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

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

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

Approximately 100 subsamples of the INRA, IUL SS and IUL PS soils were collected at random intervals within the surface horizon (0-20 cm) to obtain a representative sample. For the Evin and Dornach soils, samples were randomly
taken after having removed the litter layer to obtain the maximum contaminant concentration in the soil. The sample site in Mortagne is the so-called metallicolous meadow. The A horizon was characterized by a distinct layer (5 to 8 cm thick) starting at a 15 to 25 cm depth in which smelter ash and tailings had been spread out and buried at the time the smelter was closed. A representative sampling was obtained by collecting approximately 100 random subsamples between 5 and 40 cm to include the heavily polluted layer. The soil samples were well mixed, air-dried for at least one week and passed through a 2-mm sieve. Remaining plant debris was removed by hand prior to analysis. Relevant soil characteristics are listed in Table 1.

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

IE kinetic experiments were carried out using a 1:10 soil solution ratio and 2 mM CaCl₂ as described by Sinaj et al. (1999). After shaking the soil solution suspension on an end-over-end shaker for three days, the samples were removed, placed on a magnetic stirring plate and stirred at 300 rpm. The soil suspension samples were spiked with 1.3 to 2.5 kBq of carrier-free ⁶⁵Zn added as ZnCl₂ (NEN Biosciences, Boston, USA; specific activity 2.0 GBq mg⁻¹ Zn). Aliquots of the soil suspension filtered through a 0.2 μm porosity cellulose acetate membrane (Minisart, Sartorius) were removed at 1, 3, 10, 30 and 60 minutes, and at 1, 7 and 14 days. After the first 60 minutes of IE the flasks were left on the bench and they were put back on the stirring plate one hour before sampling at 1, 7 and 14 days so as to minimize the dispersion of soil aggregates that would have been caused by a continuous stirring. The concentration of Zn in the solution (C_Zn) was measured after 60 minutes, 1, 7 and 14 days by ion chromatography as proposed by Sinaj et al. (1999). This method
measures the oxalate complexable Zn in the filtered soil suspension which approximates the concentration of the hydrated and weakly complexed Zn species (Cardellicchio et al., 1999) present in the solution. $^{65}$Zn activity in the solution was measured at all sampling times by $\beta$ liquid scintillation detection (Packard 2500) at an emission energy of 325 keV. We measured the $\beta$-counts using 1 ml of filtrate with 5 ml of scintillation liquid (Packard Ultima Gold) and corrected them for quenching effects.

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

$$\frac{r_t}{R} = A + \sum_{i=1}^{N-1} B_i e^{-c_i t}$$

(1)
Where $r_t/R$ is the fraction of $^{65}$Zn remaining in solution at the time of sampling, $A$, $B_i$ and $C_i$ are constants, $N$ is the total number of compartments.

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

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

$$E_t = \left( \frac{v}{m} \right) \times C_{Zn} \times \frac{R}{r_t}$$

(2)

Where $v/m$ is the solution to soil ratio (1 kg$^{-1}$), $C_{Zn}$ is the Zn concentration (mg l$^{-1}$) in solution and $r_t/R$ is the fraction of $^{65}$Zn remaining in solution at the time $t$ of sampling. The amount of Zn present in the pool 1 ($E_{pool1}$) is calculated considering an exchange time of 1 minute, the amount of Zn present in the pool 2 ($E_{pool2}$) is the difference between the amount of Zn that has been exchanged at apparent isotopic equilibrium and the amount of Zn exchangeable within one minute. The amount of
Zn present in the pool 3 (\(E_{\text{pool3}}\)) is calculated as the difference between soil total Zn and the amount of Zn that has been exchanged at apparent isotopic equilibrium.

Pot experiment with Thlaspi caerulescens, calculation of the L value

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

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

After 80 days growth, the plants were harvested by cutting shoots at the soil surface. The plant aerial biomass was washed with de-ionized water, dried at 85°C for 24 hours and the dry mass was measured. Plant Zn concentrations were obtained.
by grinding the plant material with an agate ball mill and using a dry-ash digestion method adapted from Chapman and Pratt (1961). The method was performed by incinerating a 1 g sample at 500° C for 8 hours, dissolving the residual ashes with 2 ml of 5.8 M analytical grade HCl that was further diluted to 50 ml with de-ionized water before element and isotope measurements. Zn concentrations were determined by ICP-OES (Varian Liberty 220) and $^{65}$Zn was measured using high purity Ge bore-hole gamma detector (EAWAG, Dübendorf, Switzerland). All $^{65}$Zn measurements were corrected back to the date of soil labeling.

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

$$L = \frac{(Zn_{plant} - Zn_{seed})}{\frac{65Zn_{plant}}{65Zn_{introduced}}}$$

(3)

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

Selective Sequential Extraction and Total Digestion

Before conducting the selective sequential extraction (SSE), soils were labeled with $^{65}$Zn and incubated for 20, 85 and 120 days at 21° C. At the time of labeling, a carrier-free $^{65}$Zn solution (NEN Biosciences, Boston, USA; specific activity: 2.0...
GBq mg⁻¹ Zn) was added as ZnCl₂ to de-ionized water and mixed well with the soil samples at the rate of 22.5 to 86.0 MBq kg⁻¹ soil. Soils were maintained at 50% water holding capacity and well aerated during the incubation period.

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

As a final step (F7), the F6 residue was placed in an open-vessel microwave digester (MX 350 Prolabo, France) and the procedure of Lorentzen and Kingston (1996) was used to extract the residual Zn. After digestion, the sample was cooled to room temperature filtered with a 0.45 µm porosity filter prior to ICP-OES (Varian 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 ($Zn_{\text{total}}$ mg Zn kg$^{-1}$ soil) as shown in equation 4.

$$SA = \frac{65Zn_{Fx}^{\text{introduced}}}{Zn_{Fx}^{\text{total}}}$$  (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 CaCl₂ extracts (CZn)

We present the average CZn values measured during the IE kinetic experiments for each soil (Table 3). The highest values were observed in the Evin and Mortagne
soils and the lowest values were observed in the IUL SS soil (Table 3). The logarithm of $C_{Zn}$ was highly significantly related to soil pH and to the logarithm of the total soil Zn content of the 6 soils.

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

This result confirms that $C_{Zn}$ increases with Zn inputs and decreases when soil pH increases as noted by Arias et al. (2005). The very low $C_{Zn}$ values observed in the IUL SS soil can be explained by its high amorphous iron oxide content (Table 1) acting as a strong sorbent for Zn. The elevated amorphous iron oxide content of this soil is related to the repeated additions of FeCl$_3$ treated sewage sludge in this field experiment.

Decrease of radioactivity in dilute CaCl$_2$ extracts with time during the isotopic exchange kinetic experiments

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

It was possible to fit the curves describing the decrease of $r_t/R$ with exchange time by the equation 1 by a sum of two exponential terms and a constant for the Dornach soil and by a sum of three exponential terms and a constant for the other soils (data not shown). These results suggest that the $^{65}\text{Zn}$ added in the solution exchanged with Zn located in 3 compartments in the Dornach soil and in 4
compartments in the other soils. These compartments corresponded to the following exchange times: 0-1 min, 1-30 min, 30 min-7 days and > 7 days in INRA and IUL SS; 0-1 min, 1-10 min, 10 min to 14 days and >14 days in IUL PS; 0-1 min, 1-30 min, 30 min to 14 days and > 14 days in Evin; 0-1 min, 1 min to 1 day and > 1 day in Dornach; and 0-1 min, 1-30 min, 30 min to 7 days and > 7 days in Mortagne. Models with two exponential terms and a constant were tried for all soils but gave a proper fit only for Dornach (results not shown). This analysis suggests the presence in all soils of a compartment of Zn exchangeable during the first minute, while other compartments differed from soil to soil.

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

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

Because of the different number of compartments observed between soils we prefer to summarize the information given by the compartmental analysis by considering only three pools of exchangeable Zn for each soil: the amount of Zn exchangeable within 1 minute which is observed in all soils (pool 1), the amount of Zn
exchangeable that is exchangeable on the medium term (pool 2), and the amount of Zn that is very slowly or not exchangeable (pool 3). The pool 2 corresponds to the fraction of soil Zn that is exchangeable between 1 minute and apparent isotopic equilibrium, i.e. between 1 minute and 1 day in Dornach, between 1 minute and 7 days in INRA, IUL SS and Mortagne, and between 1 minute and 14 days in IUL PS and Evin. The pool 3 corresponds to the amount of Zn that could not be isotopically exchanged within 1 day in Dornach, within 7 days in INRA, IUL SS and Mortagne and within 14 days in Evin and IUL PS.

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

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

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

These equations show that the amount of Zn present in the 1st pool increases with Zn inputs and decreases with pH, while the amount of Zn present in the 2nd pool increases with Zn inputs and pH.

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

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

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

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

Selective sequential extraction of $^{65}$Zn and Zn from incubated soil

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

The normalized specific activities observed for each fraction are presented in Table 6. A monotonous decrease down to null activities was expected from the most exchangeable (F1) to the residual (F7) fraction. However, the specific activities values obtained in F1 were often lower than in F2. We suggest that the relatively low Zn and $^{65}$Zn concentrations extracted by the water (F1) and their high variability
might explain the difficulties in calculating correct specific activities values. If we do not consider F1, a monotonous decrease is observed from F2 to F7, except for the F3 extraction for the Evin and Mortagne soils. The activity is very low in the F6 extract and close to 0 in the F7 residual, as expected. This $^{65}\text{Zn}$ tracing of the SSE confirms that the chosen extractants induced a progressive removal of Zn from highly exchangeable to recalcitrant species.

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

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

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

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

This suggests that these 3 first steps have extracted the entire quantity of Zn isotopically exchangeable within 1 minute, and that F3 extracted a fraction of the Zn...
exchangeable on the medium term. Highly significant correlations were also found between ln($Zn_{F5+F6+F7}$) and ln($E_{pool3}$) (equations 12 and 13).

\[
\begin{align*}
\ln(Zn_{F5+F6+F7}) &= 1.20 + 0.73\ln(E_{pool3}), \quad n=6, \quad R^2=0.87, \quad SEE=0.35 \quad (12) \\
\ln(E_{pool3}) &= -0.65 + 1.19\ln(Zn_{F5+F6+F7}), \quad n=6, \quad R^2=0.87, \quad SEE=0.44 \quad (13)
\end{align*}
\]

The lower amount of Zn recovered in F5+F6+F7 compared to $E_{pool3}$ suggests that a fraction of very slowly or not exchangeable Zn had already been extracted in F4.

Since F1+F2+F3 extracted the Zn present in the pool 1 and some of the Zn present in pool 2 and F5+F6+F7 extracted a fraction of the pool 3, we conclude that the 4th step of the SSE solubilized both moderately and slowly exchangeable forms of Zn, i.e. Zn from pools 2 and 3.

**Zn K-edge EXAFS spectroscopy**

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

Figure 2 shows the Zn K-edge EXAFS spectra for some reference compounds used in the linear combination fits, including franklinite, Zn-sorbed birnessite (Mn oxide), Zn-substituted kerolite as a proxy for Zn-substituted phyllosilicate, Zn/Al hydrotalcite, a zinc-aluminum hydroxycarbonate, Zn-sorbed ferrihydrite, Zn-humic acid complexes (Zn-HA) at high and low Zn loading, and aqueous Zn$^{2+}$ as a proxy for outer sphere complexes. Franklinite is easily identified by the high amplitude and multiple frequencies of its spectrum. The spectra for Zn-kerolite and Zn/Al hydrotalcite present some similarities, which makes their distinction difficult in a mixture (Panfili et al., 2005). In the LCFs, these two compounds and Zn-sorbed hectorite were grouped as "Zn-phyllosilicate". Similarly,
the spectra for Zn-HA at low Zn loading and Zn-sorbed ferrihydrite look similar because Zn is 4-fold coordinated to oxygen atoms and the second shell contribution weak in the two references. Zn-HA at low Zn loading is a proxy for strongly bound inner sphere Zn-organic complexes in tetrahedral configuration (Sarret et al., 1997).

In the LCFs, the two tetrahedral species were grouped as "tetrahedral Zn-HA and/or Zn-sorbed ferrihydrite". Another pair of similar spectra is Zn-HA at high Zn loading and aqueous Zn\(^{2+}\). Because Zn is octahedrally coordinated, and the second shell contribution either weak (Zn-HA) or absent (aqueous Zn). These species are considered as representatives for less-strongly to weakly bound inner sphere Zn-organic complexes and outer sphere organic and inorganic complexes (Sarret et al., 1997). In the LCFs, these species were grouped as "weakly bound octahedral Zn".

Figure 3a shows the EXAFS spectra for the untreated soil from Mortagne and the two residues, and their reconstructions with four component spectra. In the untreated soil (MRef), Zn is distributed as 60 ± 10 % weakly bound octahedral Zn complexes, 17 ± 10 % Zn-phyllosilicate, 16 ± 10 % tetrahedral Zn-HA and/or Zn-sorbed ferrihydrite and 7% franklinite (Figure 3b). The detection limit for this last species is less than 10% because its spectrum has a high amplitude. Zn-sorbed goethite and Zn-sorbed hematite spectra were tested, but neither of them are component species to the data. In a previous study on the same soil (Manceau et al., 2000), Zn-phyllosilicate, Zn-sorbed birnessite and Zn sorbed on iron oxyhydroxides were identified as the main Zn species. Another study on a tilled soil near the Mortagne area concluded to the presence of Zn outer-sphere complexes, Zn-organic matter inner-sphere complexes, Zn/Al-hydrotalcite, Zn-phyllosilicate, and magnetite-franklinite solid solutions (Juillot et al., 2003). The F2 extraction removed 41 % of the soil Zn, and most of the weakly bound octahedral Zn pool. The
F3 extraction removed 15% of the initial soil Zn, the rest of the weakly bound pool, and some of the tetrahedral pool. The Zn-phyllosilicate and franklinite pools were marginally affected. The occurrence of 10-14% Zn as franklinite in the MF2 and MF3 samples is attested by the sharpening of the second oscillation centered at 6 Å. In this soil the F2 and F3 extractions are relatively specific, affecting mostly the weakly bound octahedral Zn pool.

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

A different behavior is observed for the Dornach soil (Figure 4). Satisfactory fits were obtained with three components for the untreated soil (DRef) and F3 and F4 residues (DF3 and DF4), and with four components for the F5 residue (DF5). The weakly bound octahedral Zn are predominant in the Dornach soil (57 ± 10 %) followed by Zn-phyllosilicate (27 ± 10 %), and tetrahedral Zn-HA and/or Zn-sorbed ferrihydrite (16 ± 10 %). The proportions of Zn species did not change statistically in DF3 and DF4, which suggests that all species were affected to a similar extent by the F3 and F4 treatments (removal of 19 and 41 % of total soil Zn, respectively).

The F5 extraction (removal of 19% total soil Zn) targeted preferentially the "weakly bound octahedral Zn" pool. As a consequence, Zn-phyllosilicate was the major species in the DF5 residue. The DF5 spectrum was simulated with Zn- Hectorite, whereas DRef, DF3 and DF4 were simulated with Zn-kerolite and/or Zn/Al...
hydrotalcite. Indeed, the third oscillation of the DF5 and Zn-hectorite spectra have the same shape, whereas the shoulder between 7.0 and 7.5 Å⁻¹ in DRef corresponds to a high amplitude feature in Zn-kerolite and/or Zn/Al hydrotalcite spectra (Figures 2 and 4a). Therefore, the local environment of Zn seems to have evolved during the selective sequential extraction. A finer description of the nature and structure of these species would require studying the < 2 µm or < 0.2 µm soil fractions by polarized EXAFS (Manceau et al., 2000). A new species is detected in DF5, Zn-sorbed birnessite. This species was probably present in the previous samples including those from Mortagne as shown by micro-EXAFS (Manceau et al., 2000), but as a minor component masked by the predominant species. Chemical extractions lacked selectivity in the Dornach soil because the fractional amount of the major species remained unchanged in DF3 and DF4 despite a removal of 19 and 41% of the soil Zn. The weakly bound octahedral Zn complexes which were extracted completely after the third extraction step in Mortagne soil remained predominant in DF3 and DF4.

In Dornach the weakly bound octahedral Zn measured in the untreated sample (962 mg Zn kg⁻¹ soil) was similar to the Zn extracted by the four first steps of the SSE (F1+F2+F3+F4, 1023 mg Zn kg⁻¹ soil), but was much higher than the IE exchangeable Zn ($E_{pool1}+E_{pool2}$, 549 mg Zn kg⁻¹ soil), which itself was also higher than the amount of Zn extracted by the three first steps of the SSE (F1+F2+F3, 326 mg Zn kg⁻¹ soil) (Table 7). Although Dornach exhibited a high proportion of weakly bound octahedral Zn, only a small proportion of it was extracted by the 3 first extractions of the SSE (22%). We suggest that the IE Zn and the Zn extracted by the 3 first steps of the SSE was indeed present as weakly bound octahedral Zn, 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 (1\(^{st}\) pool), the amount of Zn exchangeable between 1 minute and apparent isotopic equilibrium (2\(^{nd}\) pool) and the amount of Zn that could not be exchanged during the IE kinetic experiment (3\(^{rd}\) 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 1\(^{st}\) and 2\(^{nd}\) pools, i.e. that this plant had only access the IE forms of Zn.
The use of the SSE on $^{65}\text{Zn}$ 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 $\text{Zn}^{2+}$ 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$_2$O$_4$), 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$_4$O$_{10}$(OH)$_2$, nH$_2$O, Schlegel and Manceau, 2006 and Zn-substituted hectorite, Schlegel et al., 2001), Zn/Al hydrotalcite (Zn$_2$Al(OH)$_6$(CO$_3$)$_{0.5}$, n H$_2$O), 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
after the third (DF3), fourth (DF4) and fifth (DF5) extraction of the SSE. b) Distribution of Zn species derived from the fits. The error bars correspond to 10% of total Zn content.
Figure 1

**Fig 1.a INRA soil**

**Fig 1.b IUL SS soil**

**Fig 1.c IUL PS soil**
Figure 2
Figure 3

(a) Graph showing the variation of $k^2 \chi(k)$ with $k (\text{Å}^{-1})$ for different samples: MF3, MF2, and MRef.

(b) Bar graph showing the percentage of Zn adsorption forms at different Zn concentrations (mg kg$^{-1}$ soil):
- 14% Franklinite
- 44% Zn-phylosilicates
- 7% Weakly bound octahedral Zn complexes
- 17% Tetrahedral Zn-HA and/or Zn-sorbed ferrihydrite

Legend:
- □ Franklinite
- ■ Zn-phylosilicates
- ● Weakly bound octahedral Zn complexes
- ✡ Tetrahedral Zn-HA and/or Zn-sorbed ferrihydrite
Figure 4

![Graph showing the distribution of Zn forms in soil samples.](image)

- **Zn-phyllosilicates**
- **Weakly bound octahedral Zn complexes**
- **Tetrahedral Zn-HA and/or Zn-sorbed ferrihydrite**
- **Zn-sorbed birnessite**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Zn-phyllosilicates</th>
<th>Weakly bound octahedral Zn complexes</th>
<th>Tetrahedral Zn-HA and/or Zn-sorbed ferrihydrite</th>
<th>Zn-sorbed birnessite</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF5</td>
<td>27% 57% 16%</td>
<td>10%</td>
<td>11%</td>
<td></td>
</tr>
<tr>
<td>DF4</td>
<td>20% 66% 14%</td>
<td>20%</td>
<td>14%</td>
<td></td>
</tr>
<tr>
<td>DF3</td>
<td></td>
<td>28%</td>
<td>55%</td>
<td>17%</td>
</tr>
<tr>
<td>DRtef</td>
<td>27% 57% 16%</td>
<td>28%</td>
<td>57%</td>
<td>16%</td>
</tr>
</tbody>
</table>
Table 1 Selected characteristics of the six soils polluted with heavy metals

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>INRA</th>
<th>IUL SS</th>
<th>IUL PS</th>
<th>Evin</th>
<th>Dornach</th>
<th>Mortagne</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil type</td>
<td>Eutric Fluvisol</td>
<td>Orthic Luvisol</td>
<td>Orthic Luvisol</td>
<td>Gleyic Luvisol</td>
<td>Calcaric Regosol</td>
<td>Dystric Cambisol</td>
</tr>
<tr>
<td>Pollutant source</td>
<td>Sewage Sludge</td>
<td>Sewage Sludge</td>
<td>Pig Slurry</td>
<td>Pb/Zn Smelter</td>
<td>Cu/Ni/Zn Smelter</td>
<td>Zn Smelter</td>
</tr>
<tr>
<td>Land use</td>
<td>arable</td>
<td>Arable</td>
<td>Arable</td>
<td>forest</td>
<td>grassland</td>
<td>grassland</td>
</tr>
<tr>
<td>Sampling depth / cm</td>
<td>0-20</td>
<td>0-20</td>
<td>2-5</td>
<td>2-5</td>
<td>5-40</td>
<td>5-40</td>
</tr>
<tr>
<td>Sand / %&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80</td>
<td>57</td>
<td>58</td>
<td>28</td>
<td>15</td>
<td>66</td>
</tr>
<tr>
<td>Clay / %&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7</td>
<td>15</td>
<td>15</td>
<td>20</td>
<td>37</td>
<td>7</td>
</tr>
<tr>
<td>Organic matter / %&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>CaCO₃ / %&lt;sup&gt;c&lt;/sup&gt;</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>5</td>
<td>n.d.</td>
</tr>
<tr>
<td>Oxalate extractable Fe / g kg⁻¹ soil&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.14</td>
<td>11.11</td>
<td>5.11</td>
<td>3.17</td>
<td>2.70</td>
<td>1.88</td>
</tr>
<tr>
<td>EDTA extractable Zn / mg kg⁻¹ soil&lt;sup&gt;e&lt;/sup&gt;</td>
<td>166</td>
<td>15</td>
<td>9</td>
<td>435</td>
<td>349</td>
<td>406</td>
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<tr>
<td>pH&lt;sup&gt;f&lt;/sup&gt;</td>
<td>5.7</td>
<td>6.0</td>
<td>4.6</td>
<td>5.0</td>
<td>6.7</td>
<td>5.1</td>
</tr>
<tr>
<td>CEC / mmol kg⁻¹&lt;sup&gt;g&lt;/sup&gt;</td>
<td>101</td>
<td>164</td>
<td>161</td>
<td>185</td>
<td>432</td>
<td>67</td>
</tr>
<tr>
<td>Base saturation / %&lt;sup&gt;h&lt;/sup&gt;</td>
<td>47.8</td>
<td>17.5</td>
<td>45.2</td>
<td>85.6</td>
<td>42.2</td>
<td>18.8</td>
</tr>
<tr>
<td>Total Zn / mg kg⁻¹ soil&lt;sup&gt;i&lt;/sup&gt;</td>
<td>680 (5.4)</td>
<td>162 (0.5)</td>
<td>87.9 (1.7)</td>
<td>1647 (3.1)</td>
<td>1687 (3.1)</td>
<td>1307 (8.7)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Soil texture was measured via sedimentation with (NaPO₄)₆ as a dispersion agent (FAL, RAC & FAW, 1996).
<sup>b</sup> Organic matter was measured by titration (FAL, RAC & FAW, 1996).
<sup>c</sup> CaCO₃ was quantified using concentrated HCl (FAL, RAC & FAW, 1996).
<sup>d</sup> Oxalate extractable Fe was determined according to Loeppert and Inskeep (1996).
<sup>e</sup> EDTA-NH₄Ac extractions were performed using 50ml of extractant added to 10g dry soil (FAL, RAC & FAW, 1996).
<sup>f</sup> pH was measured using a 1:2.5 soil solution ratio of 0.01M CaCl₂ after 24 hours of gentle shaking.
<sup>g</sup> CEC and base saturation were determined using BaCl₂ method (FAL, RAC & FAW, 1996).
<sup>h</sup> 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)

<table>
<thead>
<tr>
<th>Extraction step</th>
<th>Reagents</th>
<th>Procedure</th>
<th>Proposed binding mechanism targeted&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>H₂O</td>
<td>1 hours at 20° C</td>
<td>Water soluble</td>
</tr>
<tr>
<td>F2</td>
<td>1 M NH₄OAc, pH 7</td>
<td>2 hours at 20° C</td>
<td>Reversible physisorption</td>
</tr>
<tr>
<td>F3</td>
<td>1 M NH₄OAc, pH 5 (HNO₃)</td>
<td>2 hours at 20° C</td>
<td>Reversible electrosorption</td>
</tr>
<tr>
<td>F4</td>
<td>0.04 M NH₂OH-HCl in 4.4 M CH₃COOH</td>
<td>6 hours at 80° C</td>
<td>Irreversible chemisorption / reduction</td>
</tr>
<tr>
<td></td>
<td>9.7 M H₂O₂, pH 2 (HNO₃)</td>
<td>5.5 hours at 80° C</td>
<td></td>
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<tr>
<td>F5</td>
<td>followed by</td>
<td>3.2 M NH₄OAc in 4.4 M HNO₃</td>
<td>30 minutes at 20° C</td>
</tr>
<tr>
<td></td>
<td>7 M HNO₃</td>
<td>6 hours at 20° C</td>
<td>Chemisorption / crystalline</td>
</tr>
<tr>
<td><strong>Total Digestion</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>F7</td>
<td>HNO₃, H₂O₂ and HCl Open-Microwave Digestion</td>
<td>45 minutes at 70°, 90° C</td>
<td>Residue</td>
</tr>
</tbody>
</table>

<sup>a</sup> Proposed by Salbu et al. (1998)
Table 3 Average Zn concentration in dilute CaCl₂ 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.

<table>
<thead>
<tr>
<th>Soil</th>
<th>( C_{Zn} ) Average /mg Zn l⁻¹</th>
<th>( E_{pool1} ) Average se/mg Zn kg⁻¹soil</th>
<th>( E_{pool2} ) Average se/mg Zn kg⁻¹soil</th>
<th>( E_{pool3} ) Average se/mg Zn kg⁻¹soil</th>
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</thead>
<tbody>
<tr>
<td>INRA</td>
<td>0.50 0.030</td>
<td>50.7 (3.0)</td>
<td>167 (5.9)</td>
<td>463 (8.8)</td>
</tr>
<tr>
<td>IUL SS</td>
<td>0.02 0.003</td>
<td>5.7 (0.6)</td>
<td>28.6 (2.5)</td>
<td>128 (3.1)</td>
</tr>
<tr>
<td>IUL PS</td>
<td>0.38 0.006</td>
<td>10.5 (0.3)</td>
<td>12.6 (0.9)</td>
<td>64.8 (0.7)</td>
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<tr>
<td>Evin</td>
<td>12.4 0.149</td>
<td>697 (11.6)</td>
<td>305 (14.4)</td>
<td>645 (10.6)</td>
</tr>
<tr>
<td>Dornach</td>
<td>0.15 0.007</td>
<td>181 (1.9)</td>
<td>368 (18.5)</td>
<td>1138 (18.6)</td>
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<tr>
<td>Mortagne</td>
<td>29.9 0.429</td>
<td>612 (10.2)</td>
<td>174 (4.1)</td>
<td>521 (7.0)</td>
</tr>
</tbody>
</table>
Table 4 Average values for plant aerial dry matter, Zn content of aerial parts and $L$ value measured with *Thlaspi caerulescens* in six soils polluted with heavy metals. The standard errors (se) are given between parentheses.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Yield Average / g DM kg$^{-1}$ soil</th>
<th>Plant Zn content Average / g kg$^{-1}$ DM</th>
<th>$L$ value Average / mg Zn kg$^{-1}$ soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>se (0.5)</td>
<td>se (0.1)</td>
<td>se (14.0)</td>
</tr>
<tr>
<td>INRA</td>
<td>16.5</td>
<td>0.4</td>
<td>224</td>
</tr>
<tr>
<td>IUL SS</td>
<td>23.4 (0.3)</td>
<td>1.2 (0.1)</td>
<td>36</td>
</tr>
<tr>
<td>IUL PS</td>
<td>20.6 (1.1)</td>
<td>1 (0.1)</td>
<td>22.6 (0.7)</td>
</tr>
<tr>
<td>Evin</td>
<td>21.7 (0.4)</td>
<td>4 (0.5)</td>
<td>828 (14.1)</td>
</tr>
<tr>
<td>Dornach</td>
<td>20.4 (0.8)</td>
<td>2.2 (0.5)</td>
<td>564 (0.8)</td>
</tr>
<tr>
<td>Mortagne</td>
<td>21.7 (0.7)</td>
<td>5.9 (0.3)</td>
<td>737 (4.7)</td>
</tr>
</tbody>
</table>
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.

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

<table>
<thead>
<tr>
<th>Fraction</th>
<th>INRA Average</th>
<th>IUL SS Average</th>
<th>IUL PS Average</th>
<th>Evin Average</th>
<th>Dornach Average</th>
<th>Mortagne Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>se</td>
<td>se</td>
<td>se</td>
<td>se</td>
<td>se</td>
<td>se</td>
</tr>
<tr>
<td>F1</td>
<td>1.95 (0.21)</td>
<td>1.15 (0.16)</td>
<td>2.42 (0.86)</td>
<td>1.32 (0.38)</td>
<td>1.22 (0.43)</td>
<td>1.10 (0.16)</td>
</tr>
<tr>
<td>F2</td>
<td>2.51 (0.05)</td>
<td>4.24 (0.12)</td>
<td>3.54 (0.28)</td>
<td>1.53 (0.12)</td>
<td>2.10 (0.29)</td>
<td>1.25 (0.05)</td>
</tr>
<tr>
<td>F3</td>
<td>2.26 (0.11)</td>
<td>3.48 (0.26)</td>
<td>3.10 (0.17)</td>
<td>1.65 (0.10)</td>
<td>1.80 (0.06)</td>
<td>1.27 (0.05)</td>
</tr>
<tr>
<td>F4</td>
<td>1.67 (0.10)</td>
<td>1.89 (0.19)</td>
<td>2.27 (0.17)</td>
<td>1.00 (0.11)</td>
<td>0.70 (0.04)</td>
<td>0.58 (0.08)</td>
</tr>
<tr>
<td>F5</td>
<td>0.49 (0.06)</td>
<td>0.38 (0.01)</td>
<td>0.47 (0.02)</td>
<td>0.37 (0.06)</td>
<td>0.42 (0.06)</td>
<td>0.17 (0.03)</td>
</tr>
<tr>
<td>F6</td>
<td>0.01 (0.01)</td>
<td>0.07 (0.02)</td>
<td>0.08 (0.02)</td>
<td>0.12 (0.03)</td>
<td>0.22 (0.08)</td>
<td>0.04 (0.01)</td>
</tr>
<tr>
<td>F7</td>
<td>0.01 (0.01)</td>
<td>0.02 (0.01)</td>
<td>0.02 (0.01)</td>
<td>0.04 (0.02)</td>
<td>0.08 (0.02)</td>
<td>0.01 0.00</td>
</tr>
</tbody>
</table>
### Table 7 Comparison of the data obtained by the various techniques

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

\(^a\) Determined only for Dornach and Mortagne soils.