolites, and detection by HPLC-MS to confirm the

157 identity of these compounds was carried out by monitoring the positive molecular ion

158 (m/z) 238.2 for alachlor, 280.3 for metalaxyl, 266.2 for CGA62826 and 194.2 for

159 CGA92370 and the negative molecular ion (m/z) 314 for alachlor ESA.

160

161 *2.5. Soil dehydrogenase activity*

162   Soil dehydrogenase activity considered as overall soil microbial activity was

163   measured using the Tabatabai method (Tabatabai, 1994).

164

165   *2.6. Statistical analysis of the data*

166   The data obtained were subjected to analysis of variance. Standard deviation

167   (SD) was used to indicate variability among replicates in the determination of pesticides

168   or their metabolites and the least significant difference (LSD), at a confidence level of

169   95%, was determined to evaluate the effects of different soil treatments on

170   dehydrogenase activity. The statistical software Statgraphics Plus version 5.1

171   (Statgraphics Plus Corp., Princenton, NJ, USA) was used.

172

173   **3. Results and discussion**

174

175   *3.1. Degradation kinetics of alachlor and metalaxyl and metabolite formation*

176   Figure 1 shows the degradation kinetics for alachlor and metalaxyl in non-

177   amended soil (S), non-amended and sterilised soil (SS) and soil amended with pine

178   (S+P) or oak (S+O). The data are plotted as residual concentrations of the pesticide

179   (percentage of pesticide initially applied) against the time of incubation of each soil.

180   The degradation of alachlor followed first-order kinetics in the SS, S+O and S+P

181   samples, but followed a biphasic pattern in the S sample with a rapid first phase and a

182   slow second phase of degradation (Figure 1). The half-life ($t_{1/2}$) of alachlor in non-

183   amended soil was calculated in the first phase because degradation was rapid (>90% in

184   this first phase). The $t_{1/2}$ was 3.2 days and increased to a lesser extension in oak

185   amended soil (16.7 days) and to a greater extension in pine amended soil (151 days)

186   (Table 1). The degradation of alachlor in S+P was slower (<50% at the end of the

187 experiment) and the $t_{1/2}$ value should be considered with caution given that only an r^2 of

188 0.53 was reached. A slight degradation was observed in the sterilised soil, which might

189 be caused by chemical and abiotic factors other than photodegradation, as the soil

190 samples were kept in the dark during the incubation period.

191   The higher $t_{1/2}$ observed in the amended soils could be due to the higher TOC

192 content of these soils compared to the non-amended soil. Given that alachlor is

193 adsorbed mainly by the soil OM, its degradation is expected to depend on the OC

194 content of the soil. Several authors have suggested that OM could play an important role

195 in enhancing alachlor sorption by an amended soil (Dorado *et al.*, 2005). Wood residues

196 could enhance herbicide adsorption, decreasing the bioavailability and increasing the $t_{1/2}$

197 of alachlor in amended soils. In addition, a higher adsorption of alachlor by soil

198 amended with pine occurs due to the lignin content of the wood, which influences the

199 sorption of pesticides by soils (Kf values were 22.4 and 41.4 for the adsorption of

200 alachlor by oak and pine wood, respectively), as reported previously (Rodriguez-Cruz *et*

201 *al.*, 2007b). Other authors have also indicated that the addition of amendment (sewage

202 sludge) to soil led to a decrease in alachlor degradation (Rodriguez-Cruz and Lacorte,

203 2005).

204   Concentrations of alachlor ESA were detected simultaneously to parent

205 compound in non-sterile conditions. The evolution of this metabolite during the

206 incubation period is included in Figure 2. The maximum amount detected was 36.7 µg

207 kg^{-1} dry soil in S after 14 days. The amount then decreased, possibly due to the

208 degradation of alachlor ESA and the low input of new metabolite in accordance with the

209 residual parent compound (less than 20% of the original pesticide applied). In the S+O

210 sample, a relative increase in alachlor ESA was recorded at 50 days of incubation after a

211 rapid degradation of the parent compound (close to 30%) occurred between 28 and 70

212   days of incubation. This compound was not subsequently degraded at the end of the

213   experiment (70 days). Lower amounts of this metabolite were found in the S+P sample

214   according to a lower degradation of alachlor in this soil (Figure 1).

215   The degradation of metalaxyl in the non-amended and amended non-sterilised

216   soil samples studied followed first-order kinetics (Table 1). The results indicated that no

217   metalaxyl degradation occurred in the non-amended and sterilised soil (Figure 1). This

218   suggests a microbial role in degrading this fungicide. The $t_{1/2}$ values indicated a slower

219   degradation of metalaxyl in the S+P (144 days) or S+O (68.6 days) samples compared

220   with the S (29.1 days) sample. As indicated for alachlor, an increase in metalaxyl

221   adsorption by the amended soils occurs, with the fungicide being less available to

222   degradation. The adsorption by pine amended soil could be higher than by oak amended

223   soil according to the Kf values of metalaxyl by pine (8.28) and oak (4.95) wood

224   (Rodriguez-Cruz *et al.*, 2007b). Similarly, Fernandes *et al.* (2006) reported an increase

225   in the $t_{1/2}$ of metalaxyl in a sandy soil with different organic amendments due to the

226   increase in sorption, with the fungicide being protected from degradation.

227   The two metalaxyl metabolites studied (CGA62826 and CGA92370) were

228   detected in the non-sterilised non-amended and amended soils (Figure 2).

229   Concentrations of CGA62826 (acid metabolite) were higher than those of CGA92370.

230   The maximum amounts of CGA62826 metabolite were 91.5 (S), 175 (S+O) and 240

231   (S+P) µg kg^{-1} dry soil at 51, 70 and 98 days, respectively. For CGA92370 metabolite,

232   the maximum amounts of 23.5 (S), 2.25 (S+O) and 2.61 (S+P) µg kg^{-1} dry soil were

233   recorded at 70, 51 and 6 days, respectively (Figure 2). The faster degradation of

234   metalaxyl in the non-amended soil gave rise to a higher production of CGA92370 in

235   comparison with the amended soils.

236

237   *3.2. Soil dehydrogenase activity*

238   The dehydrogenase activity of non-amended and amended soils treated with

239   alachlor or metalaxyl for different incubation times was monitored as an indicator of the

240   overall microbial activity of the soils to determine the pesticide´s possible side effects

241   on microbiological activity in the soil (Sukul, 2006) (Figure 3).

242   Soil dehydrogenase activity recorded a similar behaviour in non-amended and

243   amended soil for soil samples treated with alachlor. The values were not significantly

244   different (LSD=36.0, p<0.1). However, significant differences were noted for time

245   (LSD=55.0, p<0.05). A maximum value of dehydrogenase activity was detected on day

246   14 for all soils and this was followed by a decrease from day 28 up to the end of the

247   experiment on day 70. The increase was sharper in wood amended soils than in non-

248   amended soil reaching values of 162, 227 and 277 µg TPF g^{-1} dry soil for S, S+P and

249   S+O, respectively (Figure 3). The increase in the dehydrogenase activity observed at 14

250   days may correspond to a maximum activity of microorganisms to degrade alachlor in

251   non-amended soil. A peak in the amount of its metabolite alachlor ESA was detected at

252   this time (Figure 2), indicating that microbial growth was stimulated by the use of

253   alachlor as a source of energy. Sukul (2006) reported that soil dehydrogenase activity is

254   likely to be affected by pesticides. However the activity of microorganisms in amended

255   soils might also be affected by the wood amendment as an additional carbon source.

256   Some authors have noted the positive influence of organic amendments on the

257   dehydrogenase activity of the microbial community (Moorman *et al.*, 2001; Delgado-

258   Moreno and Peña, 2007; Grenni *et al.*, 2009).

259   The soil dehydrogenase activity in amended and non-amended soils treated with

260   metalaxyl increased in the order S≤S+P<S+O (LSD=14.8, p<0.001). However, this

261   activity was not significantly different over the entire incubation period for either non-

262   amended or amended soils (Figure 3). In a previous study, Pesaro *et al.* (2004) reported

263   that changes in specific activities (e.g., pesticide degradation) are not necessarily

264   reflected by bulk microbial activities such as dehydrogenase activity.

265

266   **4. Conclusions**

267   The use of wood residues to immobilize pesticides in soils affects the

268   degradation kinetics of these compounds as shown in this study. The addition of a pine

269   or oak wood amendment to the soil caused a significant reduction in the degradation

270   rate of alachlor and metalaxyl. The specific activity of the microorganisms degrading

271   the pesticides was negatively affected by the wood amendments due to the increased

272   adsorption of pesticides by woods and the decreased bioavailability of the pesticide to

273   be degraded. The results indicate that the use of wood amendments could be effective

274   for limiting the leaching of pesticides in the soil, although the adsorption capacity of the

275   amended soils should be taken into account, since it could decrease the degradation rate

276   of these compounds. Further knowledge on the role wood residues play in influencing

277   the degradation of pesticides in the soil will provide a better understanding of the

278   bioavailability and potential toxicity of these contaminants and their metabolites.

279

285

286   **References**

287 Andrades, M.S., Sánchez-Martín, M.J., Sánchez-Camazano, M., 2001. Significance of

288   soil properties in the adsorption and mobility of the fungicide metalaxyl in vineyard

289   soils. J. Agric. Food Chem. 49, 2363-2369.

290 Briceño, G., Palma, G., Durán, N., 2007. Influence of organic amendment on the

291   biodegradation and movement of pesticides. Crit. Rev. Environ. Sci. Technol. 37,

292   233-271.

293 Castillo, M.P., Torstensson, L., Stenström, J., 2008. Biobeds for environmental

294   protection from pesticide use-A review. J. Agric. Food Chem. 56, 6206-6219.

295 De Wilde, T., Spanoghe, P., Debaer, C., Ryckeboer, J., Springael, D., Jaeken, P., 2007.

296   Overview of on-farm bioremediation systems to reduce the occurrence of point source

297   contamination. Pest Manag. Sci. 63, 111-128.

298 Delgado-Moreno, L., Peña, A., 2007. Organic amendments from olive cake as a strategy

299   to modify the degradation of sulfonylurea herbicides in soil. J. Agric. Food Chem. 55,

300   6213-6218.

301 Dorado, J., Lopez-Fando, C., Zancada, M.C., Almendros, G., 2005. Sorption-desorption

302   of alachlor and linuron in a semiarid soil as influenced by organic matter properties

303   alter 16 years of periodic inputs. J. Agric. Food Chem. 53, 5359-5365.

304 Fait, G., Nicelli, M., Fragoulis, G., Trevisan, M., Capri, E., 2007. Reduction of point

305   contamination sources of pesticide from a vineyard farm. Environ. Sci. Technol. 41,

306   3302-3308.

307 Fernandes, M.C., Cox, L., Hermosin, M.C., Cornejo, J., 2006. Organic amendments

308   affecting sorption, leaching and dissipation of fungicides in soils. Pest Manag. Sci. 62,

309   1207-1215.

310   Grenni, P., Barra Caracciolo, A., Rodríguez-Cruz, M.S., Sánchez-Martín, M.J., 2009.

311   Changes in the microbial activity in a soil amended with oak and pine residues and

312   treated with linuron herbicide. Appl. Soil Ecol. 41, 2-7.

313   Guo, L., Bicki, T.J., Felsot, A., Hinesly, T.D., 1993. Sorption and movement of alachlor

314   in soil modified by carbon-rich wastes. J. Environ. Qual. 22, 186-194.

315   Gupta, V.K., Suhas, 2009. Application of low-cost adsorbents for dye removal.-A

316   review. J. Environ. Manag. 90, 2313-2342.

317   Hildebrandt, A., Guillamón, M., Lacorte, S., Tauler, R., Barcelo, D., 2008. Impact of

318   pesticides used in agriculture and vineyards to surface and groundwater quality (North

319   Spain). Water Res. 42, 3315-3326.

320   Lynch, M.R., 1995. Procedures for Assessing the Environmental Fate and Ecotoxicity

321   of Pesticides. Society of Environmental Toxicology and Chemistry (SETAC).

322   Moorman, T.B., Cowan, J.K., Arthur, E.L., Coats, J.R., 2001. Organic amendments to

323   enhance herbicide biodegradation in contaminated soils. Biol. Fertil. Soils 33, 541-

324   545.

325   Pesaro, M., Nicollier, G., Zeyer, J., Widmer, F., 2004. Impact of soil drying-rewetting

326   stress on microbial communities and activities on degradation of two crop protection

327   products. Appl. Environ. Microb. 70, 2577-2587.

328   Rodríguez-Cruz, S., Lacorte, S., 2005. Degradation of alachlor in natural and sludge-

329   amended soils, studied by gas and liquid chromatography coupled to mass-

330   spectrometry (GC-MS and HPLC-MS). J. Agric. Food Chem. 53, 9571-9577.

331   Rodríguez-Cruz, S., Sánchez-Martín, M.J., Andrades, M.S., Sánchez-Camazano, M.,

332   2007a. Modification of clay barriers with a cationic surfactant to improve the

333   retention of pesticides in soils. J. Hazar. Mat. 139, 363-372.

334   Rodríguez-Cruz, S., Andrades, M.S., Sánchez-Camazano, M., Sánchez-Martín, M.J.,

335   2007b. Relationship between the adsorption capacity of pesticides by wood residues

336   and the properties of woods and pesticides. Environ. Sci. Technol. 41, 3613-3619.

337   Sánchez-Camazano, M., Lorenzo, L.F., Sánchez-Martín, M.J., 2005. Atrazine and

338   alachlor inputs to surface and ground waters in irrigated corn cultivation areas of

339   Castilla-Leon region, Spain. Environ. Monit. Assess. 105, 11-24.

340   Shukla, A., Zhang, Y.-H., Dubey, P., Margrave, J.L., Shukla, S.S., 2002. The role of

341   sawdust in the removal of unwanted materials from water. J. Hazar. Mat. 95, 137-152.

342   Stamper, D.M., Tuovinen, O.H., 1998. Biodegradation of the acetanilide herbicides

343   alachlor, metolachlor, and propachlor. Crit. Rev. Microb. 24, 1-22.

344   Sukul, P. 2006. Enzymatic activities and microbial biomass in soil as influenced by

345   metalaxyl residues. Soil Biol. Biochem. 38, 320-326.

346   Sukul, P., Spiteller, M., 2000. Metalaxyl: Persistence, degradation, metabolism, and

347   analytical methods. Rev. Environ. Contam. Toxicol. 164, 1-26.

348   Tabatabai, M.A., 1994. Soil enzymes, in: Weaver, R.W. (Ed.), Methods of Soil

349   Analysis: Part 2. Microbiological and Biochemical Properties. Soil Science Society of

350   America, Madison, Wisconsin, USA, pp. 903–947.

351   Vryzas, Z., Vassiliou, G., Alexoudis, C., Papadopoulou-Mourkidou, E., 2009. Spatial

352   and temporal distribution of pesticide residues in surface waters in northeastern

353   Greece. Water Res. 43, 1-10.

354   **Table captions**

355

356   **Table 1.** Kinetic equation, correlation coefficient and half-life values of alachlor and

357   metalaxyl in non-amended (sterilised-SS and non-sterilised-S) and pine (S+P) or oak

358   (S+O) amended soils.

359

360

361

362   **Figure captions**

363

364   **Figure 1.** Degradation kinetics of alachlor and metalaxyl in sterilised soil (SS), non-

365   sterilised soil (S) and soil amended with pine (S+P) or oak (S+O) woods. Bars indicate

366   the standard deviation of the replicates (n = 4).

367

368   **Figure 2.** Formation of alachlor ESA and metalaxyl metabolites CGA 62826 and CGA

369   92370 in non-sterilised soil (S) and soil amended with pine (S+P) or oak (S+O) woods.

370   Bars indicate the standard deviation of the replicates (n = 4).

371

372   **Figure 3.** Dehydrogenase activity of non-amended (S) and amended soils (S+P and

373   S+O) treated with alachlor and with metalaxyl at different incubation times. Bars

374   indicate the standard error of the replicates (n = 2).

375

1

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.

Pesticide/Treatment	Kinetic equation	r^2	$t_{1/2}$ (d)
Alachlor			
SS	y = 98.935 e (-0.0035x)	0.78	198
S[a]	y = 101.65 e (-0.2187x)	0.99	3.17
S+P	y = 76.54 e (-0.0046x)	0.53	151
S+O	y = 90.738 e (-0.0414x)	0.98	16.7
Metalaxyl			
SS	-	-	nd[b]
S	y = 108.75 e (-0.0238x)	0.98	29.1
S+P	y = 95.073 e (-0.0048x)	0.95	144
S+O	y = 94.613 e (-0.0101x)	0.96	68.6

[a]First-order equation calculated for the first degradation phase (0-6 days); [b]nd, no degradation.

8

9

1

2

3

4
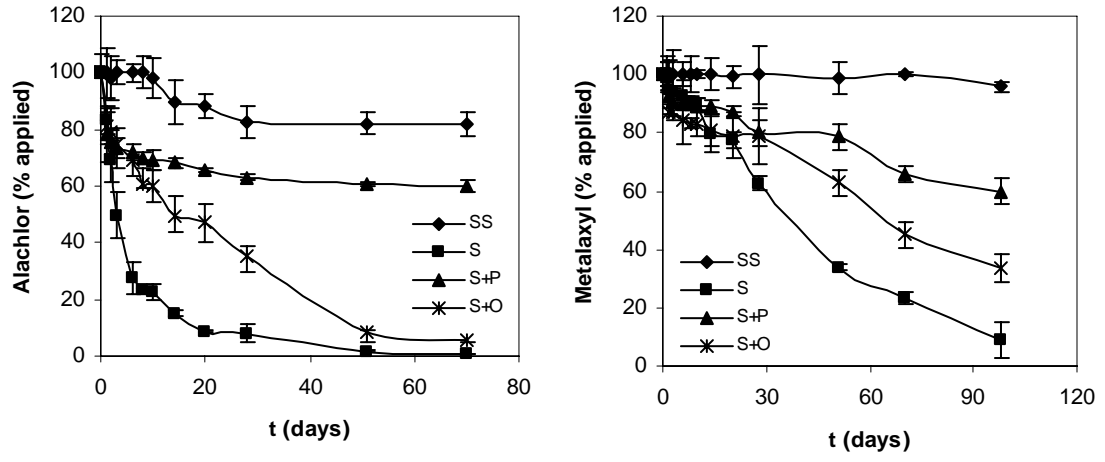
5

6

7

8

9

10

11

12

13   Figure 1

14

15

16

17

18

19

1

2

3

4

5

6

7

8

9

10
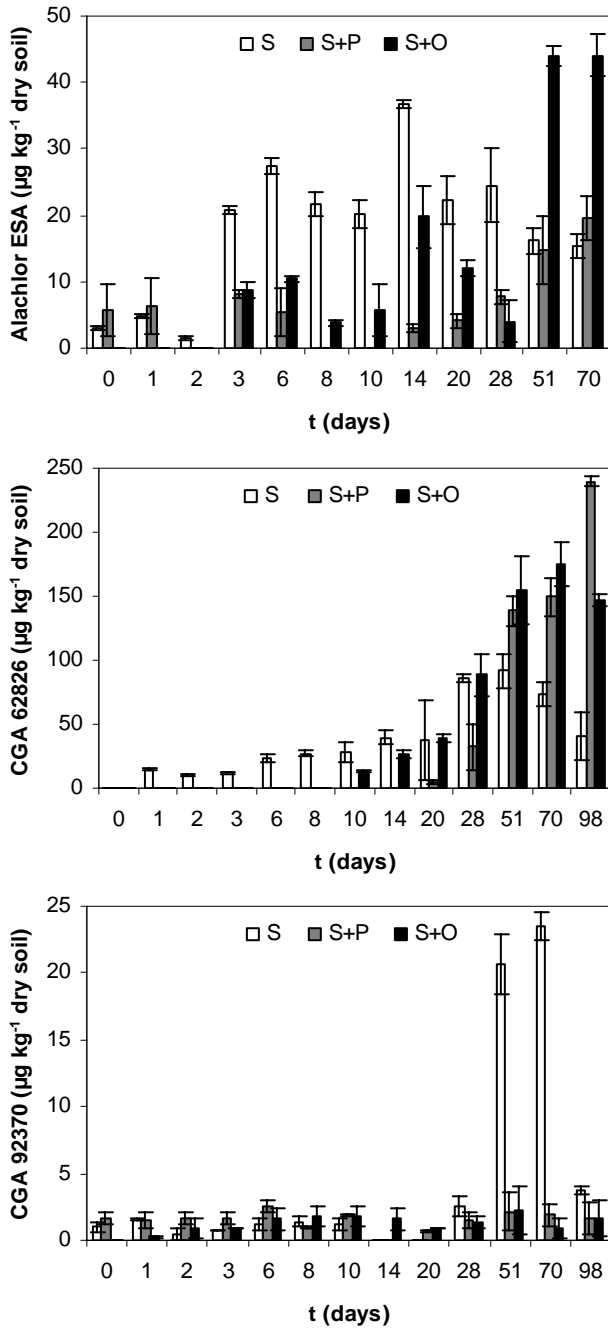
11

12

13

14

15

16

17

18

19

20   Figure 2.

21

1

2

3

4

5

6

7

8

9

10   **Fig. 3.**

11

12

13

14

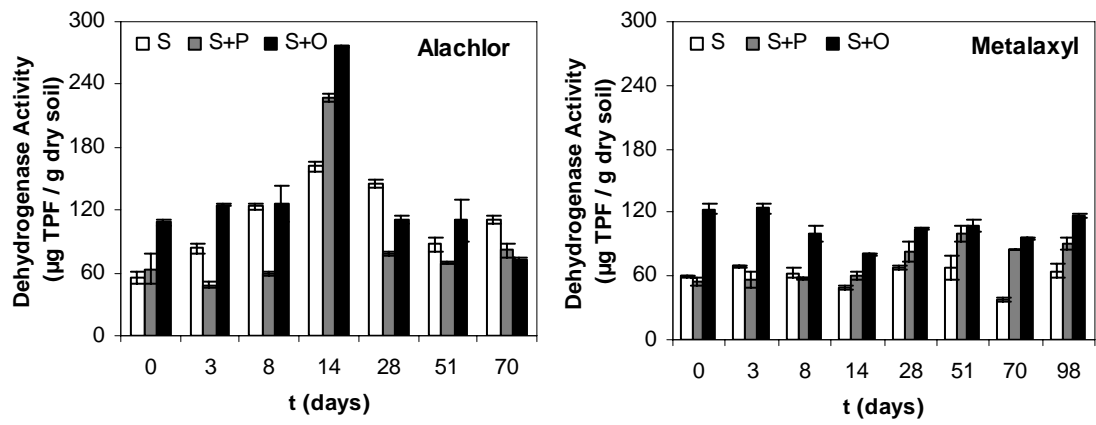15

16

17

18

19

20

21

22