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5 **VERTICAL SMALL SCALE VARIATIONS OF SORPTION**
6 **AND MINERALIZATION OF THREE HERBICIDES IN**
7 **SUBSURFACE LIMESTONE AND SANDY AQUIFER**

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Abstract

Vertical variation in sorption and mineralization potential of mecoprop (MCP), isoproturon and acetochlor was investigated at low concentrations (μg -range) at the cm-scale in unsaturated sub-surface limestone samples and saturated sandy aquifer samples from an agricultural catchment in Brévilles, France. From two intact core drills, four heterogenic limestone sections were collected from 4.50-26.40 m below surface (mbs) and divided into 12 sub-samples of 8-25 cm length, and one sandy aquifer section from 19.20-19.53 m depth divided into 7 sub-samples of 4-5 cm length. In the sandy aquifer section acetochlor and isoproturon sorption increased substantially with depth; in average 78 % (acetochlor) and 61 % (isoproturon) per 5 cm. Also the number of acetochlor and isoproturon degraders (most-probable-number) was higher in the bottom half of the aquifer section ($93 \rightarrow 16\,000$ /g) than in the upper half (4-71 /g). One 50 cm long limestone section with a distinct shift in color showed a clear shift in mineralization, number of degraders and sorption: In the two brown, uppermost samples, up to 31% mecoprop and up to 9% isoproturon was mineralized during 231 days, the numbers of mecoprop and isoproturon degraders were 1 300 to $>16\,000$ /g, and the sorption of both isoproturon and acetochlor was more than three times higher, compared to the two deeper, grayish samples just below where mineralization (≤ 4 %) and numbers of degraders (1-520 /g) were low for all three herbicides. In both unsaturated limestone and sandy aquifer, variations and even distinct shifts in both mineralization, number of specific degraders and sorption were seen within just 4-15 cm of vertical distance. A simple conceptual model of herbicides leaching to groundwater through a 10 m unsaturated limestone was established, and calculations showed that a 30 cm active layer with the measured sorption and mineralization values hardly impacted the fate of the investigated herbicides, whereas a total thickness of layers of 1 m would substantially increase natural attenuation.

50 **Keywords:** Isoproturon; mecoprop (MCP); acetochlor; degradation; pesticide; fate.

51 **1 Introduction**

52 Pesticides and their degradation products are often detected in surface water and
53 groundwater (Battaglin et al., 2000; Kolpin et al., 1997; Thorling, 2009) even above the EU
54 groundwater guideline of 0.1µg/L (European Parliament and Council, 2006) and they are in
55 this way posing a substantial risk to drinking water resources. This diffuse pesticide pollution
56 has become one of the major environmental challenges for using groundwater as drinking
57 water, and to predict the fate of pollutants in groundwater the entire catchment has to be
58 considered (Turner et al., 2006).

59 Knowledge on sorption and degradation is essential for evaluating risks of pesticides
60 contamination of groundwater since these two processes are the major drivers in natural
61 attenuation. Degradation of pesticides is often considered to decrease with depth (Fomsgaard,
62 1995) but degradation can be faster in sub-soil than in top-soil (Mills et al., 2001). Sorption
63 may also decrease with depth because of decreasing organic matter content which on the
64 other hand may increase the bioavailability (Bending and Rodriguez-Cruz, 2007). Spatial
65 variability in herbicide degradation and sorption has mainly been studied in topsoil or the
66 upper meter of agricultural fields (e.g. Bending et al., 2001, 2003; Charnay et al., 2005;
67 Jacques et al., 1999; Fredslund et al., 2008; Vinther et al., 2008; Walker et al., 2001, 2002)
68 and even at the millimeter scale (Gonod et al., 2003).

69 Although several studies have focused on pesticide fate in subsurface materials and
70 aquifers only few have studied the vertical variability in these environments (Albrechtsen et
71 al., 2001; Charnay et al., 2005; Coquet et al., 2004; Johnson et al., 2000; Larsen et al., 2000;
72 Wood et al., 2002). Even fewer have considered chalk or limestone, probably because of the
73 complexity and the difficulties in sampling such geological settings. Vertical variation in
74 limestone of atrazine mineralization was investigated in seven samples collected from 4.6-

75 10.0 mbs (Charnay et al, 2005), and small-scale vertical variation in isoproturon degradation
76 was investigated in chalk (four 5-cm core sections from 4.42-4-88 mbs) (Johnson et al.,
77 2000), but since degradation was negligible in both studies, their contribution to insight in the
78 spatial variation was limited. In limestone (Charnay) the sorption of atrazine, isoproturon and
79 metamiltron varied vertically only to a limited degree in 10 out of 11 samples (3-12 mbs), but
80 one sample had significantly higher K_d -values which could not be explained by the content of
81 clay or organic matter (Coquet et al., 2004). Hence, only vertical variation in sorption but not
82 in degradation of herbicides in subsurface carbonate rock was reported.

83 If sediment layers with increased mineralization could be identified it would be a step
84 towards better understanding the interaction between sediment and biodegradation, which
85 may improve the predictions and modeling of herbicides fate. Such sediment layers may in
86 fact be "ecotones" where the microbial activity is high, found at the interface between
87 different lithological layers (Goldscheider et al., 2006).

88 Spatial variability at the cm-scale in natural mineralization potential and sorption
89 capacity of herbicides has to our knowledge not been studied before in deep subsurface
90 samples (below 5 mbs), and especially not in limestone. The aim of this current work was to
91 investigate i) if there is a vertical small scale spatial variability in sorption and in
92 mineralization potential of the herbicides mecoprop, isoproturon and acetochlor in sediments
93 from subsurface limestone and sandy aquifer; ii) if the variations could be related to a simple
94 geochemical parameter; and iii) at which such a variation occurs and becomes important for
95 the whole system.

96 **2 Materials and Methods**

97 **2.1 Sediment sampling and characterization**

98 Sediment was collected as intact cores in 2005 (Janniche et al., *submitted*), from a 4.5
99 km² agricultural catchment with diffuse pesticide pollution. The unsaturated zone consists of

100 lutetian heterogeneous limestone, and the sandy aquifer is unconfined and aerobic with the
101 groundwater table between 13.3 and 41.9 m below surface (mbs) (Morvan et al., 2006). Five
102 sections were collected from two cores (Pz14 and Pz17c); four from the unsaturated
103 limestone (sections A to D) and one from the saturated sand (section E). The sections were
104 sampled from 4.50 to 26.40 mbs (Table 2). The sections were further divided vertically in 4
105 to 25 cm long subsamples. The four limestone sections were selected based on a visual
106 inspection: three with rather consistent color and texture over depth, and one section with a
107 distinct shift in color that divided the section in a brownish upper part and a grayish lower
108 part. The sand section had a color gradient over the section length; ranging from brown in
109 the upper samples to dark grey especially in the two bottom samples.

110 Water content of the sediment samples was measured as weight loss after 24 h at 105
111 °C. Sediment pH_w was measured by electrode in 10 g sediment mixed with 10 mL deionized
112 (Bigham et al., 1996). Total inorganic carbon (TIC) was calculated as the difference between
113 total carbon content (TC) and Total organic (TOC). TOC and TC were measured based on the
114 method by Heron et al. (1997) by a total elemental carbon analyzer (LECO CS-225) in
115 triplicates (0.2000 g) where carbonates were removed by adding 2 mL 6 % sulphurous acid
116 (H_2SO_3) six times to ensure that no bubble formation as released CO_2 could be observed.
117 Grain size (only for unconsolidated samples) was characterized by sieving (sieves of 0.063-
118 2.0 mm) and, for the particles below 63 μm , by particle distribution (Sedigraph 5100,
119 Micromeritics Gemini). The specific surface area was measured by Multipoint N_2 -BET
120 analysis (Micromeritics, Gemini III 2375 surface area analyzer), after being outgassed
121 (Micromeritics, FlowPrep 060 Degasser) for 4h at 70 °C.

122 **2.2 Herbicides**

123 Isoproturon and acetochlor are hydrophobic, non-polar organic herbicides whereas
124 mecoprop is a hydrophilic phenoxy acid with a pK_a value of 3.78 and at near-neutral pH it is
125 therefore negatively charged (Table 1). The three herbicides have all been used in the field

126 site to a wide extent. The experiments were carried out with [Ring-¹⁴C]-pesticides (Table 1).
127 In the MPN also unlabelled acetochlor with 99.4 % purity was used.

128 **Table 1**

129 **2.3 Sorption investigations**

130 Sorption was investigated by a batch equilibrium technique based on the OECD
131 guideline 106 (1993) at initial pesticide concentrations of 1 and 50 µg/L. Stock solutions of
132 ¹⁴C-labeled pesticide was prepared with ¹⁴C-labelled pesticides in sterile filtered MilliQ water
133 (Clausen et al., 2001). The sediment samples were crushed (only limestone), freeze-dried and
134 sieved (<2 mm) (Madsen et al., 2000). The investigations were carried out in glass tubes (10
135 mL) with Teflon caps at 10°C in the dark and previous tests excluded adsorption on those
136 materials (Madsen et al., 2000). The water from the Brévilles Spring in the catchment was
137 used as equilibrium liquid in the investigations as representative for the groundwater after
138 treatment with activated carbon to remove pesticides and sterile filtering. Triplicate 5 ±
139 0.0005 g of sediment was pre-equilibrated with 4 mL sterile filtered (0.2 µm Minisart,
140 Sartorius) water for 20 h, then 1 mL pesticide solution was added resulting in an initial
141 concentration of 1 or 50 µg/L (these low concentrations were chosen to mimic
142 environmentally relevant concentrations), and each test tube was placed in a vertical rotator.
143 Based on preliminary investigations (Mouvet et al., 2004) 168 h (for mecoprop and
144 acetochlor) or 336 h (for isoproturon) was sufficient to reach equilibrium, and after this
145 equilibrations time the suspension was centrifuged and 1 mL of supernatant collected for
146 quantification of ¹⁴C-activity (Clausen et al., 2001). Reference samples were prepared in
147 triplicate without sediment but otherwise handled identically to the other samples. The
148 amount of adsorbed pesticide was calculated as the difference between the ¹⁴C-activity in the
149 supernatant solution at equilibrium and the ¹⁴C-activity in the reference solution (detection
150 limit of 1 %). To check if the herbicides were degraded during the sorption equilibration 1
151 mL of supernatant was transferred to a "double-vial" system; 20 mL polyethylene

152 scintillation vial containing a 6 mL scintillation vial with 1 mL 2.5 M NaOH to trap $^{14}\text{CO}_2$
153 after acidification of the subsample (0.1 mL 37%). The inner-vial was removed after 48
154 hours, added scintillation cocktail and the ^{14}C -activity in e.g. the $^{14}\text{CO}_2$ -fraction was
155 quantified.

156 **2.4 Mineralization**

157 Mineralization was investigated in laboratory batch incubations set up under unsaturated
158 or saturated conditions depending on the origin of each sample (Janniche et al., *submitted*)
159 and incubated aerobically in the dark at 10°C for up to 251 days. Autoclaved (20 min at 1.5
160 bars and 120°C three times with one-day intervals) control incubations and duplicates were
161 set up for selected sediments.

162 Unsaturated sediment was set up with 40 g (wet weight) sediment (limestone samples
163 were “gently” crushed prior to a size that could pass the bottleneck with a diameter of 3 cm)
164 in a 100 mL DURAN[®] bottle with Teflon inlayer caps and spiked with 0.5 mL of pesticide
165 stock solution to reach initial concentrations of 1 µg/kg. The produced $^{14}\text{CO}_2$ was trapped in a
166 test tube placed inside each bottle with 2 mL 0.5 M NaOH. When sampled, the sodium
167 hydroxide was replaced and mixed with 10 mL scintillation fluid and the ^{14}C -activity
168 quantified. The bottles were left open for 5 minutes in a laminar flow bench to replenish
169 oxygen. Mineralization was calculated as the cumulated $^{14}\text{CO}_2$ evolution over time as a
170 percentage of initial ^{14}C -pesticide e.g. $^{14}\text{CO}_2/^{14}\text{C}_0$ (%).

171 Aquifer sediment was set-up with 40 g (wet weight) sediment and 60 mL groundwater
172 in a 118 mL glass serum bottle, sealed with a 1 cm thick butyl rubber stopper and crimp caps.
173 1 mL stock solution of ^{14}C labeled pesticide was added through the stopper resulting in an
174 initial concentration in the water phase of approximately 1 µg/L of each pesticide. As for the
175 sorption investigations, the groundwater was collected from the Brévilles spring, treated with
176 activated carbon and sterile filtered. When sampling, 2 mL filtered (0.2 µm PTFE-filter;
177 Advantec/MFS 13HP) subsamples were acidified to strip off (from the water phase) $^{14}\text{CO}_2$

178 that then was captured by NaOH in a "double-vial" system as described earlier. Pesticide
179 content over time was calculated as the fraction of initial ^{14}C -pesticide corrected for pesticide
180 removed during sampling. Mineralization over time was calculated as $^{14}\text{CO}_2$ evolution
181 (corrected for loss to headspace) as a percentage of the corrected initial ^{14}C -pesticide e.g.
182 $^{14}\text{CO}_2/^{14}\text{C}_0$ (%).

183 **2.5 Enumeration of specific pesticide degraders**

184 The specific pesticide degraders was enumerated by a modified (Janniche et al.,
185 *submitted*) most-probable-number method (^{14}C -MPN) (Lehmicke et al., 1979). For each
186 pesticide, sediment samples were mixed with pyrophosphate-buffer (1.2 mM $\text{Na}_4\text{P}_2\text{O}_7$, pH =
187 7.0) and 10-fold serial dilutions ($10^0 - 10^{-4}$) were prepared from which five replicates were
188 made, resulting in 25 MPN-tubes to achieve 1 enumeration. The dilutions were added a
189 complex mineral medium (ISO 7827, 1984) with pH = 6.8 and 25 $\mu\text{g/L}$ ^{14}C -mecoprop or ^{14}C -
190 isoproturon, or 10 $\mu\text{g/L}$ ^{14}C -acetochlor and 20 $\mu\text{g/L}$ acetochlor (due to late receipt of the
191 acetochlor isotope the MPN-assay was set up with unlabeled acetochlor, and 3 month later
192 was isotope added). The MPN-tubes were incubated 11-23 months in the dark at 15 °C and
193 the $^{14}\text{CO}_2$ evolved during incubation was trapped and measured by liquid scintillation
194 counting. Controls were set up with sterile pyrophosphate-buffer without sediment
195 suspension.

196 The MPN values and 95 % confides intervals were calculated by the computer software
197 MPN calculator (Curiale, 2000) based on the number of positive scored tubes in each of the
198 five dilutions. A positive score means that the MPN-tube contained bacteria capable of
199 mineralizing the specific herbicide. The MPN-tubes were scored positive if the trapped $^{14}\text{CO}_2$
200 was greater than the average of 10 sterile controls plus three times the standard deviation
201 (Lehmicke et al. 1979). The MPNs containing mecoprop were scored as positive if the
202 radioactivity in the basetrap was >2.7% of the initially added amount of labeled; the values
203 for isoproturon was 1.2% and 2.0% for acetochlor.

204 **3 Results and Discussion**

205 **3.1 Sediment characterization**

206 Core sections A-D were taken from the heterogeneous unsaturated limestone and
207 section E from the sandy aquifer (Table 2). The heterogeneous limestone samples had
208 relatively low values of total inorganic carbon (TIC): 17-69 % CaCO_3 with the lowest values
209 found in the samples with marls. Though high TIC content may interfere with the total
210 organic carbon (TOC) measuring, the results have high reproducibility, and are equivalent to
211 reports for similar settings, e.g. 0.1-2.9 % TOC in limestone and 0-5.9 % in marlstone
212 (Coquet et al., 2004), who also reported CaCO_3 content of 69-88 % in limestone and 69-74 %
213 in marlstone. The heterogeneous nature of our limestone may explain the relatively low TIC
214 content compared to samples of pure limestone.

215 The limestone sections from Pz14 (A and C) had in general higher contents of water,
216 TOC and TIC but a lower specific surface area than those from Pz17c (B and D). Besides
217 differences observed between sections, differences were also observed at cm scale within a
218 given section. Thus, limestone section D had a clear visual color shift and a clear shift in
219 specific surface area with much higher specific surface area in the two uppermost samples
220 (D1 and D2) compared to the two bottom samples (D3 and D4). Also the TIC content shifted
221 with a lower TIC content in D1 and D2 compared to D3 and D4 expressing more pure
222 limestone in D3-D4 than in D1-D2. Besides, TOC was much lower in the first sample, D1,
223 than the other samples D2-D4 within this section.

224 Aquifer section (E) from Pz17c had high specific surface area but low TOC (Table 2).
225 The color gradually changed across the whole section from brownish to grayish and TIC
226 slightly increased with depth; samples E1 and E2 had approximately half the TIC content of
227 E3-E7.

228 In summary, the five core sections represented two major geological settings and
229 different limestone settings; the limestone cores varied in textures, but the geology of the

230 individual sections was very similar at the macro geological scale. Section D and E had clear
231 small-scale variations at the micro scale in the sediment characterization parameters.

232 **Table 2**

233

234 **3.2 Sorption**

235 The sorption investigations included two concentrations (1 and 50 $\mu\text{g/L}$) and linear
236 distribution coefficients (K_d) were calculated. The different concentrations only to a limited
237 degree affected the K_d -values (Fig. 1), and only noteworthy for isoproturon in the aquifer
238 samples E3, E4 and E5, where sorption at the high concentration surprisingly was clearly
239 higher (0.78-1.69 L/kg) than at the low concentration (0.52-0.87 L/kg). However, all
240 isoproturon K_d values from section E followed the same trend of increased sorption over
241 depth (Fig. 1).

242 **Fig. 1**

243 In limestone samples, sorption was in general low for all three herbicides and so was the
244 standard deviation reflecting high reproducibility (Fig. 1 A-D). In all limestone sections,
245 isoproturon sorption was at or below the detection limit of 0.010 L/kg except in section D
246 (K_d : 0.03-0.25 L/kg). These findings are within the range reported for isoproturon sorption to
247 limestone and chalk: <0.01-0.55 L/kg (Besien et al., 2000; Coquet et al., 2004; Johnson et al.,
248 1998; Madsen et al., 2000; Mouvet et al., 2004). The K_d values for acetochlor in the
249 limestone was <0.01-0.34 L/kg; literature K_d values for acetochlor, in the range of 0.48-7.11
250 L/kg (Hiller et al., 2009; Lengyel and Foldenyi, 2003; Ma et al., 2004), are predominantly
251 reported for topsoil which explains their substantially higher K_d values. The mecoprop
252 sorption to limestone was also low (≤ 0.15 L/kg) and in the range (0-0.17 L/kg) reported in
253 the literature for chalk and limestone (Clausen et al., 2001; Kristensen et al., 2001; Madsen et
254 al., 2000).

255 The sorption of isoproturon and acetochlor to limestone was highest in D1 and D2
256 with higher specific surface area, and lower TOC than in the other limestone samples (Table
257 2). This indicated that isoproturon and acetochlor sorption in heterogenic limestone to a
258 higher degree was controlled by mineralogy than by organic carbon content. Isoproturon
259 sorbs to kaolinite, but not to quartz, calcite, alumina (Clausen et al., 2001) or iron oxides
260 (Clausen and Fabricius, 2001), and then sorption is strongly related to the content of organic
261 matter and clay (Coquet et al., 2004; Madsen et al., 2000). This supports that the increased
262 isoproturon sorption was caused by the increased clay content within the limestone marls.
263 Sorption of acetochlor is likely to be controlled by the same parameters, but comparable
264 literature data are limited for acetochlor sorption in subsurface sediments, low in TOC.
265 Sorption of mecoprop was not increased in the same sections as isoproturon and acetochlor
266 because the sorption mechanisms of the anionic mecoprop differ from the nonionic herbicides
267 isoproturon and acetochlor; mecoprop sorbs to positive sites on the mineral surface (Clausen
268 et al., 2001).

269 In sandy aquifer samples sorption was in general substantial for acetochlor (0.25-5.12
270 L/kg) and isoproturon (<0.01-1.69 L/kg) except in E1 (Fig. 1), whereas mecoprop sorption
271 was very low (≤ 0.12 L/kg) in the two investigated samples (E2 and E3). The sorption could
272 not be explained solely by variations in the investigated geochemical parameters (clay
273 content, organic and inorganic carbon content). The grayish color observed in the bottom two
274 subsamples (E6 and E7) of the aquifer section indicated reduced conditions which may,
275 however, explain the increased sorption capacity over depth because reduced organic matter
276 induce a more hydrophobic surface whereto nonionic compounds better sorbs (Clausen et al.,
277 2004). K_d values of 0-0.4 L/kg have been reported for mecoprop sorption onto sand (Madsen
278 et al., 2000; Pedersen, 2000; Tuxen et al., 2000), and our finding is within this range. K_d
279 values for isoproturon are reported to be 0.007-1 L/kg in sand (Coquet et al., 2004; Madsen et

280 al., 2000; Pedersen, 2000; Tuxen et al., 2000); our results are in close agreement with these
281 values.

282 **3.3 Mineralization potential**

283 Mecoprop, isoproturon and acetochlor degraders were detected in many samples (Fig. 2)
284 which indicated a degradation potential of these compounds in the catchment. Furthermore,
285 this suggested that the catchment was polluted with all three herbicides since another study
286 only detected phenoxy acid (mecoprop, dichlorprop and 2,4-D) degraders in the part of the
287 aquifer exposed to phenoxy acids (<40 µg/L) but not in the upstream pristine aquifer (de
288 Liphay et al., 2003). The number of mecoprop degraders found in our investigation was at
289 same level as observed by de Liphay et al. (2003) but the number of isoproturon degraders
290 was higher than reported by Bending & Rodriguez-Cruz (2007).

291 **Fig. 2**

292 In all the 12 limestone samples mineralization of acetochlor was below 2%. Isoproturon
293 was mineralized (up to 9%) in D1 and D2; in the remaining samples <1% was mineralized.
294 19-31 % mecoprop was mineralized in D1 and D2 and 4 % in D3 and C4 (average of two
295 replicates with 6 and 2 %). The other samples exhibited mecoprop mineralization below 1 %.
296 The number of degraders was in general high in section D, but also in sample C3 the number
297 of isoproturon degraders was extremely high (>16 000 /g). Some samples had high number of
298 degraders without subsequent high mineralization (e.g. D1 (acetochlor), C3 (isoproturon)).

299 In the sandy aquifer (E), mecoprop was also mineralized substantially; 10-22% was
300 recovered as ¹⁴CO₂ with only 49-790 /g mecoprop degraders. The mineralization of both
301 isoproturon (1-5%) and acetochlor (3-6%) was markedly lower than for mecoprop, despite
302 higher numbers of isoproturon (27-2 200 /g) and acetochlor degraders (4-16 000 /g).

303 **3.4 Small scale variability**

304 The 19 samples from the 5 sections fell into 3 different cases: 1) In unsaturated
305 limestone sections A, B and C sorption, mineralization and MPN were in general limited for

306 all three studied herbicides (one exception was mecoprop sorption which was ≤ 0.01 L/kg in
307 sample A1 but 10-fold higher (0.11-0.15 L/kg) in A2 – the sample just below). 2)
308 Unsaturated limestone section D clearly showed small scale variations with a clear shift in
309 sorption, mineralization and MPN. 3) Sandy aquifer section E had evident small scale
310 variation in sorption but without a clear shift; it was rather a gradient, but also with variation
311 in especially MPN.

312 **3.4.1 Unsaturated limestone from section D**

313 Sorption of isoproturon and acetochlor, and mineralization of mecoprop and acetochlor
314 as well as number of mecoprop and acetochlor degraders were increased in the upper part of
315 section D (Fig. 1 and 2). A shift in color divided the section in two: the two brownish
316 uppermost samples (D1 and D2) with high sorption (isoproturon: 0.17-0.25 L/kg and
317 acetochlor: 0.22-0.34 L/kg), mineralization (mecoprop: 19-31 % and isoproturon: 9 %), and
318 number of herbicide degraders (mecoprop: 1300-1400 /g and isoproturon: 4300->16000 /g)
319 and on the other hand the grayish, lower samples (D3 and D4) with lower sorption
320 (isoproturon: 0.03-0.07 L/kg and acetochlor: <0.01-0.11 L/kg), mineralization (mecoprop:
321 0.5-4 % and isoproturon: 0.2-0.5 %), and number of herbicide degraders (mecoprop: 1-6 /g
322 and isoproturon: 3-520 /g). Already after 14 days of incubation the mineralization in D1 and
323 D2 was noticeable higher than in D3 and D4 (Fig. 3). Furthermore, the $^{14}\text{CO}_2$ evolution in D4
324 was as low as in the autoclaved incubations with sediment from D1-D4. Hence, a clear small
325 scale shift in sorption and mineralization potential within only 25 cm was evident in the
326 limestone.

327 **Fig. 3**

328 **3.4.2 Sandy aquifer section E**

329 In the sandy aquifer samples sorption was low in the two uppermost samples, and
330 increased gradually with depth. Acetochlor sorption increased in average 78 % pr every 5 cm

331 in the sandy aquifer section (E). Also the isoproturon sorption increased gradually with depth
332 (in average 61 % pr 5 cm) in the sandy aquifer (E) and hence the small scale variance in
333 acetochlor and isoproturon sorption within these 33 cm was substantial. Despite the
334 acetochlor mineralization in the sandy aquifer was rather constant, the number of acetochlor
335 degraders increased dramatically in the three bottom samples; from 4-9/g in E1-E4 to 120-16
336 000/g in E5-E7. Also the number of isoproturon degraders was higher in the bottom samples
337 (93-2 200/g in E4-E7) than in the top samples (27-71/g in E1-E3), and mineralization was
338 faster in E1-E4 (2-5 %) than in E5-E7 (<1 %). For mecoprop however, the middle part (E3-
339 E5) had the highest number of degraders; 490-790/g compared to 49-93/g, and mineralization
340 was also substantially faster in E5 (22 %) compared to the rest of the aquifer (10-15 %).
341 Mineralization of acetochlor and isoproturon in the saturated sandy aquifer samples (Fig. 2)
342 could very well be negatively affected by the increased sorption with depth, leaving less
343 herbicide available for mineralization, and could explain the lack of faster mineralization,
344 despite very high number of degraders in some samples. This inverse relationship between
345 sorption strength and mineralization was reported for MCPA (Jensen et al., 2004). This could
346 explain why isoproturon was mineralized (1.7-4.7 %) in the uppermost samples (E2-E4) but
347 not in the bottom samples (<1 % in E5-E7). However, acetochlor which sorbed stronger did
348 not follow that trend.

349 Conclusively, sorption and mineralization potential varied substantially within just a
350 few cm in both limestone and sand. These small scale variations were found in sections with
351 shifts in color, but could not be strongly related to the investigated geochemical parameters.

352 **3.5 Importance of an active limestone layer on groundwater** 353 **contamination**

354 The investigated four limestone sections revealed an overall picture of very low
355 herbicide sorption and mineralization, except in the upper two 15 cm samples of section D,
356 where both sorption and mineralization potential was increased. This section contained marls

357 (Table 2) and upper Lutetian marly layers are ubiquitous in the catchment, and may reduce
358 the flow rate and subsequent solute transport (Gutierrez and Baran, 2009). This marly layer
359 with increased water content may be an environment favoring microbial activity.

360 To evaluate the relative impact on mecoprop, isoproturon and acetochlor of such an
361 active layer with respect to sorption and mineralization in the unsaturated limestone a simple
362 conceptual model was set up. Although both matrix flow and fracture flow may occur in
363 heterogeneous unsaturated limestone matrix flow has in several studies shown to be
364 predominant in deep groundwater sites and at steady-state flow (Haria et al., 2003; Mathias et
365 al., 2005; Wellings, 1984). Slow transfer velocities of 0.5 to 1 m/yr have earlier been
366 determined for our catchment based on profiles of water contents, tritium activities, and
367 nitrate concentrations (Gutierrez and Baran, 2009). We therefore assumed that the dominant
368 flow occurred in matrix in the calculations of the conceptual model consisting of a 10 m
369 unsaturated heterogeneous limestone profile with an active layer of a certain thickness (x_a).
370 Sorption and mineralization was confined to this active layer and hence microbial activity is
371 assumed in the matrix. Such an active layer with sorption will cause retardation (R) of the
372 herbicides which was calculated assuming matrix flow as:

$$373 \quad R = \frac{x_w}{x_s} = \frac{v_w}{v_s} = \frac{\rho_b}{\varepsilon} K_d + 1$$

374 where v_s is the solute velocity [m/y], ρ_b is the bulk density [kg/L] calculated as $\rho_b = (1 - \varepsilon)\rho$, ε
375 is the porosity [-], and K_d is the linear sorption coefficient [L/kg]. The delay in break through
376 due to sorption of the herbicides in the active layer was calculated as:

$$377 \quad Delay = \frac{x_a}{v_w} (R - 1)$$

378 Mineralization of herbicide will reduce the risk of groundwater contamination in
379 accordance with the observed mineralization patterns (Fig. 3) and the removal of herbicides
380 due to mineralization was calculated based on first order mineralization kinetics as:

381
$$removal = \left(1 - \exp\left(k \frac{x_a R}{v_w} \right) \right) \times 100\%$$

382 where k is the first order mineralization constant. K_d -values and k-values are mean values as
383 observed for D1 and D2 (Fig. 1 and 2). Unfortunately, density (ρ) and porosity (ϵ) were not
384 measured specifically for D1 and D2 due to lack of sample material but in 7 other samples
385 from the catchment; ρ ranged from 2.69 to 2.73 kg/L and ϵ from 0.08 to 0.37 (Janniche et al.,
386 *submitted*). We have used the mean value of 0.18 for ϵ and 2.72 kg/L for ρ in this simple
387 conceptual model calculation which results in a ρ_b of 2.2 kg/L. If a lower porosity (ϵ) and
388 hence a higher bulk density (ρ_b) was applied the retardation would have increased, and vice
389 versa.

390 Three scenarios were set up: 1) a reference scenario with no active layer, 2) an active
391 layer of 30 cm thickness, and 3) an active layer of 1 m thickness (Fig. 4). In the reference
392 scenario the herbicides are transported at the same velocity as water (v_w), and with a water
393 velocity of 1 m/yr it will take 10 years before breakthrough of the herbicides through the 10
394 m long profile. Presence of a 30 cm thick layer (scenario 2) will only to a very limited degree
395 cause delay. It would take only 10.2 year for mecoprop to reach the groundwater (bottom of
396 profile), 10.7 year for isoproturon and 11.0 year for acetochlor (Fig. 4). However, the
397 mineralization in the layer would remove approximately 24 % mecoprop, 10 % isoproturon
398 and 3 % acetochlor. The heterogeneous limestone could contain several active layers or one
399 thicker layer. This may be realistic since the limestone settings in the field site are more than
400 30 m thick in 61 % and less than 10 m thick in 7 % of the surface of the catchment (Morvan
401 et al., 2006). Assuming the summed extent of active layers to be 1 m over the whole 10 m
402 profile (scenario 3), then the breakthrough times would be 10.6 years (mecoprop), 12.4 years
403 (isoproturon) and 13.3 years (acetochlor) respectively, and the proportion of mineralized
404 herbicide would then become 59 % mecoprop, 31 % isoproturon and only 9 % acetochlor
405 (Fig. 4).

406 **Fig. 4**

407 In conclusion, if only one active layer of 30 cm thickness is present pr 10 m the
408 retardation due to sorption will be negligible for all three herbicides and the removal will
409 negligible for acetochlor but not for isoproturon and mecoprop, whereas the effect of both
410 retardation and removal will be notable for all three herbicides if the layer is 3 times thicker.
411 Considering the thickness of unsaturated limestone in the investigated field site is up to 42 m
412 thick it is not unlikely that the summed extend of an active layer will be greater than the
413 observed 30 cm. Hence the importance of such active layers in the unsaturated limestone
414 depends on their magnitude and consequently a very detailed knowledge of the limestone
415 characteristics of the site is paramount.

416 **4 Conclusions**

417 Within few centimeters small scale vertical variability was present beyond measuring
418 and sampling uncertainties showing clear shifts in sorption and microbial activity in both
419 heterogeneous unsaturated limestone and sandy aquifer. None of the investigated parameters
420 (TOC; TIC; specific surface area; sand, silt and clay content; water content; pH_w)
421 individually could explain the variations. These variations followed a color shift or a color
422 gradient in the core section.

423 The significance of these layers, or ecotones, with increased sorption and microbial
424 mineralization depends on the specific herbicide and the extent of these layers, and it is
425 therefore important to determine this. Such a detailed knowledge will improve the predictions
426 of herbicide fate in the catchment if the geology is well described and included in reactive
427 geochemical models.

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