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## Zinc speciation and isotopic exchangeability in soils polluted with heavy metals

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1 *Zn exchangeability in soils*

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4 **Zinc speciation and isotopic exchangeability in soils polluted with**  
5 **heavy metals**

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## 25 **Summary**

26 A correct characterization of heavy metal availability is a prerequisite for the  
27 management of polluted soils. Our objective was to describe zinc (Zn) availability in  
28 polluted soils by measuring the isotopic exchangeability of Zn in soil/solution (*E*  
29 value) and in soil/plant systems (*L* value), by assessing the transfer of Zn and <sup>65</sup>Zn in  
30 the fractions of a six-step selective sequential extraction (SSE) in incubated soils  
31 and by identifying Zn forms in soils using extended X-ray absorption fine structure  
32 (EXAFS) spectroscopy. We distinguished 3 pools of exchangeable Zn: the pool of  
33 Zn exchangeable within 1 minute which is observed in all soils, Zn exchangeable on  
34 the medium term, and the slowly and not exchangeable Zn. The amount of Zn  
35 present in the 2 first pools was similar to the *L* value measured with *T. caerulea*.  
36 The 3 first steps of the SSE solubilized the 1<sup>st</sup> pool and a fraction of the 2<sup>nd</sup> pool.  
37 Most of the 2<sup>nd</sup> pool and a fraction of the 3<sup>rd</sup> pool were extracted in the 4<sup>th</sup> step of  
38 the SSE, while the rest of the 3<sup>rd</sup> pool was extracted in the final steps of the SSE.  
39 The EXAFS study conducted on two soils showed that more than half of the Zn was  
40 present in species weakly bound to organic compounds and/or outer sphere  
41 inorganic and organic complexes. Other species included strongly sorbed Zn species  
42 and Zn species in crystalline minerals. The EXAFS study of selected SSE residues  
43 showed that the specificity and the efficiency of the extractions depended on the  
44 properties of the soil studied.

45

## 46 **Introduction**

47 An accurate assessment of heavy metal availability is essential for the proper  
48 management of polluted soils. Young *et al.* (2006) reviewed the use of selective  
49 sequential extractions (SSE) and isotope exchange (IE) techniques to characterize

50 heavy metals availability and speciation on the solid phase of soils. They concluded  
51 that although sequential extractions are rather easy to implement, their results are  
52 flawed because of the lack of specificity of the extractants for given metal species,  
53 and/or because of the adsorption or precipitation of metals occurring during the  
54 extraction. IE techniques conducted in soil/solution systems allow the quantification  
55 of ions located on the solid phase of the soil that can exchange with the same ion  
56 present in the soil solution within a given exchange time ( $E$  value) (Sinaj *et al.*,  
57 1999). Ayoub *et al.* (2003) and Sinaj *et al.* (2004) showed that  $E$  values measured  
58 after a long IE time in acidic soils are identical to the amount of isotopically  
59 exchangeable Zn measured in pot experiments with different plant species ( $L$   
60 values) demonstrating that Zn that is isotopically exchangeable within a time frame  
61 relevant for plant growth is the main source of Zn for plant nutrition. In most studies  
62  $E$  values are only measured after a single time of exchange (for instance 24h; Young  
63 *et al.*, 2000; Degryse *et al.*, 2003; Nolan *et al.*, 2005) although according to Young  
64 *et al.* (2006) the study of the kinetics of IE has a lot of potential for assessing Zn  
65 forms and availability in soils.

66 To assess whether a SSE could deliver relevant results on cadmium (Cd)  
67 availability, Ahnstrom and Parker (2001) carried out a sequential extraction on soils  
68 that had been labeled with a stable isotope of Cd ( $^{111}\text{Cd}$ ). They measured in all  
69 extracts the total Cd content and the abundance of  $^{111}\text{Cd}$  and then compared these  
70 results with the amount of soil isotopically exchangeable Cd. They concluded that  
71 no single fraction of the sequential extraction or a combination of fractions  
72 corresponded to the size of the isotopically labile Cd pool.

73 Extended X-ray Absorption Fine Structure (EXAFS) spectroscopy is also  
74 used to assess the forms of heavy metals in soils (Manceau *et al.*, 2002). Sarret *et al.*

75 (2004) explained the high proportion of isotopically exchangeable Zn observed in a  
76 polluted soil by its high concentration in octahedral Zn weakly bound to organic  
77 compounds identified with EXAFS. The sensitivity of EXAFS spectroscopy for  
78 exchangeable species (bound to organics or weakly sorbed on minerals) is however  
79 relatively weak as compared to precipitated and crystalline phases. On the opposite,  
80 chemical extractions allow a better quantification of easily mobilized species as  
81 compared to more recalcitrant ones due to non-specific dissolution and possible  
82 formation of new species. Therefore, the combination of IE methods, SSE and  
83 EXAFS spectroscopy may provide a better picture of exchangeable and non-  
84 exchangeable metal species (Scheinost *et al.*, 2002; Sarret *et al.*, 2004).

85         We analyzed Zn exchangeability and speciation in six polluted soils with IE  
86 methods, both in soil/water systems (*E* values) and in soil/plant systems (*L* values),  
87 SSE and Zn K-edge EXAFS spectroscopy. Our purpose was to establish  
88 relationships between the Zn pools determined by the various techniques, and  
89 between these pools and soil parameters such as total Zn content and pH. Moreover,  
90 the specificity of SSE steps for extracting real Zn chemical species was tested by  
91 comparing the distribution of Zn species determined by EXAFS spectroscopy in the  
92 soil and in selected extraction residues. To test whether the extraction steps released  
93 Zn species with a specific exchangeability, soils were labeled with <sup>65</sup>Zn before the  
94 SSE, and the specific activity was measured in each residue.

95

## 96 **Materials and methods**

### 97 *Soils*

98 We studied six soils that had been polluted with heavy metals. The soil from the  
99 Institut National de la Recherche Agronomique (INRA soil) was sampled from a

100 field experiment performed near Bordeaux, France (44° 51' N 00° 32' W). This soil  
101 had received 100 t of municipal digested and dehydrated sewage sludge per hectare  
102 every second year between 1974 and 1993. A description of the experiment can be  
103 found in Weissenhorn *et al.* (1995). Two soils from the Institut für Umweltschutz  
104 und Landwirtschaft (IUL soils) were collected in a field experiment conducted near  
105 Bern, Switzerland (46° 55' N 07° 25' E). Aerobically digested and dehydrated  
106 sewage sludge had been applied to the IUL SS soil, while pig slurry had been  
107 applied to the IUL PS soil. The amendment-loading rate for both soils was 5 t ha<sup>-1</sup>  
108 year<sup>-1</sup> from 1976 to 1996. A description of this field experiment is given in  
109 Siegenthaler *et al.* (1999). The three remaining soils were collected in the vicinity of  
110 industrial metal smelting facilities located close to Dornach, Switzerland (47° 25' N  
111 07° 35' E), and in Evin (50° 25' N 03° 01' E) and Mortagne (50° 30' N 03° 27' E),  
112 France. The Dornach soil has accumulated Cd, Cu, Ni and Zn from the deposition of  
113 about 700 t dust year<sup>-1</sup> from brass smelting that began in 1895 and continued into  
114 the 1980s before the installation of emission filters and scrubbers (Geiger *et al.*,  
115 1993). The smelting facility in Evin began operations in 1894 and grew to become  
116 the largest Pb and Zn ore processing plant in Europe before its closure in 2003. Until  
117 1970, the Evin facility emitted approximately 5 t smelter dust day<sup>-1</sup> (LASIR, 2000).  
118 The Mortagne soil is heavily polluted with metal dust and slag from a Pb and Zn  
119 smelter in operation between 1906 and 1968 (Manceau *et al.*, 2000). Thiry *et al.*  
120 (2002) estimate that 15'000 t of metals have been dispersed over 25 hectares  
121 surrounding the smelter.

122         Approximately 100 subsamples of the INRA, IUL SS and IUL PS soils were  
123 collected at random intervals within the surface horizon (0-20 cm) to obtain a  
124 representative sample. For the Evin and Dornach soils, samples were randomly

125 taken after having removed the litter layer to obtain the maximum contaminant  
126 concentration in the soil. The sample site in Mortagne is the so-called metallicolous  
127 meadow. The A horizon was characterized by a distinct layer (5 to 8 cm thick)  
128 starting at a 15 to 25 cm depth in which smelter ash and tailings had been spread out  
129 and buried at the time the smelter was closed. A representative sampling was  
130 obtained by collecting approximately 100 random subsamples between 5 and 40 cm  
131 to include the heavily polluted layer. The soil samples were well mixed, air-dried for  
132 at least one week and passed through a 2-mm sieve. Remaining plant debris was  
133 removed by hand prior to analysis. Relevant soil characteristics are listed in Table 1.

134

135 *Isotopic exchange kinetics, compartmental analysis and determination of*  
136 *isotopically exchangeable Zn*

137 IE kinetic experiments were carried out using a 1:10 soil solution ratio and 2 mM  
138 CaCl<sub>2</sub> as described by Sinaj *et al.* (1999). After shaking the soil solution suspension  
139 on an end-over-end shaker for three days, the samples were removed, placed on a  
140 magnetic stirring plate and stirred at 300 rpm. The soil suspension samples were  
141 spiked with 1.3 to 2.5 kBq of carrier-free <sup>65</sup>Zn added as ZnCl<sub>2</sub> (NEN Biosciences,  
142 Boston, USA; specific activity 2.0 GBq mg<sup>-1</sup> Zn). Aliquots of the soil suspension  
143 filtered through a 0.2 μm porosity cellulose acetate membrane (Minisart, Sartorius)  
144 were removed at 1, 3, 10, 30 and 60 minutes, and at 1, 7 and 14 days. After the first  
145 60 minutes of IE the flasks were left on the bench and they were put back on the  
146 stirring plate one hour before sampling at 1, 7 and 14 days so as to minimize the  
147 dispersion of soil aggregates that would have been caused by a continuous stirring.  
148 The concentration of Zn in the solution ( $C_{Zn}$ ) was measured after 60 minutes, 1, 7  
149 and 14 days by ion chromatography as proposed by Sinaj *et al.* (1999). This method

150 measures the oxalate complexable Zn in the filtered soil suspension which  
151 approximates the concentration of the hydrated and weakly complexed Zn species  
152 (Cardellicchio *et al.*, 1999) present in the solution. <sup>65</sup>Zn activity in the solution was  
153 measured at all sampling times by β liquid scintillation detection (Packard 2500) at  
154 an emission energy of 325 keV. We measured the β-counts using 1 ml of filtrate  
155 with 5 ml of scintillation liquid (Packard Ultima Gold) and corrected them for  
156 quenching effects.

157         The decrease of the fraction of radioactivity remaining in the solution ( $r_t/R$ )  
158 where  $r_t$  is the radioactivity remaining in the solution expressed in Bq after  $t$   
159 minutes, and  $R$  the total introduced radioactivity expressed in Bq) was analyzed in  
160 each soil with a compartmental analysis to assess the number of Zn containing  
161 compartments (a compartment is defined as an amount of material that acts as  
162 though it is well-mixed and kinetically homogeneous, Cobelli *et al.* (2000)). We  
163 proceeded as proposed by Cobelli *et al.* (2000). If we consider that for a given soil  
164 an apparent isotopic equilibrium is reached before or at 14 days of exchange (i.e.  
165 that the fraction of radioactivity remaining in the solution has reached a constant  
166 value), we can subtract the fraction of radioactivity remaining at equilibrium from  
167 the  $r_t/R$  values measured at earlier times during the experiment and analyze the  
168 resulting curve. If a finite number of compartments can be identified it is possible to  
169 separate the obtained curve in a sum of exponential terms that are a function of  
170 exchange time. The final equation describing the change of radioactivity in solution  
171 with time can then be written as follows:

172 
$$\frac{r_t}{R} = A + \sum_{i=1}^{N-1} B_i \times e^{-C_i \times t} \quad (1)$$



173 Where  $r_t/R$  is the fraction of  $^{65}\text{Zn}$  remaining in solution at the time of sampling,  $A$ ,  $B_i$   
174 and  $C_i$  are constants,  $N$  is the total number of compartments.

175 The theory of the compartmental analysis states that when the system is  
176 closed, at a steady-state for the element studied, and when the tracer (here  $^{65}\text{Zn}$ ) is  
177 introduced in one injection within a very short time, then the number of total  
178 compartment ( $N$ ) is equal to the number of exponential terms ( $N-1$ ) plus 1 (Cobelli  
179 *et al.*, 2000).

180 This analysis suggests the presence in all soils of a compartment of Zn  
181 exchangeable during the first minute of exchange while other compartments differed  
182 from soil to soil in their time limits and/or in their total numbers. To simplify the  
183 subsequent analysis of the results we decided to consider only three pools of Zn (a  
184 pool being defined as a group of compartments, Cobelli *et al.*, 2000) in the rest of  
185 the paper: the pool of Zn exchangeable within 1 minute which is observed in all  
186 soils (pool 1), the pool of Zn that is exchangeable between 1 minute and apparent  
187 isotopic equilibrium (pool 2) and the pool of Zn that can not be exchanged or that  
188 exchanges very slowly (pool 3). The amount of Zn isotopically exchangeable within  
189 a given time ( $E_t$  value,  $\text{mg kg}^{-1}$ ) is calculated using the following equation:

$$190 \quad E_t = \left[ \left( \frac{v}{m} \right) \times C_{\text{Zn}} \right] \times \frac{R}{r_t} \quad (2)$$

191 Where  $v/m$  is the solution to soil ratio ( $\text{l kg}^{-1}$ ),  $C_{\text{Zn}}$  is the Zn concentration ( $\text{mg l}^{-1}$ ) in  
192 solution and  $r_t/R$  is the fraction of  $^{65}\text{Zn}$  remaining in solution at the time  $t$  of  
193 sampling. The amount of Zn present in the pool 1 ( $E_{\text{pool1}}$ ) is calculated considering  
194 an exchange time of 1 minute, the amount of Zn present in the pool 2 ( $E_{\text{pool2}}$ ) is the  
195 difference between the amount of Zn that has been exchanged at apparent isotopic  
196 equilibrium and the amount of Zn exchangeable within one minute. The amount of

197 Zn present in the pool 3 ( $E_{\text{pool3}}$ ) is calculated as the difference between soil total Zn  
198 and the amount of Zn that has been exchanged at apparent isotopic equilibrium.

199

200 *Pot experiment with *Thlaspi caerulescens*, calculation of the L value*

201 The most readily exchangeable Zn pools were labeled with carrier-free  $^{65}\text{Zn}$  (NEN  
202 Biosciences, Boston, USA; specific activity:  $2.0 \text{ GBq mg}^{-1} \text{ Zn}$ ). De-ionized water,  
203 nutrient solution and  $^{65}\text{Zn}$  were mixed well in to the soils to bring the soil moisture  
204 content to 50% water holding capacity (WHC), to provide basal nutrients and to  
205 obtain an activity of  $2.4 \text{ MBq kg}^{-1}$  soil. The activity was raised to  $4.7 \text{ MBq kg}^{-1}$  soil  
206 in the Dornach soil due its high Zn fixing capacity. The nutrient solution provided  
207  $120 \text{ mg K kg}^{-1}$  dry soil as  $\text{K}_2\text{SO}_4$  and  $\text{KH}_2\text{PO}_4$ ,  $30 \text{ mg Mg kg}^{-1}$  dry soil as  $\text{MgSO}_4$ ,  
208  $140 \text{ mg N kg}^{-1}$  dry soil as  $\text{NH}_4\text{NO}_3$ ,  $60 \text{ mg P kg}^{-1}$  dry soil as  $\text{KH}_2\text{PO}_4$  and  $58 \text{ mg S}$   
209  $\text{kg}^{-1}$  dry soil as  $\text{K}_2\text{SO}_4$  and  $\text{MgSO}_4$ . Incubation of the soils for 40 days at  $21^\circ \text{ C}$   
210 under aerobic conditions was performed to allow the  $^{65}\text{Zn}$  to label the most readily  
211 exchangeable pools. Each pot was filled with 400 g dry mass soil and the water  
212 content was raised to 75% water holding capacity (WHC) before sowing the seeds.

213 The pot experiment consisted of a randomized block design of four  
214 replicates. Ten seeds of *T. caerulescens* (Ganges ecotype) were sown and after  
215 germination (~14 days) the plants were thinned to four plants per pot. Pots were  
216 watered daily with de-ionized water to maintain 75% soil WHC. The plants were  
217 grown under a controlled environment of  $16^\circ\text{C}$  /8 h night and  $20^\circ\text{C}$  /16 h day, at  
218 70% relative humidity and a light intensity of  $280 \mu\text{mol m}^{-2}\text{s}^{-1}$ .

219 After 80 days growth, the plants were harvested by cutting shoots at the soil  
220 surface. The plant aerial biomass was washed with de-ionized water, dried at  $85^\circ \text{ C}$   
221 for 24 hours and the dry mass was measured. Plant Zn concentrations were obtained

222 by grinding the plant material with an agate ball mill and using a dry-ash digestion  
223 method adapted from Chapman and Pratt (1961). The method was performed by  
224 incinerating a 1 g sample at 500° C for 8 hours, dissolving the residual ashes with 2  
225 ml of 5.8 M analytical grade HCl that was further diluted to 50 ml with de-ionized  
226 water before element and isotope measurements. Zn concentrations were determined  
227 by ICP-OES (Varian Liberty 220) and <sup>65</sup>Zn was measured using high purity Ge  
228 bore-hole gamma detector (EAWAG, Dübendorf, Switzerland). All <sup>65</sup>Zn  
229 measurements were corrected back to the date of soil labeling.

230 Calculation of the *L* value (mg Zn kg soil<sup>-1</sup>) was performed with the  
231 following equation proposed by Smith (1981):

$$232 \quad L = \frac{(Zn_{plant} - Zn_{seed})}{\frac{{}^{65}Zn_{plant}}{{}^{65}Zn_{introduced}}} \quad (3)$$

233 where  $Zn_{plant}$  (mg Zn plant<sup>-1</sup>) is the amount of Zn in the aerial parts of the plant,  
234  $Zn_{seed}$  (mg Zn plant<sup>-1</sup>) is the amount of Zn in the seed,  ${}^{65}Zn_{plant}$  (Bq plant<sup>-1</sup>) is the  
235 amount of <sup>65</sup>Zn in the aerial parts of the plant, and  ${}^{65}Zn_{introduced}$  the total amount of  
236 <sup>65</sup>Zn introduced to the soil (Bq kg<sup>-1</sup> soil). This equation provides the most  
237 conservative calculation of *L* since it assumes that all the seed Zn was redistributed  
238 to the aerial portions of the plant. The concentration of Zn present in the seeds  
239 ( $Zn_{Seed}$ ) was 34.0 ng Zn plant<sup>-1</sup> (standard error: 0.1 ng Zn plant<sup>-1</sup>).

240

#### 241 *Selective Sequential Extraction and Total Digestion*

242 Before conducting the selective sequential extraction (SSE), soils were labeled with  
243 <sup>65</sup>Zn and incubated for 20, 85 and 120 days at 21° C. At the time of labeling, a  
244 carrier-free <sup>65</sup>Zn solution (NEN Biosciences, Boston, USA; specific activity: 2.0

245 GBq mg<sup>-1</sup> Zn) was added as ZnCl<sub>2</sub> to de-ionized water and mixed well with the soil  
246 samples at the rate of 22.5 to 86.0 MBq kg<sup>-1</sup> soil. Soils were maintained at 50%  
247 water holding capacity and well aerated during the incubation period.

248 The selective sequential extraction used in this study was a six-step  
249 procedure (F1-F6) developed by Salbu *et al.* (1998) and modified using a 1:10 soil  
250 to extractant ratio. A description of the analytical grade reagents, procedures and the  
251 proposed binding mechanisms are provided in Table 2. The extraction procedure is  
252 designed to extract metals in a step-wise fashion first from weak outer sphere bound  
253 forms (F1-F3), then from tightly bound outer and inner sphere complexes (F4 and  
254 F5) and finally from crystalline metal forms (F6). Prior to performing extractions,  
255 the soil samples were ground to a fine powder to homogenize and increase the  
256 surface area exposed to the extractants during the extraction process. For each  
257 extraction step the samples were shaken on a horizontal shaker at 100 rpm for the  
258 times listed in the procedure. Following each extraction, the samples were  
259 centrifuged at 11'000 g for 30 minutes, the solution was filtered through a 0.45 µm  
260 porosity filter and the filtrate was analyzed for Zn and <sup>65</sup>Zn. Measurements for Zn  
261 were performed on an ICP-OES (Varian Liberty 220) and <sup>65</sup>Zn was measured using  
262 high purity Ge bore-hole gamma detector (EAWAG, Dübendorf, Switzerland). All  
263 <sup>65</sup>Zn measurements were corrected back to the date of soil labeling.

264 As a final step (F7), the F6 residue was placed in an open-vessel microwave  
265 digester (MX 350 Prolabo, France) and the procedure of Lorentzen and Kingston  
266 (1996) was used to extract the residual Zn. After digestion, the sample was cooled to  
267 room temperature filtered with a 0.45 µm porosity filter prior to ICP-OES (Varian  
268 Liberty 220) analysis. In addition, a single step total digestion was conducted on all

269 soils to obtain total Zn and  $^{65}\text{Zn}$  quantities as a comparison to the total quantities  
270 recovered by the SSE.

271 The fraction of Zn that had undergone isotopic exchange in each fraction of  
272 the SSE was assessed by calculating the specific activity (SA) of Zn in each fraction  
273 normalized by the quantity of isotopes introduced during labeling ( $^{65}\text{Zn}_{\text{introduced}}$  Bq  
274  $\text{kg}^{-1}$  soil) and the total soil Zn ( $\text{Zn}_{\text{total}}$  mg Zn  $\text{kg}^{-1}$  soil) as shown in equation 4.

$$275 \quad SA = \frac{\left( \frac{^{65}\text{Zn}_{F_x}}{^{65}\text{Zn}_{\text{introduced}}} \right)}{\left( \frac{\text{Zn}_{F_x}}{\text{Zn}_{\text{total}}} \right)} \quad (4)$$

276 In this equation the subscript  $F_x$  is the extraction number,  $^{65}\text{Zn}_{F_x}$  represents the  
277 radioisotope concentration (Bq  $\text{kg}^{-1}$  soil) in this extract and  $\text{Zn}_{F_x}$  the total Zn  
278 concentration (mg Zn  $\text{kg}^{-1}$  soil) in the same extract.

279

#### 280 *Statistics*

281 All soil analyses were conducted in triplicate while the plant analyses were made  
282 with four replicates. Mean values are presented with the standard errors.  
283 “Statgraphics plus for Windows” was used for both linear and nonlinear regressions.  
284 The standard error of estimate (SEE) and the coefficient of determination are given  
285 for each regression. The validity of regressions was evaluated by comparing the  
286 predicted and experimental values and by looking at the residuals.

287

#### 288 *Extended X-ray Absorption Fine Structure (EXAFS) Spectroscopy*

289 Untreated reference samples of the Dornach and Mortagne soils and residues  
290 obtained from the selective sequential extraction were air-dried at 35° C ground and  
291 pressed into 5 mm diameter pellets for EXAFS analysis.

292 Experiments were conducted in 2003 at the European Synchrotron Radiation  
293 Facility (ESRF, Grenoble, France) on beamline ID-26. The electron storage ring was  
294 operating in 16 bunch mode at 6 GeV and current ranging from 70 to 90 mA. The  
295 monochromator was a pair of Si(220) flat crystals. Spectra were collected in  
296 fluorescence mode using a photo-diode detector and aluminum filters. For each  
297 sample 10-20 scans of 40 minutes were averaged. Data extraction was done using  
298 WinXAS (version 2.0, Ressler, 2000).

299 Data analysis was done by linear combination fits (LCFs) without principal  
300 component analysis since this latter approach is not adapted to small sets of spectra.  
301 The LCFs were conducted using a reference Zn K-edge reference spectra library  
302 described previously (Manceau *et al.*, 2003; Sarret *et al.*, 2004). The maximum  
303 number of components for the fit of the extraction residues and soil spectra was  
304 limited to four since the precision of the method does not enable a reliable  
305 quantification of more complex mixtures. From these four components,  
306 concentrations of Zn species (mg Zn kg<sup>-1</sup> soil) in each sample were calculated by  
307 multiplying the percentage of each component by the total Zn concentration in the  
308 sample.

309

## 310 **Results and discussion**

### 311 *Zn concentration in dilute CaCl<sub>2</sub> extracts (C<sub>Zn</sub>)*

312 We present the average C<sub>Zn</sub> values measured during the IE kinetic experiments for  
313 each soil (Table 3). The highest values were observed in the Evin and Mortagne

314 soils and the lowest values were observed in the IUL SS soil (Table 3). The  
315 logarithm of  $C_{Zn}$  was highly significantly related to soil pH and to the logarithm of  
316 the total soil Zn content of the 6 soils.

$$317 \ln(C_{Zn})=4.81-3.06pH+1.86\ln(Zn_{total}), n=6, R^2=0.97, SEE=0.67 \quad (5)$$

318 This result confirms that  $C_{Zn}$  increases with Zn inputs and decreases when soil pH  
319 increases as noted by Arias *et al.* (2005). The very low  $C_{Zn}$  values observed in the  
320 IUL SS soil can be explained by its high amorphous iron oxide content (Table 1)  
321 acting as a strong sorbent for Zn. The elevated amorphous iron oxide content of this  
322 soil is related to the repeated additions of FeCl<sub>3</sub> treated sewage sludge in this field  
323 experiment.

324

325 *Decrease of radioactivity in dilute CaCl<sub>2</sub> extracts with time during the isotopic*  
326 *exchange kinetic experiments*

327 The radioactivity found in the solution at a given time divided by the total amount of  
328 radioactivity added to the suspension ( $r_t/R$ ) decreased following the same pattern in  
329 all samples (Figure 1). In most soils the 2 last  $r_t/R$  values were very similar showing  
330 that an apparent isotopic equilibrium had been reached after 14 days. Only in the  
331 IUL PS and in the Evin soils was the last  $r_t/R$  value lower than the previous one  
332 indicating that the isotopic equilibrium might have not been reached after 14 days of  
333 IE.

334 It was possible to fit the curves describing the decrease of  $r_t/R$  with exchange  
335 time by the equation 1 by a sum of two exponential terms and a constant for the  
336 Dornach soil and by a sum of three exponential terms and a constant for the other  
337 soils (data not shown). These results suggest that the <sup>65</sup>Zn added in the solution  
338 exchanged with Zn located in 3 compartments in the Dornach soil and in 4

339 compartments in the other soils. These compartments corresponded to the following  
340 exchange times: 0-1 min, 1-30 min, 30 min-7 days and > 7 days in INRA and IUL  
341 SS; 0-1 min, 1-10 min, 10 min to 14 days and >14 days in IUL PS; 0-1 min, 1-30  
342 min, 30 min to 14 days and > 14 days in Evin; 0-1 min, 1 min to 1 day and > 1 day  
343 in Dornach; and 0-1 min, 1-30 min, 30 min to 7 days and > 7 days in Mortagne.  
344 Models with two exponential terms and a constant were tried for all soils but gave a  
345 proper fit only for Dornach (results not shown). This analysis suggests the presence  
346 in all soils of a compartment of Zn exchangeable during the first minute, while other  
347 compartments differed from soil to soil.

348         This compartmental analysis has some limits and its results must be  
349 interpreted with caution. More sampling points might have resulted in the  
350 determination of more compartments (Fardeau, 1993). Besides, it is extremely  
351 difficult to sample the suspension at exchange time shorter than 1 minute (Fardeau,  
352 1993). The stochastic approach used by Sinaj *et al.* (1999) was tested with our soils.  
353 This approach allowed modeling the changes of  $r_t/R$  with time as well as the sum of  
354 exponentials for all soils except for IUL PS where it led to negative values of  
355 radioactivity as time tended towards the infinity (results not shown). Besides, this  
356 stochastic approach does not allow distinguishing compartments of exchangeable  
357 elements (Fardeau, 1993).

358

### 359 *Calculation of the amount of isotopically exchangeable Zn (E values)*

360 Because of the different number of compartments observed between soils we prefer  
361 to summarize the information given by the compartmental analysis by considering  
362 only three pools of exchangeable Zn for each soil: the amount of Zn exchangeable  
363 within 1 minute which is observed in all soils (pool 1), the amount of Zn



364 exchangeable that is exchangeable on the medium term (pool 2), and the amount of  
365 Zn that is very slowly or not exchangeable (pool 3). The pool 2 corresponds to the  
366 fraction of soil Zn that is exchangeable between 1 minute and apparent isotopic  
367 equilibrium, i.e. between 1 minute and 1 day in Dornach, between 1 minute and 7  
368 days in INRA, IUL SS and Mortagne, and between 1 minute and 14 days in IUL PS  
369 and Evin. The pool 3 corresponds to the amount of Zn that could not be isotopically  
370 exchanged within 1 day in Dornach, within 7 days in INRA, IUL SS and Mortagne  
371 and within 14 days in Evin and IUL PS.

372 The amounts of Zn present in the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> pools ( $E_{\text{pool1}}$ ,  $E_{\text{pool2}}$  and  
373  $E_{\text{pool3}}$ ) are presented in Table 3. Soils polluted with organic amendments (INRA,  
374 IUL SS, IUL PS) had between 3.5 and 11.9% of the total Zn in the pool 1 and  
375 between 68.1 and 78.9% of total Zn in the pool 3. The Evin and Mortagne smelter-  
376 impacted soils had between 42.3 and 46.8% of the total Zn in the pool 1 and  
377 between 39.1 and 39.9% of total Zn in the pool 3. The Dornach smelter-impacted  
378 soil showed an intermediate result with 10.7% of Zn in the pool 1 and 67.5% in the  
379 pool 3. Highly significant relationships were observed between the logarithm of the  
380 Zn content of pool 1 ( $E_{\text{pool1}}$ ) and pool 2 ( $E_{\text{pool2}}$ ) and the logarithm of total Zn and  $pH$   
381 (equations 6 and 7).

$$382 \ln(E_{\text{pool1}}) = -0.51 - 1.03pH + 1.67\ln(Zn_{\text{total}}), n=6, R^2=0.98, SEE=0.39 \quad (6)$$

$$383 \ln(E_{\text{pool2}}) = -2.92 + 0.19pH + 1.03\ln(Zn_{\text{total}}), n=6, R^2=0.98, SEE=0.23 \quad (7)$$

384 These equations show that the amount of Zn present in the 1<sup>st</sup> pool increases with Zn  
385 inputs and decreases with pH, while the amount of Zn present in the 2<sup>nd</sup> pool  
386 increases with Zn inputs and pH.

387

388 *Pot experiment with T. caerulescens, calculation of the L value*

389 The results are presented in Table 4. The biomass production of *T. caerulescens* was  
390 similar in all soils, but the Zn content in the plant increased with soil total Zn  
391 content. The *L* values varied between 22.2 and 32.9% of the total soil Zn content in  
392 the three soils that had been polluted by organic amendments and between 33.4 and  
393 56.4% of the total soil Zn content in the three soils that had been polluted by smelter  
394 emissions. The *L* values were numerically very similar to the sum of the Zn content  
395 present in the 2 first pools of the isotope exchange kinetic analysis (equations 8 and  
396 9).

$$397 \ln(L)=0.13+0.97\ln(E_{\text{pool1}}+E_{\text{pool2}}), n=6, R^2=0.99, \text{SEE}=0.08 \quad (8)$$

$$398 \ln(E_{\text{pool1}}+E_{\text{pool2}})=-0.12+1.03\ln(L), n=6, R^2=0.99, \text{SEE}=0.08 \quad (9)$$

399 This result shows that pools 1 and 2 contain the soil Zn that can be accessed by *T.*  
400 *caerulescens* through diffusion and desorption.

401

#### 402 *Selective sequential extraction of <sup>65</sup>Zn and Zn from incubated soil*

403 No significant shifts in <sup>65</sup>Zn concentration between the extracted fractions were  
404 noted for all soils between 20, 85 and 120 days of incubation, with the exception of  
405 the Evin and Mortagne soils in which <sup>65</sup>Zn concentrations significantly decreased in  
406 F2 while the concentration of <sup>65</sup>Zn increased in F3 and F4. This coincides with the  
407 findings of Almås *et al.* (1999, 2000) in which measurable levels of <sup>65</sup>Zn were found  
408 among all fractions within 7 days of soil labeling.

409 The normalized specific activities observed for each fraction are presented in  
410 Table 6. A monotonous decrease down to null activities was expected from the most  
411 exchangeable (F1) to the residual (F7) fraction. However, the specific activities  
412 values obtained in F1 were often lower than in F2. We suggest that the relatively  
413 low Zn and <sup>65</sup>Zn concentrations extracted by the water (F1) and their high variability

414 might explain the difficulties in calculating correct specific activities values. If we  
415 do not consider F1, a monotonous decrease is observed from F2 to F7, except for the  
416 F3 extraction for the Evin and Mortagne soils. The activity is very low in the F6  
417 extract and close to 0 in the F7 residual, as expected. This <sup>65</sup>Zn tracing of the SSE  
418 confirms that the chosen extractants induced a progressive removal of Zn from  
419 highly exchangeable to recalcitrant species.

420 The average concentrations of Zn in the different fractions of the SSE are  
421 provided in Table 5. The total amount of Zn recovered from this sequential  
422 extraction ranged between 90.9 and 116% of the total Zn content measured after  
423 direct digestion. Between 41.0 and 49.6% of the total Zn was extracted in the 6<sup>th</sup>  
424 step (F6) in INRA, IUL SS and IUL PS soils while in the Evin and Mortagne soils  
425 between 45.4 and 53.6% of the Zn was extracted in the 2<sup>nd</sup> and 3<sup>rd</sup> steps (F2-F3  
426 fractions). This predominance of exchangeable species is most likely due to the  
427 dissolution of smelter-inherited primary minerals (franklinite, sphalerite, willemite)  
428 and redistribution in the exchangeable fractions as described by various authors  
429 (Juillot et al, 2003, Manceau et al., 2000, Roberts et al., 2002).

430 Comparison between the amounts of Zn recovered in the different fractions  
431 of the SSE (Table 5) and the amount of isotopically exchangeable Zn (Table 3),  
432 shows that the total amount of Zn extracted during the 3 first steps ( $Zn_{F1+F2+F3}$ ) is  
433 slightly higher than the amount of very rapidly exchangeable Zn ( $E_{pool1}$ ) (equations  
434 10 and 11).

$$435 \ln(Zn_{F1+F2+F3})=0.78+0.92\ln(E_{pool1}), n=6, R^2=0.97, SEE= 0.38 \quad (10)$$

$$436 \ln(E_{pool1})=-0.68+1.05\ln(Zn_{F1+F2+F3}), n=6, R^2=0.97, SEE=0.40 \quad (11)$$

437 This suggests that these 3 first steps have extracted the entire quantity of Zn  
438 isotopically exchangeable within 1 minute, and that F3 extracted a fraction of the Zn

439 exchangeable on the medium term. Highly significant correlations were also found  
440 between  $\ln(Zn_{F5+F6+F7})$  and  $\ln(E_{pool3})$  (equations 12 and 13).

441  $\ln(Zn_{F5+F6+F7})=1.20+0.73\ln(E_{pool3})$ , n=6,  $R^2=0.87$ , SEE=0.35 (12)

442  $\ln(E_{pool3})=-0.65+1.19\ln(Zn_{F5+F6+F7})$ , n=6,  $R^2=0.87$ , SEE=0.44 (13)

443 The lower amount of Zn recovered in F5+F6+F7 compared to  $E_{pool3}$  suggests that a  
444 fraction of very slowly or not exchangeable Zn had already been extracted in F4.  
445 Since F1+F2+F3 extracted the Zn present in the pool 1 and some of the Zn present  
446 in pool 2 and F5+F6+F7 extracted a fraction of the pool 3, we conclude that the 4<sup>th</sup>  
447 step of the SSE solubilized both moderately and slowly exchangeable forms of Zn,  
448 *i.e.* Zn from pools 2 and 3.

449

#### 450 *Zn K-edge EXAFS spectroscopy*

451 Zinc K-edge EXAFS analysis was conducted on the Dornach and Mortagne  
452 untreated samples and on the F2 and F3 residues for the Mortagne soil, and the F3,  
453 F4 and F5 residues for the Dornach soil.

454 Figure 2 shows the Zn K-edge EXAFS spectra for some reference  
455 compounds used in the linear combination fits, including franklinite, Zn-sorbed  
456 birnessite (Mn oxide), Zn-substituted kerolite as a proxy for Zn-substituted  
457 phyllosilicate, Zn/Al hydrotalcite, a zinc-aluminum hydroxycarbonate, Zn-sorbed  
458 ferrihydrite, Zn-humic acid complexes (Zn-HA) at high and low Zn loading, and  
459 aqueous  $Zn^{2+}$  as a proxy for outer sphere complexes. Franklinite is easily identified  
460 by the high amplitude and multiple frequencies of its spectrum. The spectra for Zn-  
461 kerolite and Zn/Al hydrotalcite present some similarities, which makes their  
462 distinction difficult in a mixture (Panfili *et al.*, 2005). In the LCFs, these two  
463 compounds and Zn-sorbed hectorite were grouped as "Zn-phyllosilicate". Similarly,

464 the spectra for Zn-HA at low Zn loading and Zn-sorbed ferrihydrite look similar  
465 because Zn is 4-fold coordinated to oxygen atoms and the second shell contribution  
466 weak in the two references. Zn-HA at low Zn loading is a proxy for strongly bound  
467 inner sphere Zn-organic complexes in tetrahedral configuration (Sarret *et al.*, 1997).  
468 In the LCFs, the two tetrahedral species were grouped as "tetrahedral Zn-HA and/or  
469 Zn-sorbed ferrihydrite". Another pair of similar spectra is Zn-HA at high Zn loading  
470 and aqueous Zn<sup>2+</sup>. Because Zn is octahedrally coordinated, and the second shell  
471 contribution either weak (Zn-HA) or absent (aqueous Zn). These species are  
472 considered as representatives for less-strongly to weakly bound inner sphere Zn-  
473 organic complexes and outer sphere organic and inorganic complexes (Sarret *et al.*,  
474 1997). In the LCFs, these species were grouped as "weakly bound octahedral Zn".

475 Figure 3a shows the EXAFS spectra for the untreated soil from Mortagne  
476 and the two residues, and their reconstructions with four component spectra. In the  
477 untreated soil (MRef), Zn is distributed as  $60 \pm 10$  % weakly bound octahedral Zn  
478 complexes,  $17 \pm 10$  % Zn-phyllsilicate,  $16 \pm 10$  % tetrahedral Zn-HA and/or Zn-  
479 sorbed ferrihydrite and 7% franklinite (Figure 3b). The detection limit for this last  
480 species is less than 10% because its spectrum has a high amplitude. Zn-sorbed  
481 goethite and Zn-sorbed hematite spectra were tested, but neither of them are  
482 component species to the data. In a previous study on the same soil (Manceau *et al.*,  
483 2000), Zn- phyllsilicate, Zn-sorbed birnessite and Zn sorbed on iron oxyhydroxides  
484 were identified as the main Zn species. Another study on a tilled soil near the  
485 Mortagne area concluded to the presence of Zn outer-sphere complexes, Zn-organic  
486 matter inner-sphere complexes, Zn/Al-hydrotalcite, Zn-phyllsilicate, and  
487 magnetite-franklinite solid solutions (Juillot *et al.*, 2003). The F2 extraction  
488 removed 41 % of the soil Zn, and most of the weakly bound octahedral Zn pool. The

489 F3 extraction removed 15% of the initial soil Zn, the rest of the weakly bound pool,  
490 and some of the tetrahedral pool. The Zn-phyllsilicate and franklinite pools were  
491 marginally affected. The occurrence of 10-14% Zn as franklinite in the MF2 and  
492 MF3 samples is attested by the sharpening of the second oscillation centered at 6 Å<sup>-1</sup>.  
493 In this soil the F2 and F3 extractions are relatively specific, affecting mostly the  
494 weakly bound octahedral Zn pool.

495 In this soil the weakly bound octahedral Zn measured in the untreated  
496 sample (784 mg Zn kg<sup>-1</sup> soil) was identical to the IE exchangeable Zn ( $E_{\text{pool1}}+E_{\text{pool2}}$ ,  
497 786 mg Zn kg<sup>-1</sup> soil) and to the amount of Zn extracted by the three first steps of the  
498 SSE (F1+F2+F3, 729 mg Zn kg<sup>-1</sup> soil) (Table 7). These observations suggest that  
499 weakly bound octahedral Zn is the main source of available Zn in this soil which is  
500 consistent with our previous EXAFS and isotopic exchange study (Sarret *et al.*,  
501 2004).

502 A different behavior is observed for the Dornach soil (Figure 4). Satisfactory  
503 fits were obtained with three components for the untreated soil (DRef) and F3 and  
504 F4 residues (DF3 and DF4), and with four components for the F5 residue (DF5).  
505 The weakly bound octahedral Zn are predominant in the Dornach soil ( $57 \pm 10 \%$ )  
506 followed by Zn-phyllsilicate ( $27 \pm 10 \%$ ), and tetrahedral Zn-HA and/or Zn-sorbed  
507 ferrihydrite ( $16 \pm 10 \%$ ). The proportions of Zn species did not change statistically  
508 in DF3 and DF4, which suggests that all species were affected to a similar extent by  
509 the F3 and F4 treatments (removal of 19 and 41 % of total soil Zn, respectively).  
510 The F5 extraction (removal of 19% total soil Zn) targeted preferentially the "weakly  
511 bound octahedral Zn" pool. As a consequence, Zn-phyllsilicate was the major  
512 species in the DF5 residue. The DF5 spectrum was simulated with Zn-hectorite,  
513 whereas DRef, DF3 and DF4 were simulated with Zn-kerolite and/or Zn/Al

514 hydrotalcite. Indeed, the third oscillation of the DF5 and Zn-hectorite spectra have  
515 the same shape, whereas the shoulder between 7.0 and 7.5 Å<sup>-1</sup> in DRef corresponds  
516 to a high amplitude feature in Zn-kerolite and/or Zn/Al hydrotalcite spectra (Figures  
517 2 and 4a). Therefore, the local environment of Zn seems to have evolved during the  
518 selective sequential extraction. A finer description of the nature and structure of  
519 these species would require studying the < 2 µm or < 0.2 µm soil fractions by  
520 polarized EXAFS (Manceau *et al.*, 2000). A new species is detected in DF5, Zn-  
521 sorbed birnessite. This species was probably present in the previous samples  
522 including those from Mortagne as shown by micro-EXAFS (Manceau *et al.*, 2000),  
523 but as a minor component masked by the predominant species. Chemical extractions  
524 lacked selectivity in the Dornach soil because the fractional amount of the major  
525 species remained unchanged in DF3 and DF4 despite a removal of 19 and 41% of  
526 the soil Zn. The weakly bound octahedral Zn complexes which were extracted  
527 completely after the third extraction step in Mortagne soil remained predominant in  
528 DF3 and DF4.

529 In Dornach the weakly bound octahedral Zn measured in the untreated  
530 sample (962 mg Zn kg<sup>-1</sup> soil) was similar to the Zn extracted by the four first steps  
531 of the SSE (F1+F2+F3+F4, 1023 mg Zn kg<sup>-1</sup> soil), but was much higher than the IE  
532 exchangeable Zn ( $E_{\text{pool1}}+E_{\text{pool2}}$ , 549 mg Zn kg<sup>-1</sup> soil), which itself was also higher  
533 than the amount of Zn extracted by the three first steps of the SSE (F1+F2+F3, 326  
534 mg Zn kg<sup>-1</sup> soil) (Table 7). Although Dornach exhibited a high proportion of  
535 weakly bound octahedral Zn, only a small proportion of it was extracted by the 3  
536 first extractions of the SSE (22%). We suggest that the IE Zn and the Zn extracted  
537 by the 3 first steps of the SSE was indeed present as weakly bound octahedral Zn,  
538 but a large fraction of these so-called weakly bound species were neither IE

539 exchangeable nor extractable by the three first steps of the SSE. The different  
540 behavior of Zn in Dornach and Mortagne may be explained by the difference in soil  
541 pH (6.7 for Dornach and 5.1 for Mortagne) and in soil organic matter content (11%  
542 for Dornach and 1% for Mortagne) because inner-sphere mineral surface complexes  
543 and organically-bound cationic species are more strongly retained at near neutral  
544 than at acidic pH. Altogether these results suggest that isotopically exchangeable Zn  
545 and therefore available Zn is present as weakly bound octahedral Zn species but that  
546 the proportion of weakly bound octahedral Zn that can exchange with  $Zn^{2+}$  in the  
547 solution decreases when soil pH and organic matter content increase.

548

#### 549 **Conclusion**

550 The combination of techniques used in this work (isotopic exchange kinetics, pot  
551 experiment with *T. caeruleus* on soil labeled with  $^{65}Zn$ , selective sequential  
552 extraction carried out on  $^{65}Zn$  labeled soils, and EXAFS spectroscopy) gave  
553 comprehensive information on the forms and availability of Zn in these heavy metal  
554 polluted soils. The main results of this study are summarized in the Table 7.

555         Our results allowed quantifying the number of compartments containing IE  
556 Zn in these soils. Three pools were derived from this analysis, the amount of Zn  
557 exchangeable within 1 minute (1<sup>st</sup> pool), the amount of Zn exchangeable between 1  
558 minute and apparent isotopic equilibrium (2<sup>nd</sup> pool) and the amount of Zn that could  
559 not be exchanged during the IE kinetic experiment (3<sup>rd</sup> pool). The experiment  
560 conducted with *T. caeruleus* confirmed that the amount of IE Zn measured in pot  
561 experiments was similar to the sum of Zn content present in the 1<sup>st</sup> and 2<sup>nd</sup> pools, i.e.  
562 that this plant had only access the IE forms of Zn.



563 The use of the SSE on  $^{65}\text{Zn}$  labeled soils showed that the 3 first extractions  
564 (F1, F2 and F3) solubilized the amount of Zn present in the 1<sup>st</sup> pool and a slight  
565 fraction of the Zn present in the 2<sup>nd</sup> pool. The three last fractions of the SSE (F5, F6  
566 and F7) solubilized the Zn from the 3<sup>rd</sup> pool. We deduced from these observations  
567 that the 4<sup>th</sup> extraction of the SSE solubilized the Zn from the 2<sup>nd</sup> pool and a fraction  
568 of the Zn from the 3<sup>rd</sup> pool.

569 Finally, EXFAS spectroscopy showed that the 3 first extractions of the SSE  
570 solubilized all the weakly bound octahedral Zn in the Mortagne soil, i.e. that the 1<sup>st</sup>  
571 pool was dominated by these Zn species. In Dornach the 5 first extractions of the  
572 SSE were necessary to solubilize the weakly bound octahedral Zn. We suggest that  
573 in Dornach most of the weakly bound octahedral Zn was not IE nor extractable in  
574 the 3 first steps of the SSE. The difference between Mortagne and Dornach could  
575 be explained by the highest pH and soil organic matter content of the latter.  
576 Altogether these results suggest that isotopically exchangeable Zn and therefore  
577 available Zn is present as weakly bound octahedral Zn species but that the  
578 proportion of weakly bound octahedral Zn that can exchange with  $\text{Zn}^{2+}$  in the  
579 solution decreases when soil pH and organic matter content increase.

580

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591

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714 **Figure Captions**

715 **Figure 1** Change in the fraction of radioactivity remaining in solution ( $r_t/R$ ) during  
716 14 days in six soils that have been polluted with heavy metals (1a INRA soil, 1b  
717 IUL SS soil, 1c IUL PS soil, 1d Evin soil, 1e Dornach soil, 1f Mortagne soil). The  
718 points represent the experimental data, the solid line the values predicted from the  
719 model developed for each soil, and the dotted lines the 80% confidence limits of the  
720 model.

721

722 **Figure 2** Zn K-edge EXAFS spectra ( $\chi(k) k^3$ , with  $k$ : wave number) of reference Zn  
723 compounds used to model the spectra for the soils and extraction residues:  
724 Franklinite ( $ZnFe_2O_4$ ), Zn-sorbed birnessite (adsorption at pH 4, Zn/Mn = 0.134)  
725 (Manceau *et al.*, 2000), Zn-substituted phyllosilicate ((Zn, Mg) kerolite  
726  $Zn_{2.1}Mg_{0.9}Si_4O_{10}(OH)_2 \cdot nH_2O$ , Schlegel and Manceau, 2006 and Zn-substituted  
727 hectorite, Schlegel *et al.*, 2001), Zn/Al hydrotalcite ( $Zn_2Al(OH)_6(CO_3)_{0.5} \cdot n H_2O$ ),  
728 Zn-sorbed ferrihydrite containing 1500 mg kg<sup>-1</sup> Zn (Manceau *et al.*, 2000), Zn-  
729 humic acid complexes at low (600 mg kg<sup>-1</sup> Zn) and high (3.2 % Zn) Zn loading  
730 (Sarret *et al.*, 1997), and aqueous Zn (pH 4).

731

732 **Figure 3** a) Zn K-edge EXAFS spectra (solid line) and linear combination fit  
733 (dashed line) for the Mortagne untreated reference soil (MRef), for the residue left  
734 after the second (MF2) and third (MF3) extraction of the SSE. b) Distribution of Zn  
735 species derived from the fits. The error bars correspond to 10% of total Zn content.

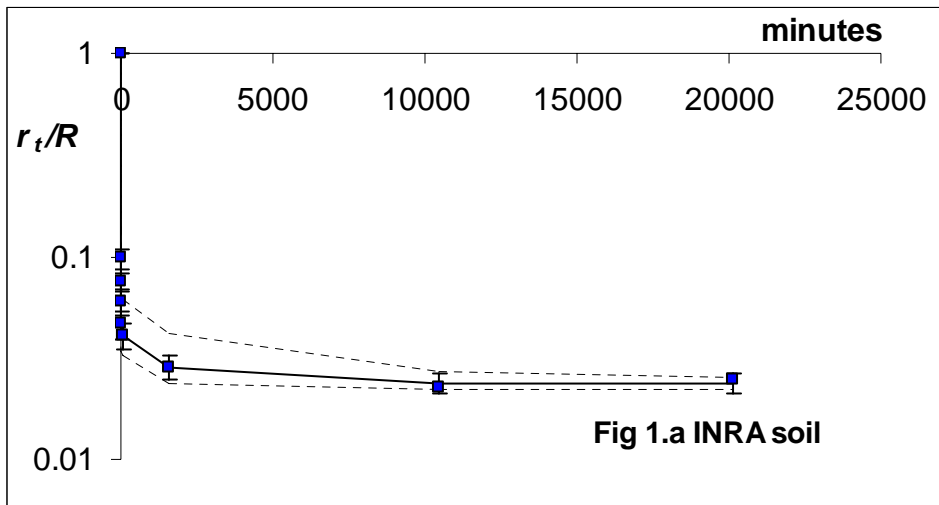
736

737 **Figure 4** a) Zn K-edge EXAFS spectra (solid line) and linear combination fits  
738 (dashed line) for the Dornach untreated reference soil (DRef), for the residue left

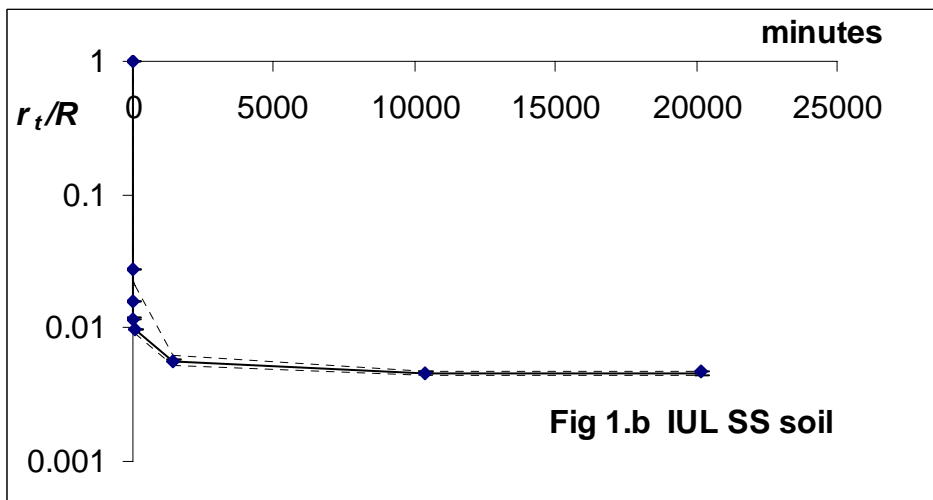


739 after the third (DF3), fourth (DF4) and fifth (DF5) extraction of the SSE. b)  
740 Distribution of Zn species derived from the fits. The error bars correspond to 10% of  
741 total Zn content.

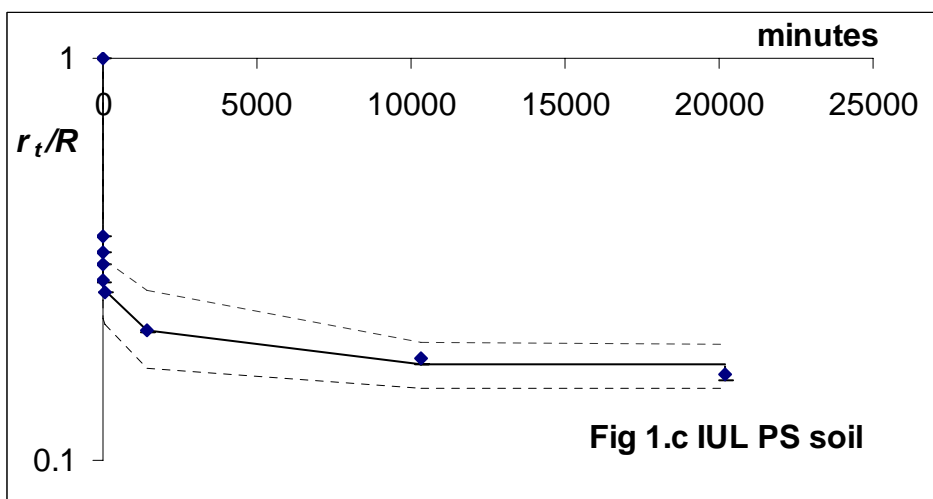
742 Figure 1



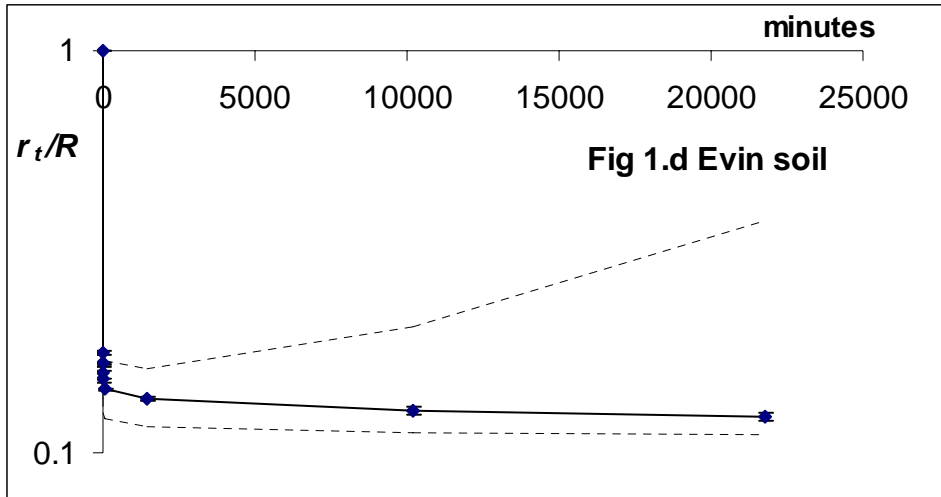
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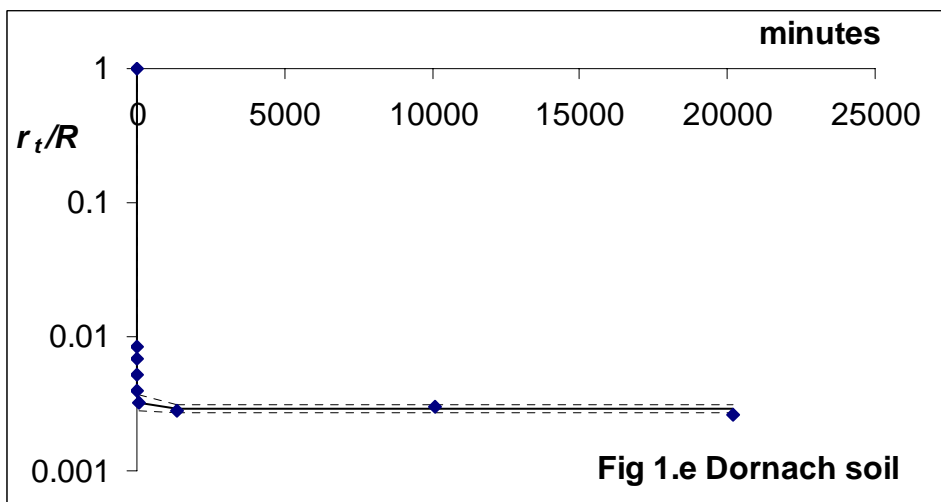
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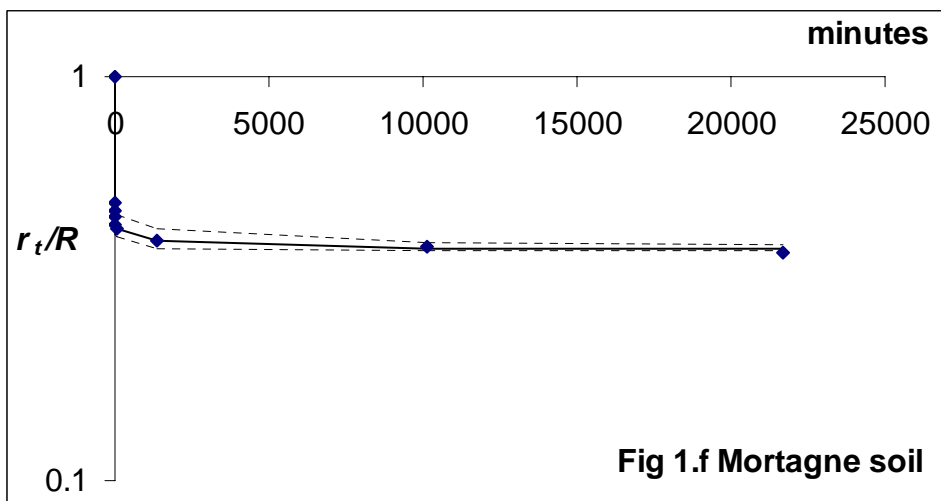
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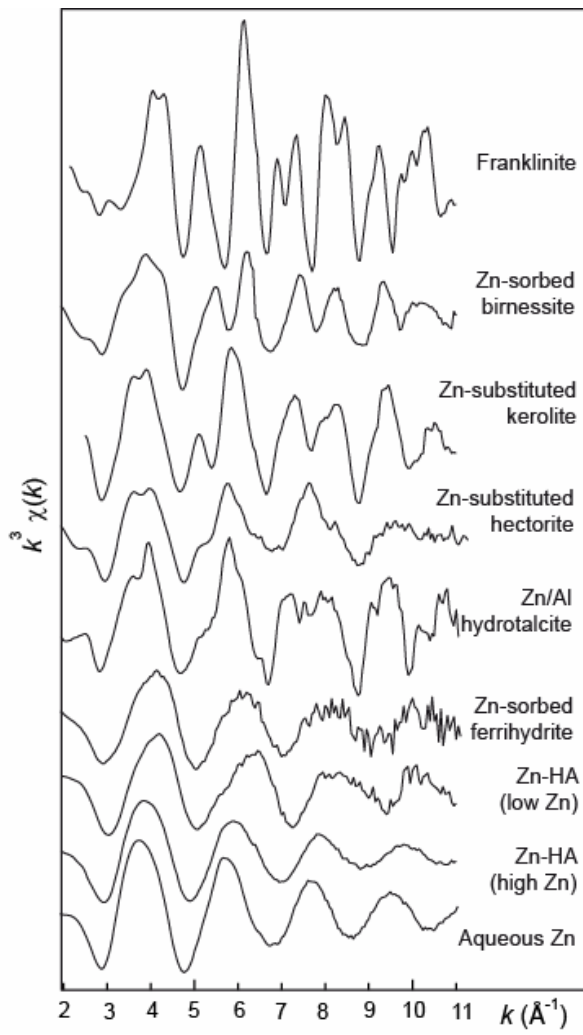


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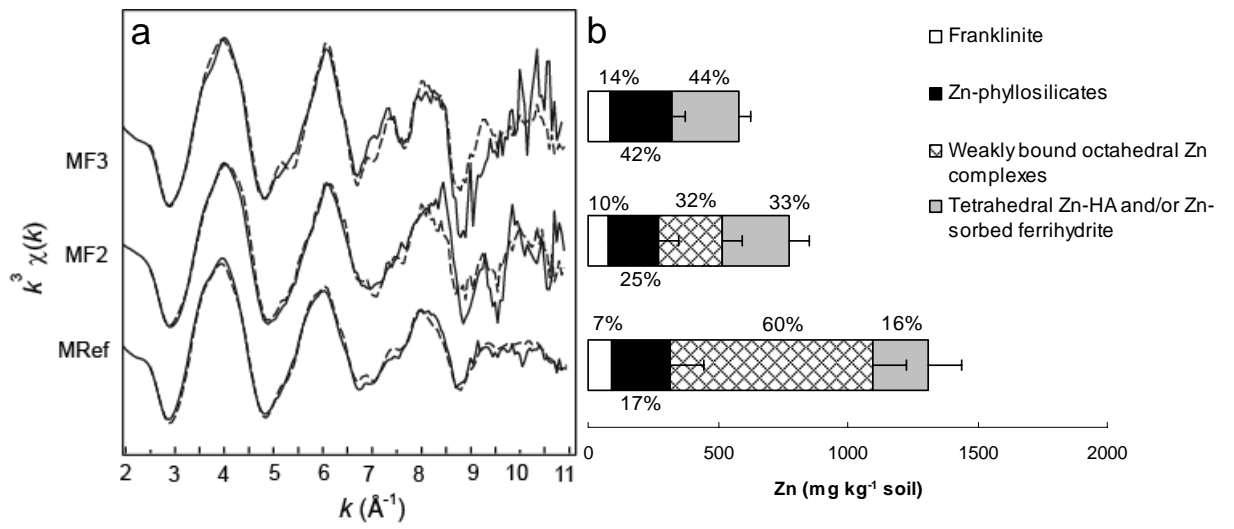
748

749 Figure 2



750

751 Figure 3

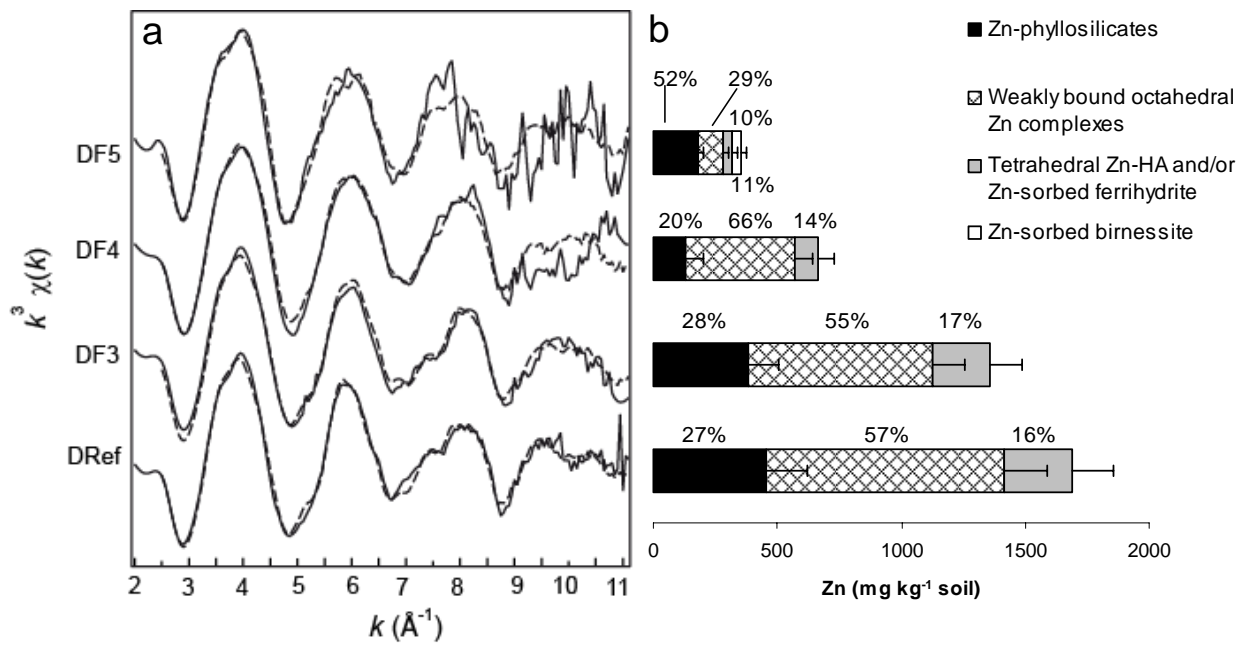


752 Figure 4

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**Table 1** Selected characteristics of the six soils polluted with heavy metals

Characteristic	INRA	IUL SS	IUL PS	Evin	Dornach	Mortagne
Soil type	Eutric Fluvisol	Orthic Luvisol	Orthic Luvisol	Gleyic Luvisol	Calcaric Regosol	Dystric Cambisol
Pollutant source	Sewage Sludge	Sewage Sludge	Pig Slurry	Pb/Zn Smelter	Cu/Ni/Zn Smelter	Zn Smelter
Land use	arable	Arable	Arable	forest	grassland	grassland
Sampling depth / cm	0-20	0-20	0-20	2-5	2-5	5-40
Sand / % <sup>a</sup>	80	57	58	28	15	66
Clay / % <sup>a</sup>	7	15	15	20	37	7
Organic matter / % <sup>b</sup>	2	3	2	4	11	1
CaCO <sub>3</sub> / % <sup>c</sup>	n.d.	n.d.	n.d.	n.d.	5	n.d.
Oxalate extractable Fe / g kg <sup>-1</sup> soil <sup>d</sup>	2.14	11.11	5.11	3.17	2.70	1.88
EDTA extractable Zn / mg kg <sup>-1</sup> soil <sup>e</sup>	166	15	9	435	349	406
pH <sup>f</sup>	5.7	6.0	4.6	5.0	6.7	5.1
CEC / mmol <sub>c</sub> kg <sup>-1g</sup>	101	164	161	185	432	67
Base saturation / % <sup>g</sup>	47.8	17.5	45.2	85.6	42.2	18.8
Total Zn / mg kg <sup>-1</sup> soil <sup>i</sup>	680 (5.4)	162 (0.5)	87.9 (1.7)	1647 (3.1)	1687 (3.1)	1307 (8.7)

n.d. non detectable

<sup>a</sup> Soil texture was measured via sedimentation with (NaPO<sub>4</sub>)<sub>6</sub> as a dispersion agent (FAL, RAC & FAW, 1996).

<sup>b</sup> Organic matter was measured by titration (FAL, RAC & FAW, 1996).

<sup>c</sup> CaCO<sub>3</sub> was quantified using concentrated HCl (FAL, RAC & FAW, 1996).

<sup>d</sup> Oxalate extractable Fe was determined according to Loeppert and Inskeep (1996).

<sup>e</sup> EDTA-NH<sub>4</sub>Ac extractions were performed using 50ml of extractant added to 10g dry soil (FAL, RAC & FAW, 1996).

<sup>f</sup> pH was measured using a 1:2.5 soil solution ratio of 0.01M CaCl<sub>2</sub> after 24 hours of gentle shaking.

<sup>g</sup> CEC and base saturation were determined using BaCl<sub>2</sub> method (FAL, RAC & FAW, 1996).

<sup>i</sup> Total Zn obtained after direct digestion (n=3) and standard error (in parentheses).

758

759 **Table 2** Selective sequential extraction used for the fractionation of Zn based on Salbu *et al.* (1998) and Lorentzen and Kingston (1996)

760

Extraction step	Reagents	Procedure	Proposed binding mechanism targeted <sup>a</sup>
F1	H <sub>2</sub> O	1 hours at 20° C	Water soluble
F2	1 M NH <sub>4</sub> OAc, pH 7	2 hours at 20° C	Reversible physisorption
F3	1 M NH <sub>4</sub> OAc, pH 5 (HNO <sub>3</sub> )	2 hours at 20° C	Reversible electrosorption
F4	0.04 M NH <sub>2</sub> OH-HCl in 4.4 M CH <sub>3</sub> COOH	6hours at 80° C	Irreversible chemisorption / reduction
F5	9.7 M H <sub>2</sub> O <sub>2</sub> , pH 2 (HNO <sub>3</sub> ) followed by 3.2 M NH <sub>4</sub> OAc in 4.4 M HNO <sub>3</sub>	5.5 hours at 80° C  30 minutes at 20° C	Chemisorption / oxidation
F6	7 M HNO <sub>3</sub>	6 hours at 20° C	Chemisorption / crystalline
<b>Total Digestion</b>			
F7	HNO <sub>3</sub> , H <sub>2</sub> O <sub>2</sub> and HCl Open- Microwave Digestion	45 minutes at 70°, 90° C	Residue

<sup>a</sup> Proposed by Salbu *et al.* (1998)



761 **Table 3** Average Zn concentration in dilute CaCl<sub>2</sub> extracts ( $C_{Zn}$ ) measured during the isotopic exchange experiments and amounts of Zn  
 762 exchangeable within 1 minute ( $E_{pool1}$ ), moderately isotopically exchangeable Zn ( $E_{pool2}$ ) and slowly or not exchangeable Zn ( $E_{pool3}$ )  
 763 calculated for six soils polluted with heavy metals. The average data is followed by the standard error (se) given between parentheses.

Soil	$C_{Zn}$		$E_{pool1}$		$E_{pool2}$		$E_{pool3}$	
	Average — /mg Zn l <sup>-1</sup> —	Se —	Average	se	Average	se	Average	se
			————— /mg Zn kg <sup>-1</sup> soil —————					
INRA	0.50	0.030	50.7	(3.0)	167	(5.9)	463	(8.8)
IUL SS	0.02	0.003	5.7	(0.6)	28.6	(2.5)	128	(3.1)
IUL PS	0.38	0.006	10.5	(0.3)	12.6	(0.9)	64.8	(0.7)
Evin	12.4	0.149	697	(11.6)	305	(14.4)	645	(10.6)
Dornach	0.15	0.007	181	(1.9)	368	(18.5)	1138	(18.6)
Mortagne	29.9	0.429	612	(10.2)	174	(4.1)	521	(7.0)

764

765 **Table 4** Average values for plant aerial dry matter, Zn content of aerial parts and *L* value measured with *Thlaspi caerulescens* in six soils  
 766 polluted with heavy metals. The standard errors (se) are given between parentheses.

767

Soil	Yield		Plant Zn content		<i>L</i> value	
	Average	se	Average	se	Average	se
	/ g DM kg <sup>-1</sup> soil		/ g kg <sup>-1</sup> DM		/ mg Zn kg <sup>-1</sup> soil	
INRA	16.5	(0.5)	0.4	(0.1)	224	(14.0)
IUL SS	23.4	(0.3)	1.2	(0.1)	36	(0.2)
IUL PS	20.6	(1.1)	1	(0.1)	22.6	(0.7)
Evin	21.7	(0.4)	4	(0.5)	828	(14.1)
Dornach	20.4	(0.8)	2.2	(0.5)	564	(0.8)
Mortagne	21.7	(0.7)	5.9	(0.3)	737	(4.7)

768 **Table 5** Average amount of Zn recovered in the different fractions of the selective sequential extraction after 20, 85 and 120 days of  
 769 incubation in six soils polluted by heavy metals. The results are expressed in mg Zn kg<sup>-1</sup> soil and the standard errors (se) are given between  
 770 parentheses.

771

Fractions	INRA		IUL SS		IUL PS		Evin		Dornach		Mortagne	
	Average	se	Average	se	Average	se	Average	se	Average	se	Average	se
/mg Zn kg <sup>-1</sup> soil												
F1	2	(0.5)	0.2	(0.1)	1.2	(0.5)	23.7	(6.8)	1.2	(0.5)	28.1	(3.8)
F2	26.7	(1.8)	1.8	(0.2)	2.5	(0.2)	499	(27.9)	78.7	(4.2)	505	(26.0)
F3	103	(11.7)	10.4	(1.6)	8	(0.9)	248	(2.9)	246	(23.4)	195	(4.8)
F4	217	(10.0)	55.1	(3.5)	19.8	(1.0)	362	(22.1)	696	(44.8)	285	(20.1)
F5	71.6	(21.5)	24.4	(2.3)	11.2	(1.6)	256	(18.1)	317	(48.9)	64	(11.0)
F6	279	(38.6)	74.9	(10.6)	43.6	(4.9)	256	(36.8)	165	(37.6)	101	(11.9)
F7	10.1	(2.2)	21.4	(2.7)	12.0	(2.9)	45.0	(10.9)	28.9	(5.1)	26.2	(4.8)
Total extracted	709	(19.5)	188	(12.6)	98.2	(6.0)	1690	(42.3)	1533	(64.5)	1205	(51.2)

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775 **Table 6** Mean specific activities calculated for each fraction of the selective sequential extraction over 120 days for 6 soils polluted with  
 776 heavy metals. The values for each fraction are normalized by the <sup>65</sup>Zn introduced and the total soil Zn. Standard errors (se) are given  
 777 between parentheses.

778

Fraction	INRA		IUL SS		IUL PS		Evin		Dornach		Mortagne	
	Average	se	Average	se	Average	se	Average	se	Average	se	Average	se
F1	1.95	(0.21)	1.15	(0.16)	2.42	(0.86)	1.32	(0.38)	1.22	(0.43)	1.10	(0.16)
F2	2.51	(0.05)	4.24	(0.12)	3.54	(0.28)	1.53	(0.12)	2.10	(0.29)	1.25	(0.05)
F3	2.26	(0.11)	3.48	(0.26)	3.10	(0.17)	1.65	(0.10)	1.80	(0.06)	1.27	(0.05)
F4	1.67	(0.10)	1.89	(0.19)	2.27	(0.17)	1.00	(0.11)	0.70	(0.04)	0.58	(0.08)
F5	0.49	(0.06)	0.38	(0.01)	0.47	(0.02)	0.37	(0.06)	0.42	(0.06)	0.17	(0.03)
F6	0.01	(0.01)	0.07	(0.02)	0.08	(0.02)	0.12	(0.03)	0.22	(0.08)	0.04	(0.01)
F7	0.01	(0.01)	0.02	(0.01)	0.02	(0.01)	0.04	(0.02)	0.08	(0.02)	0.01	(0.00)

779

780 **Table 7** Comparison of the data obtained by the various techniques  
781

Soil	INRA	IUL-SS	IUL-PS	Evin	Dornach	Mortagne
Total Zn / mg Zn kg soil <sup>-1</sup>	680	162	87.9	1647	1687	1307
pH	5.7	6.0	4.6	5.0	6.7	5.1
Concentration of Zn in CaCl <sub>2</sub> ; C <sub>Zn</sub> / mg Zn l <sup>-1</sup>	0.50	0.01	0.38	12.3	0.15	29.9
$E_{\text{pool1}}/E_{\text{pool2}}/E_{\text{pool3}}$ / mg Zn kg soil <sup>-1</sup>	50.7 / 167 / 463	5.7 / 28.6 / 128	10.5 / 12.6 / 64.8	697 / 305 / 645	181 / 368 / 1138	612 / 174 / 521
<i>L</i> values / mg Zn kg soil <sup>-1</sup>	224	36	22.6	828	564	737
Extraction steps, by decreasing order of Zn recovery	F6>F4>F3> F5>F2>F7>F1	F6>F4>F5> F7>F3>F2>F1	F6>F4>F7> F5>F3>F2>F1	F2>F4>F6- F5> F3>F7>F1	F4>F5>F3> F6>F2>F7>F1	F2>F4>F3> F6>F5>F1>F7
Distribution of Zn species in the untreated soil / % <sup>a</sup>					57% weakly bound octahedral Zn + 27% Zn- phyllosilicates + 16% tetrahedral Zn-HA and/or Zn-sorbed ferrihydrite	60% weakly bound octahedral Zn + 17% Zn- phyllosilicates + 16% tetrahedral Zn-HA and/or Zn-sorbed ferrihydrite + 7% franklinite
Comparison of results obtained by the different methods / mg Zn kg soil <sup>-1</sup>					F1+F2+F3+F4 (1023) = weakly bound octahedral Zn (962) > $E_{\text{pool1}} + E_{\text{pool2}}$ (549) > F1+F2+F3 (326)	F1+F2+F3+F4 (1014) > weakly bound octahedral Zn (784) = $E_{\text{pool1}} + E_{\text{pool2}}$ (786) = F1+F2+F3 (729)

<sup>a</sup> Determined only for Dornach and Mortagne soils.

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