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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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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 ^{65}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. caerulescens*.

36 The 3 first steps of the SSE solubilized the 1st pool and a fraction of the 2nd pool.

37 Most of the 2nd pool and a fraction of the 3rd pool were extracted in the 4th step of

38 the SSE, while the rest of the 3rd 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]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.*

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 [65]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

4

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　$\dfrac{r_t}{R} = A + \displaystyle\sum_{i=1}^{N-1} B_i \times e^{-C_i \times t}$ 　　　　　　　　　　　(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
$$L = \frac{(Zn_{plant} - Zn_{seed})}{\dfrac{^{65}Zn_{plant}}{^{65}Zn_{introduced}}} \qquad (3)$$

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, $^{65}Zn_{plant}$ (Bq plant^{-1}) is the

235   amount of ^{65}Zn in the aerial parts of the plant, and $^{65}Zn_{introduced}$ 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

269   soils to obtain total Zn and ^{65}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 ($^{65}Zn_{introduced}$ Bq

274   kg^{-1} soil) and the total soil Zn (Zn_{total} mg Zn kg^{-1} soil) as shown in equation 4.

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

276   In this equation the subscript F_X is the extraction number, $^{65}Zn_{Fx}$ represents the

277   radioisotope concentration (Bq kg^{-1} soil) in this extract and Zn_{Fx} the total Zn

278   concentration (mg Zn 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^{-1} 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$_2$ extracts (C$_{Zn}$)*

312   We present the average C_{Zn} 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   (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 CaCl₂ 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 ⁶⁵Zn 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

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. caerulescens* 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 (1st pool), the amount of Zn exchangeable between 1

558 minute and apparent isotopic equilibrium (2nd pool) and the amount of Zn that could

559 not be exchanged during the IE kinetic experiment (3rd pool). The experiment

560 conducted with *T. caerulescens* confirmed that the amount of IE Zn measured in pot

561 experiments was similar to the sum of Zn content present in the 1st and 2nd pools, i.e.

562 that this plant had only access the IE forms of Zn.

563   The use of the SSE on ^{65}Zn labeled soils showed that the 3 first extractions

564   (F1, F2 and F3) solubilized the amount of Zn present in the 1st pool and a slight

565   fraction of the Zn present in the 2nd pool. The three last fractions of the SSE (F5, F6

566   and F7) solubilized the Zn from the 3rd pool. We deduced from these observations

567   that the 4th extraction of the SSE solubilized the Zn from the 2nd pool and a fraction

568   of the Zn from the 3rd 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 1st

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 Zn^{2+} in the

579   solution decreases when soil pH and organic matter content increase.

580

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 $Zn_{2.1}Mg_{0.9}Si_4O_{10}(OH)_2$, nH_2O, Schlegel and Manceau, 2006 and Zn-substituted

727 hectorite, Schlegel *et al.*, 2001), Zn/Al hydrotalcite ($Zn_2Al(OH)_6(CO_3)_{0.5}$, n H_2O),

728 Zn-sorbed ferrihydrite containing 1500 mg kg^{-1} Zn (Manceau *et al.*, 2000), Zn-

729 humic acid complexes at low (600 mg kg^{-1} Zn) and high (3.2 % Zn) Zn loading

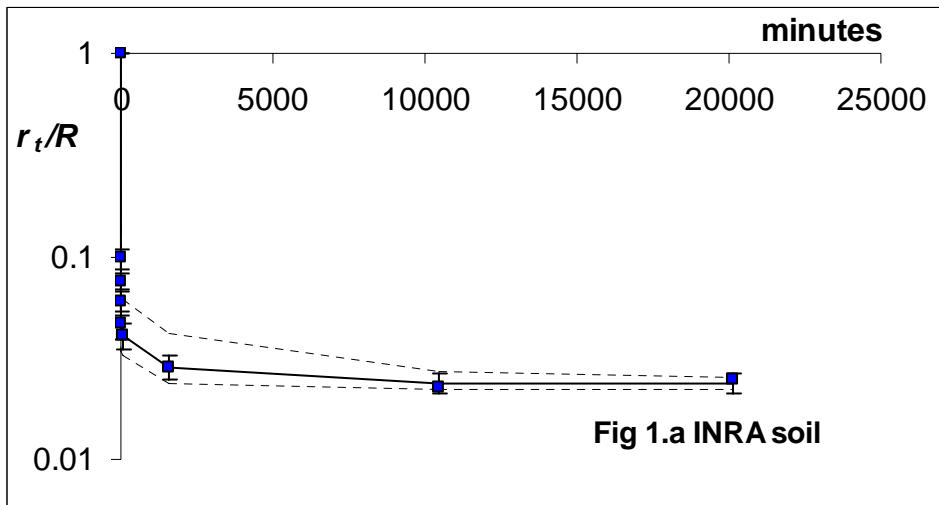730 (Sarret *et al.*, 1997), and aqueous Zn (pH 4).

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.
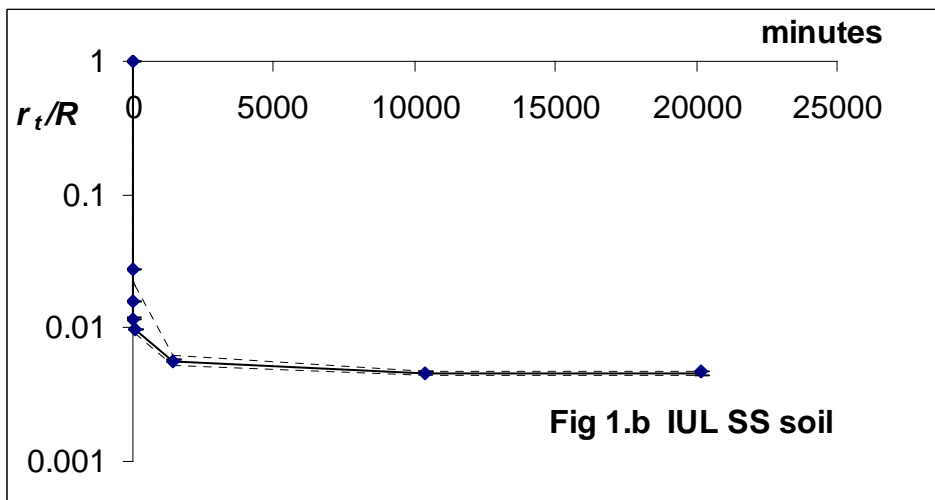
736

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.

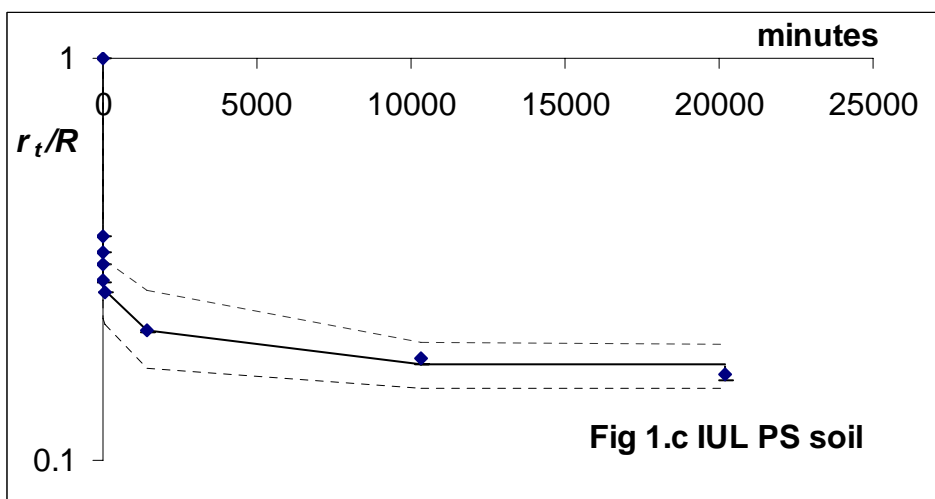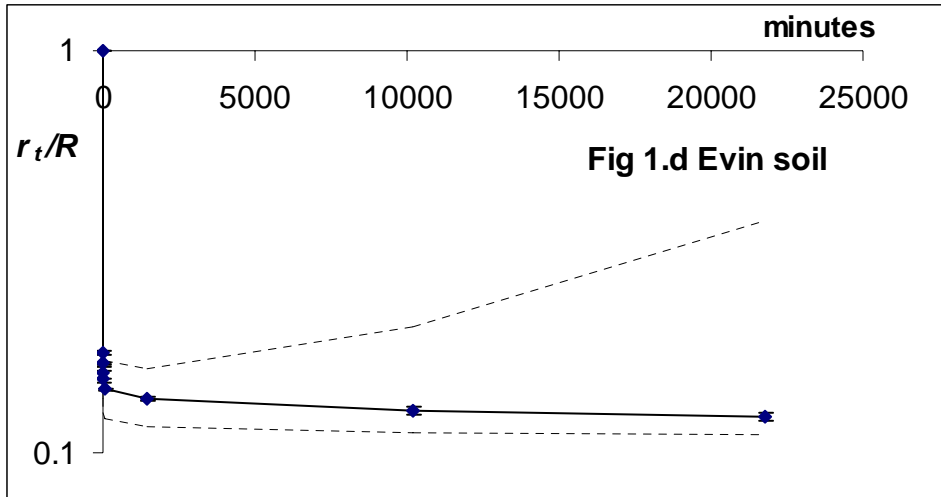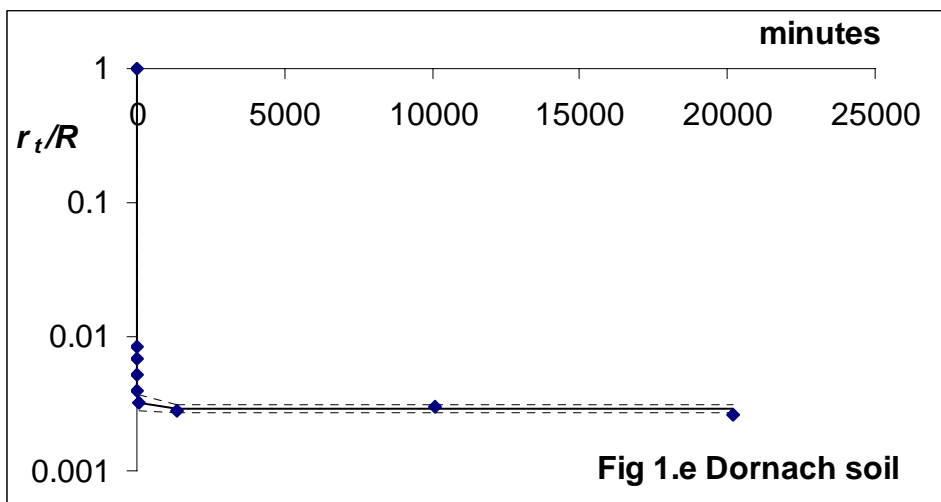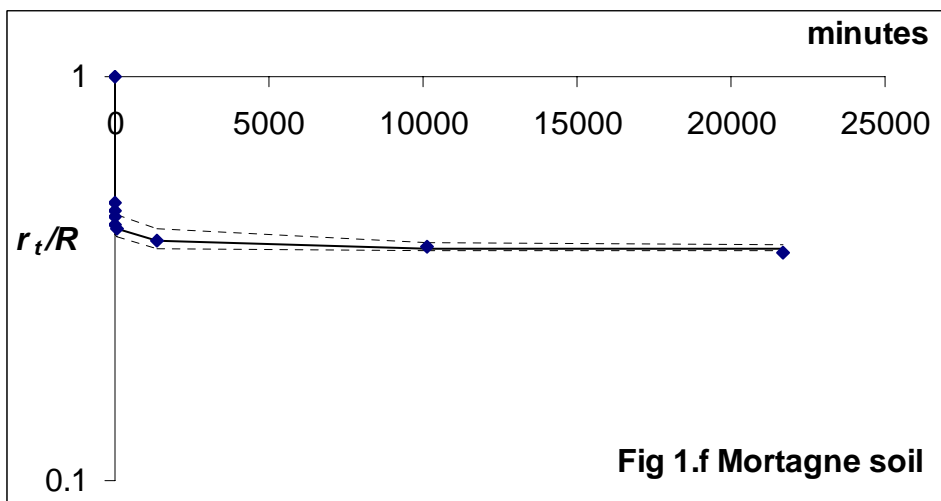742   Figure 1

Fig 1.a INRA soil

Fig 1.b IUL SS soil

Fig 1.c IUL PS soil

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Fig 1.d Evin soil

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Fig 1.e Dornach soil

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Fig 1.f Mortagne soil
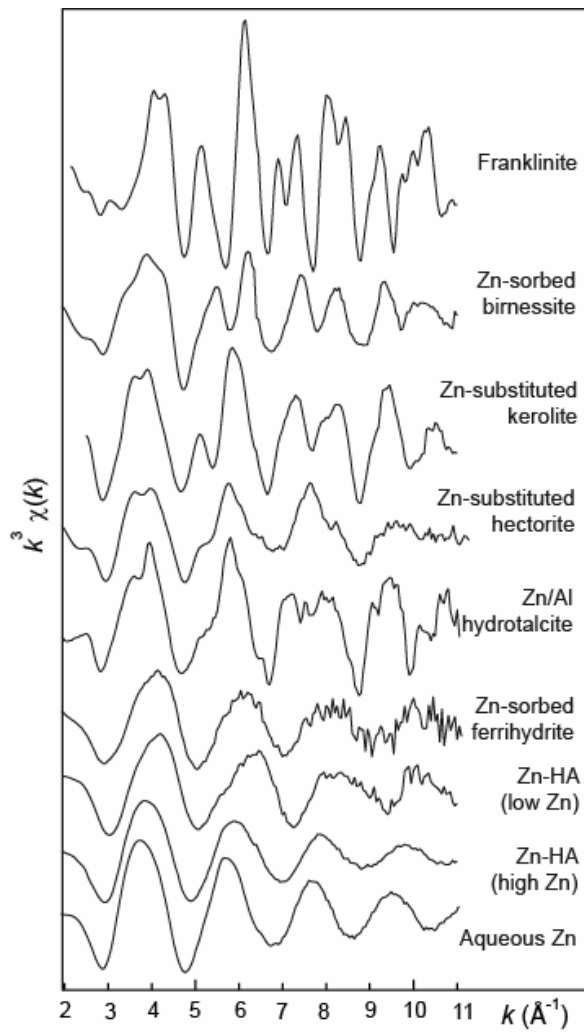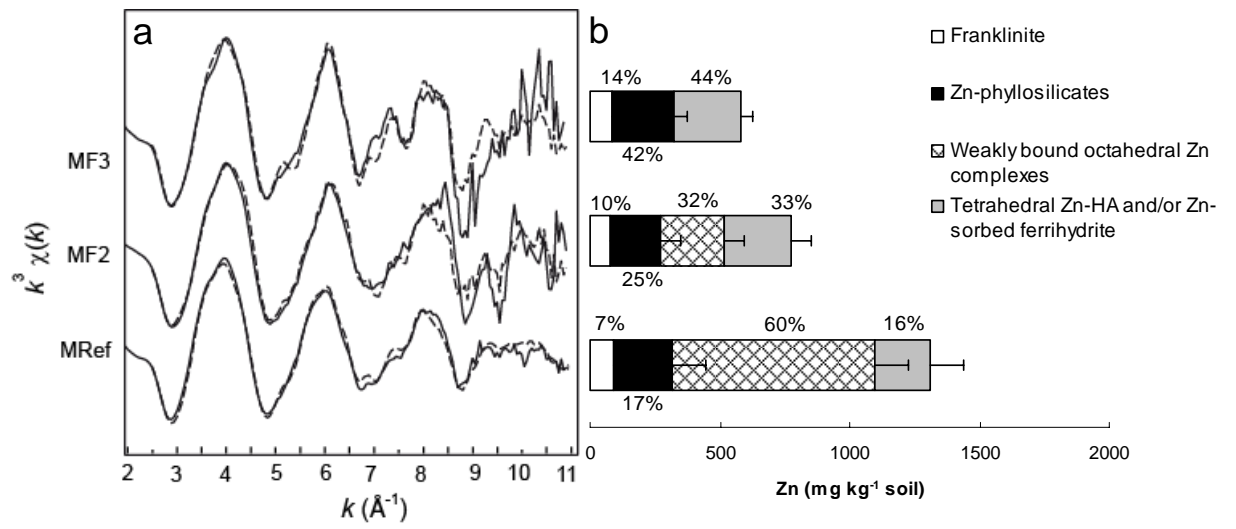
748
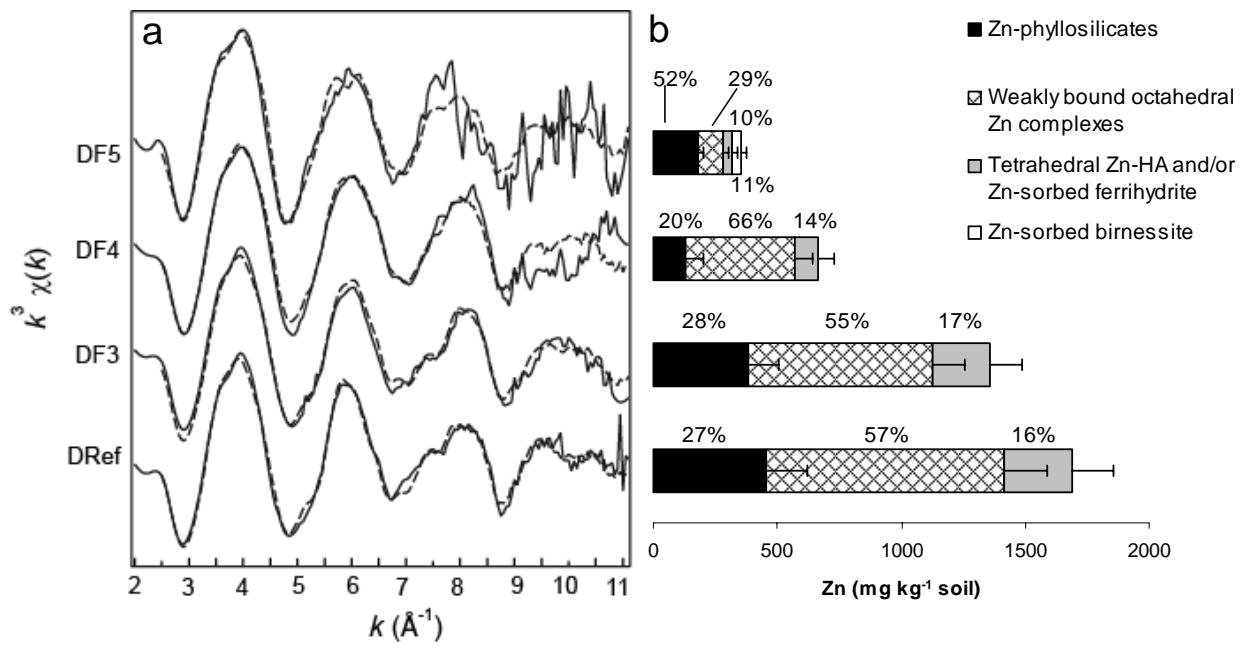
749   Figure 2

751   Figure 3

752   Figure 4

753

754

755

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

757

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).

758

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

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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
F5	9.7 M H_2O_2, pH 2 (HNO_3) followed by 3.2 M NH_4OAc in 4.4 M HNO_3	5.5 hours at 80° C 30 minutes at 20° C	Chemisorption / oxidation
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)

761 **Table 3** Average Zn concentration in dilute $CaCl_2$ 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	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)

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		*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)

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^{-1} 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^{-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)

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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 ^{65}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^{-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)

782 [a] Determined only for Dornach and Mortagne soils.

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