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Zinc speciation and isotopic exchangeability in soils polluted with heavy metals

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Summary

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

Introduction

An accurate assessment of heavy metal availability is essential for the proper management of polluted soils. Young *et al.* (2006) reviewed the use of selective 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}Cd). They measured in all
69 extracts the total Cd content and the abundance of ^{111}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.*

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

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

Materials and methods

Soils

We studied six soils that had been polluted with heavy metals. The soil from the 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^{-1}
108 $year^{-1}$ 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 $year^{-1}$ 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^{-1} (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 $CaCl_2$ 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 ^{65}Zn added as $ZnCl_2$ (NEN Biosciences,
142 Boston, USA; specific activity 2.0 GBq mg^{-1} 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. ^{65}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 $$\frac{r_t}{R} = A + \sum_{i=1}^{N-1} B_i \times e^{-C_i \times t} \qquad (1)$$

173 Where r_t/R is the fraction of ^{65}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}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, mg kg^{-1}) is calculated using the following equation:

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

191 Where v/m is the solution to soil ratio (l kg^{-1}), C_{Zn} is the Zn concentration (mg l^{-1}) in
192 solution and r_t/R is the fraction of ^{65}Zn remaining in solution at the time t of
193 sampling. The amount of Zn present in the pool 1 (E_{pool1}) is calculated considering
194 an exchange time of 1 minute, the amount of Zn present in the pool 2 (E_{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_{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}Zn (NEN
202 Biosciences, Boston, USA; specific activity: 2.0 GBq mg^{-1} Zn). De-ionized water,
203 nutrient solution and ^{65}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 MBq kg^{-1} soil. The activity was raised to 4.7 MBq kg^{-1} soil
206 in the Dornach soil due its high Zn fixing capacity. The nutrient solution provided
207 120 mg K kg^{-1} dry soil as K_2SO_4 and KH_2PO_4, 30 mg Mg kg^{-1} dry soil as $MgSO_4$,
208 140 mg N kg^{-1} dry soil as NH_4NO_3, 60 mg P kg^{-1} dry soil as KH_2PO_4 and 58 mg S
209 kg^{-1} dry soil as K_2SO_4 and $MgSO_4$. Incubation of the soils for 40 days at 21° C
210 under aerobic conditions was performed to allow the ^{65}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°C /8 h night and 20°C /16 h day, at
218 70% relative humidity and a light intensity of 280 µmol $m^{-2}s^{-1}$.
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° 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 ^{65}Zn was measured using high purity Ge
228 bore-hole gamma detector (EAWAG, Dübendorf, Switzerland). All ^{65}Zn
229 measurements were corrected back to the date of soil labeling.

230 Calculation of the *L* value (mg Zn kg soil^{-1}) was performed with the
231 following equation proposed by Smith (1981):

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

233 where Zn_{plant} (mg Zn plant^{-1}) is the amount of Zn in the aerial parts of the plant,
234 Zn_{seed} (mg Zn plant^{-1}) is the amount of Zn in the seed, $^{65}Zn_{\text{plant}}$ (Bq plant^{-1}) is the
235 amount of ^{65}Zn in the aerial parts of the plant, and $^{65}Zn_{\text{introduced}}$ the total amount of
236 ^{65}Zn introduced to the soil (Bq kg^{-1} 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^{-1} (standard error: 0.1 ng Zn plant $^{-1}$).

240

241 *Selective Sequential Extraction and Total Digestion*

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

245 GBq mg^{-1} Zn) was added as $ZnCl_2$ to de-ionized water and mixed well with the soil
246 samples at the rate of 22.5 to 86.0 MBq kg^{-1} 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 ^{65}Zn. Measurements for Zn
261 were performed on an ICP-OES (Varian Liberty 220) and ^{65}Zn was measured using
262 high purity Ge bore-hole gamma detector (EAWAG, Dübendorf, Switzerland). All
263 ^{65}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

soils to obtain total Zn and ^{65}Zn quantities as a comparison to the total quantities recovered by the SSE.

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

$$SA = \frac{\left(\dfrac{^{65}Zn_{F_X}}{^{65}Zn_{introduced}} \right)}{\left(\dfrac{Zn_{Fx}}{Zn_{total}} \right)} \qquad (4)$$

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

Statistics

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

Extended X-ray Absorption Fine Structure (EXAFS) Spectroscopy

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

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

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

Results and discussion

Zn concentration in dilute $CaCl_2$ extracts (C_{Zn})

We present the average C_{Zn} values measured during the IE kinetic experiments for 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 (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 $FeCl_3$ treated sewage sludge in this field
323 experiment.

324

325 *Decrease of radioactivity in dilute $CaCl_2$ 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 ^{65}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 1st, 2nd and 3rd pools (E_{pool1}, E_{pool2} and
373 E_{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_{pool1}) and pool 2 (E_{pool2}) and the logarithm of total Zn and *pH*
381 (equations 6 and 7).

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

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

384 These equations show that the amount of Zn present in the 1st pool increases with Zn
385 inputs and decreases with pH, while the amount of Zn present in the 2nd 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 $\ln(L)=0.13+0.97\ln(E_{pool1}+E_{pool2})$, n=6, $R^2=0.99$, SEE=0.08 (8)

398 $\ln(E_{pool1}+E_{pool2})=-0.12+1.03\ln(L)$, n=6, $R^2=0.99$, SEE=0.08 (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 ^{65}Zn and Zn from incubated soil*

403 No significant shifts in ^{65}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 ^{65}Zn concentrations significantly decreased in
406 F2 while the concentration of ^{65}Zn increased in F3 and F4. This coincides with the
407 findings of Almås *et al.* (1999, 2000) in which measurable levels of ^{65}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 ^{65}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 ^{65}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 6th
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 2nd and 3rd 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 (10)

436 $\ln(E_{pool1})=-0.68+1.05\ln(Zn_{F1+F2+F3})$, n=6, $R^2=0.97$, SEE=0.40 (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 4th
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^{2+} 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 ± 10 % weakly bound octahedral Zn
478 complexes, 17 ± 10 % Zn-phyllosilicate, 16 ± 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- phyllosilicate, 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-phyllosilicate, 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-phyllosilicate 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 $Å^{-}$
493 ^{1}. 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^{-1} soil) was identical to the IE exchangeable Zn (E_{pool1}+E_{pool2},
497 786 mg Zn kg^{-1} soil) and to the amount of Zn extracted by the three first steps of the
498 SSE (F1+F2+F3, 729 mg Zn kg^{-1} 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 ± 10 %)
506 followed by Zn-phyllosilicate (27 ± 10 %), and tetrahedral Zn-HA and/or Zn-sorbed
507 ferrihydrite (16 ± 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-phyllosilicate 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 $Å^{-1}$ 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^{-1} soil) was similar to the Zn extracted by the four first steps
531 of the SSE (F1+F2+F3+F4, 1023 mg Zn kg^{-1} soil), but was much higher than the IE
532 exchangeable Zn (E_{pool1}+E_{pool2}, 549 mg Zn kg^{-1} 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^{-1} 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

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

Conclusion

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

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

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

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

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Figure Captions

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

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

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

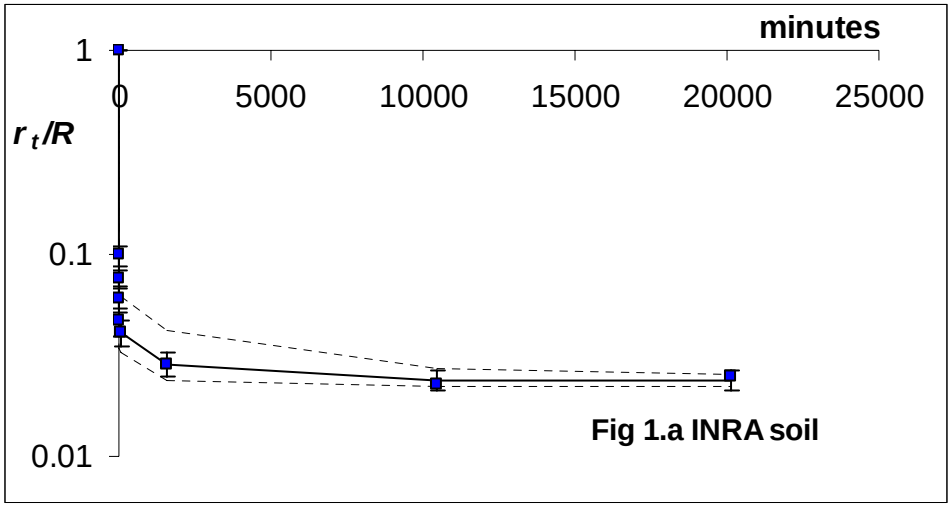
Figure 4 a) Zn K-edge EXAFS spectra (solid line) and linear combination fits (dashed line) for the Dornach untreated reference soil (DRef), for the residue left

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.

Figure 1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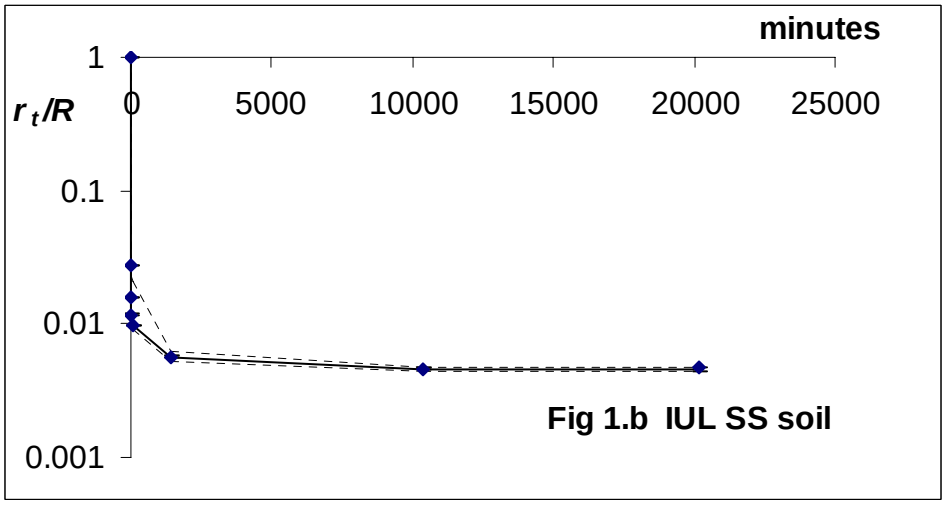
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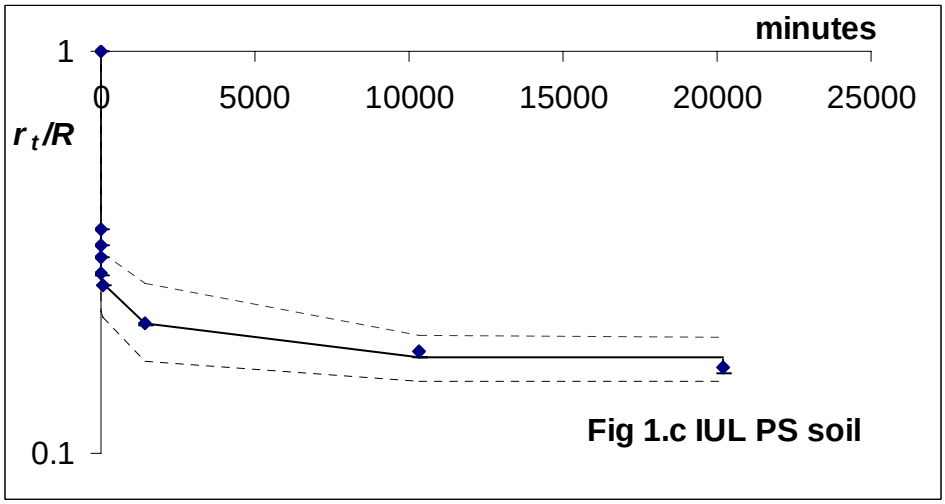
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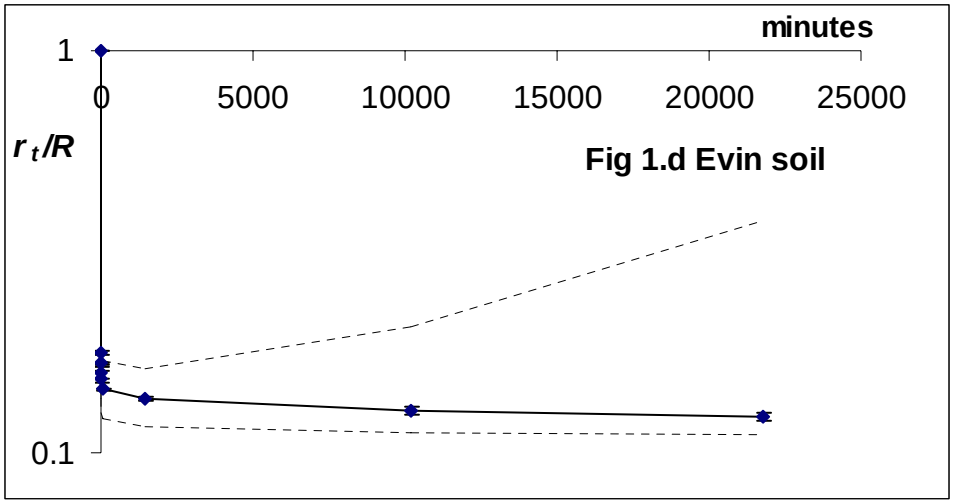


Fig 1.d Evin soil

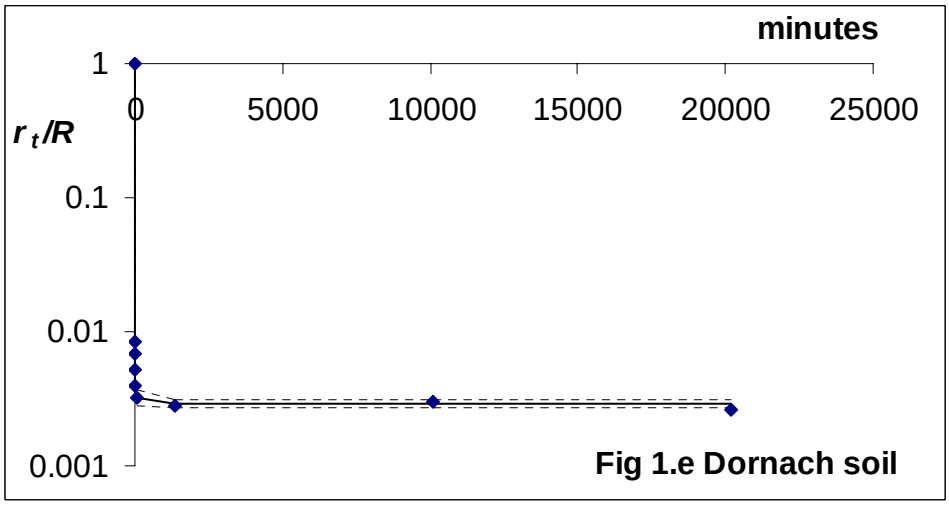


Fig 1.e Dornach soil

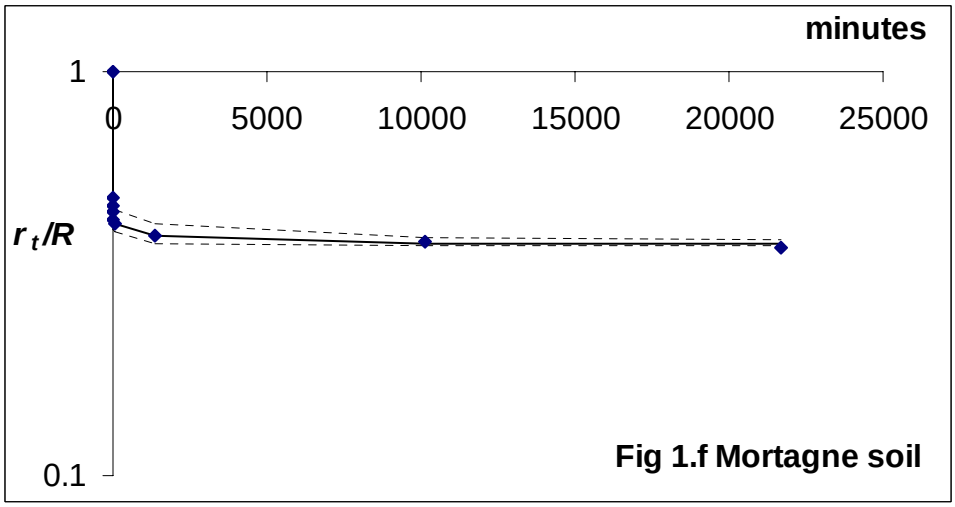
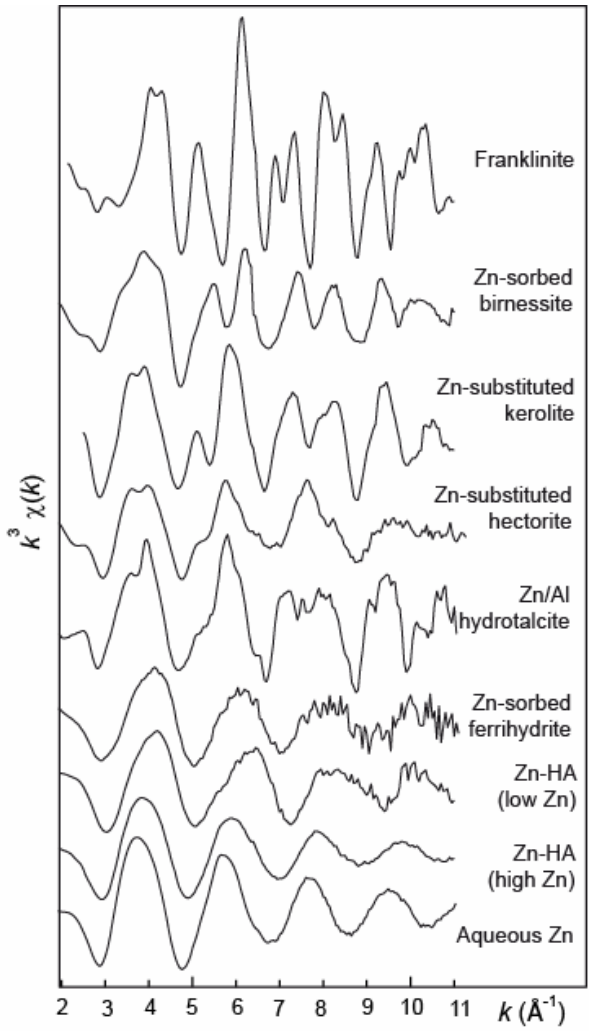


Fig 1.f Mortagne soil

749 Figure 2



750

751 Figure 3

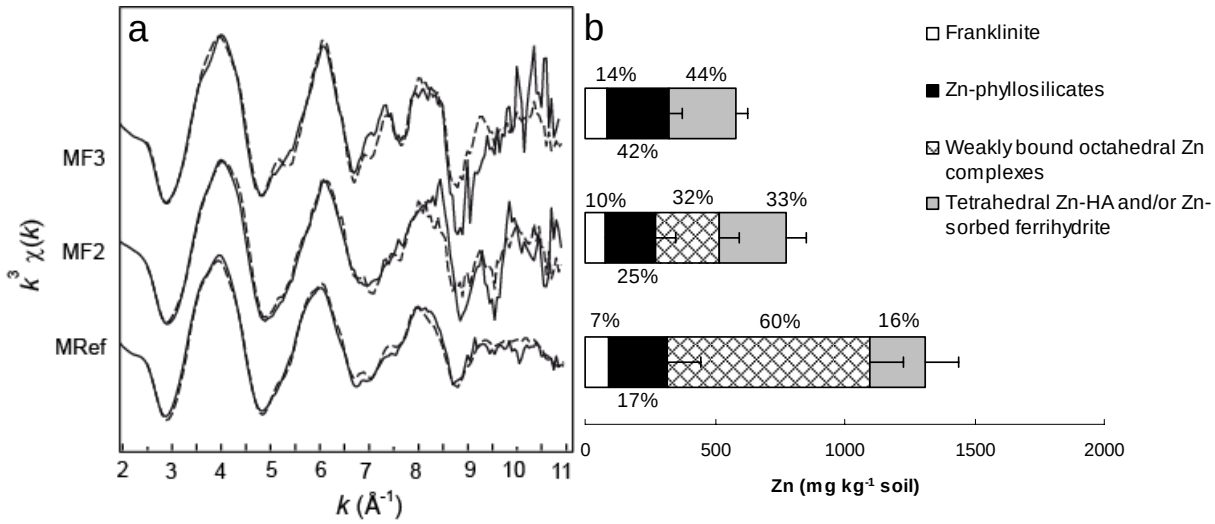

a
b
MF3
MF2
MRef
$k^3\chi(k)$
2 3 4 5 6 7 8 9 10 11
k (Å^{-1})
14%
44%
42%
10%
32%
33%
25%
7%
60%
16%
17%
0 500 1000 1500 2000
Zn (mg kg^{-1} soil)
Franklinite
Zn-phyllosilicates
Weakly bound octahedral Zn complexes
Tetrahedral Zn-HA and/or Zn-sorbed ferrihydrite

Figure 4

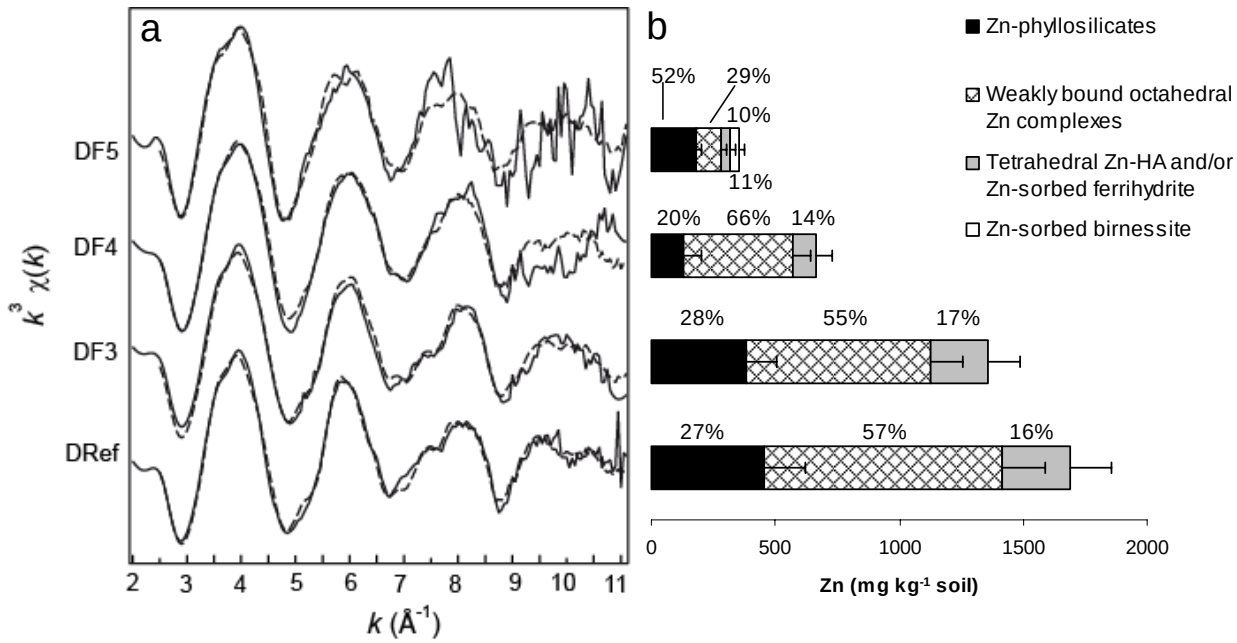

a
DF5
DF4
DF3
DRef
$k^3\chi(k)$
2 3 4 5 6 7 8 9 10 11
k (Å^{-1})
b
52% 29% 10% 11%
20% 66% 14%
28% 55% 17%
27% 57% 16%
0 500 1000 1500 2000
Zn (mg kg^{-1} soil)
Zn-phyllosilicates
Weakly bound octahedral Zn complexes
Tetrahedral Zn-HA and/or Zn-sorbed ferrihydrite
Zn-sorbed birnessite

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 / %[a]	80	57	58	28	15	66
Clay / %[a]	7	15	15	20	37	7
Organic matter / %[b]	2	3	2	4	11	1
$CaCO_3$ / %[c]	n.d.	n.d.	n.d.	n.d.	5	n.d.
Oxalate extractable Fe / g kg^{-1} soil[d]	2.14	11.11	5.11	3.17	2.70	1.88
EDTA extractable Zn / mg kg^{-1} soil[e]	166	15	9	435	349	406
pH[f]	5.7	6.0	4.6	5.0	6.7	5.1
CEC / $mmol_c$ kg^{-1}[g]	101	164	161	185	432	67
Base saturation / %[g]	47.8	17.5	45.2	85.6	42.2	18.8
Total Zn / mg kg^{-1} soil[i]	680 (5.4)	162 (0.5)	87.9 (1.7)	1647 (3.1)	1687 (3.1)	1307 (8.7)

n.d. non detectable

[a] Soil texture was measured via sedimentation with $(NaPO_4)_6$ as a dispersion agent (FAL, RAC & FAW, 1996).
[b] Organic matter was measured by titration (FAL, RAC & FAW, 1996).
[c] $CaCO_3$ was quantified using concentrated HCl (FAL, RAC & FAW, 1996).
[d] Oxalate extractable Fe was determined according to Loeppert and Inskeep (1996).
[e] EDTA-NH_4Ac extractions were performed using 50ml of extractant added to 10g dry soil (FAL, RAC & FAW, 1996).
[f] pH was measured using a 1:2.5 soil solution ratio of 0.01M $CaCl_2$ after 24 hours of gentle shaking.
[g] CEC and base saturation were determined using $BaCl_2$ method (FAL, RAC & FAW, 1996).
[i] Total Zn obtained after direct digestion (n=3) and standard error (in parentheses).

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

Extraction step	Reagents	Procedure	Proposed binding mechanism targeted [a]
F1	H_2O	1 hours at 20° C	Water soluble
F2	1 M NH_4OAc, pH 7	2 hours at 20° C	Reversible physisorption
F3	1 M NH_4OAc, pH 5 (HNO_3)	2 hours at 20° C	Reversible electrosorption
F4	0.04 M NH_2OH-HCl in 4.4 M CH_3COOH	6hours at 80° C	Irreversible chemisorption / reduction
	9.7 M H_2O_2, pH 2 (HNO_3)	5.5 hours at 80° C	
F5	followed by		Chemisorption / oxidation
	3.2 M NH_4OAc in 4.4 M HNO_3	30 minutes at 20° C	
F6	7 M HNO_3	6 hours at 20° C	Chemisorption / crystalline
Total Digestion			
F7	HNO_3 , H_2O_2 and HCl Open-Microwave Digestion	45 minutes at 70°, 90° C	Residue

[a] Proposed by Salbu *et al.* (1998)

Table 3 Average Zn concentration in dilute $CaCl_2$ extracts (C_{Zn}) measured during the isotopic exchange experiments and amounts of Zn exchangeable within 1 minute (E_{pool1}), moderately isotopically exchangeable Zn (E_{pool2}) and slowly or not exchangeable Zn (E_{pool3}) 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	Se	Average	se	Average	se	Average	se
	/mg Zn l^{-1}		/mg Zn kg^{-1}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)

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		*L* value	
	Average	se	Average	se	Average	se
	/ g DM kg^{-1} soil		/ g kg^{-1} DM		/ mg Zn kg^{-1} 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)

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

Fractions	INRA		IUL SS		IUL PS		Evin		Dornach		Mortagne	
	Average	se	Average	se	Average	se	Average	se	Average	se	Average	se
	/mg Zn kg^{-1}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)

Table 6 Mean specific activities calculated for each fraction of the selective sequential extraction over 120 days for 6 soils polluted with heavy metals. The values for each fraction are normalized by the ^{65}Zn introduced and the total soil Zn. Standard errors (se) are given between parentheses.

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)

Table 7 Comparison of the data obtained by the various techniques

Soil	INRA	IUL-SS	IUL-PS	Evin	Dornach	Mortagne
Total Zn / mg Zn kg soil^{-1}	680	162	87.9	1647	1687	1307
pH	5.7	6.0	4.6	5.0	6.7	5.1
Concentration of Zn in $CaCl_2$; C_{Zn} / mg Zn l^{-1}	0.50	0.01	0.38	12.3	0.15	29.9
E_{pool1}/ E_{pool2} /E_{pool3} / mg Zn kg soil^{-1}	50.7 / 167 / 463	5.7 / 28.6 / 128	10.5 / 12.6 / 64.8	697 / 305 / 645	181 / 368 / 1138	612 / 174 / 521
L values / mg Zn kg soil^{-1}	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 / % [a]					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^{-1}					F1+F2+F3+F4 (1023) = weakly bound octahedral Zn (962) > E_{pool1}+ E_{pool2} (549) > F1+F2+F3 (326)	F1+F2+F3+F4 (1014) > weakly bound octahedral Zn (784) = E_{pool1}+ E_{pool2} (786) = F1+F2+F3 (729)

[a] Determined only for Dornach and Mortagne soils.