The reactivity of trace metals (Cd, Pb, Zn, Cu) present in two polluted soils was studied using two extractants, EDTA and hydroxylamine. As the classical chemical speciation of trace metals in soils when using these reagents seems to be unreliable (readsoption phenomena, non-selectivity of chemical reagents, soil/solution ratio, etc.), we applied two different kinetic approaches to the results in order to determine the speciation of trace metals in the two soil samples studied. The advantages of and problems with these approaches are discussed.

**Keywords:** EDTA; hydroxylamine; trace metals; polluted soils; speciation; kinetics

Regardless of their origin and the reasons for the increase in their concentration in soils, trace metals are liable to contaminate the food chain by migrating towards ground water or by accumulating in plants. This possible mobility and bioavailability are the result of the reactivity of trace metals in soils, in other words, their localization in different soil components, which is now usually called speciation. Note that, by also using the term ‘localization’, speciation of trace elements in soils is not fully defined by Florence,1 who first distinguishes the different physico-chemical forms of the same element. Here, in our discussion of trace elements, we shall use the terms ‘speciation’ and ‘localization’ interchangeably.

Speciation of trace elements in soils may be performed using physical or chemical methods. In the first case, the possible methods2–4 are generally not sufficiently sensitive, and therefore can only be used for this purpose with severely contaminated samples. Chemical methods are more sensitive, and consist of using different chemical reagents (Table 1) for the extraction of trace elements, terminating with their quantification in the extraction phase (usually when equilibrium is reached).5

Several reagents are generally used in what are called sequential extraction protocols.6–11 However, it is now agreed that these protocols cannot supply a reliable estimate of the speciation of trace elements in soils, particularly for thermodynamic reasons (measurements made at equilibrium). This is why it appears necessary to consider other methods of determining this speciation of trace metals in soils in more detail, particularly considering kinetic aspects that also characterize the stability of the various trace metal–soil constituent associations.

The first part of this paper includes some experimental results obtained with the use of two reagents for the extraction of trace metals in soils (hydroxylamine and EDTA), for what may be called the speciation equilibrium method. In the second part, we consider the possibilities of a kinetic approach, using the results obtained with the same two reagents, in order to determine the speciation of trace metals in soil samples.

### Experimental

#### Soil samples

Soil samples were taken from polluted sites. One of them, Couhins, is located in the Bordeaux region of France and forms part of a long-term agronomic test being carried out by the INRA Agronomy Unit on the consequences of spreading sewage sludge from a treatment station with a high concentration of Cd and Ni. The second, Evin, is located in the north of France and is part of an agrosystem contaminated by atmospheric industrial fallout around a metallurgical plant. Table 2 gives the main physico-chemical characteristics of the two samples (0–20 cm layer taken in 1994) measured on the granulometric fraction smaller than 2 mm after sieving. Total concentrations in this case were determined after mineralization with hydrofluoric acid.

#### Extractions with hydroxylamine

For the kinetic study of the extraction of metal cations by hydroxylamine, polyethylene tubes washed in acid and containing 1 g of fine soil (sieved to 2 mm and coarsely ground) and 50 ml of a solution of hydroxylamine hydrochloride (0.1 mol l⁻¹) were stirred using a rotary stirrer for a given mixing time, different for each tube and ranging from 30 min to 24 h. At the end of the chosen mixing time, the tube was removed from

### Table 1 Some chemical reagents used for the localization of soil trace metals

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Soil compartments attacked</th>
<th>Chemical reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing reagents—</td>
<td>Fe, Mn oxides</td>
<td>Acidification and oxide reduction (dissolution)</td>
</tr>
<tr>
<td>Oxalate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxidizing reagents—</td>
<td>Organic matter</td>
<td>Oxidation and dissolution of organic matter</td>
</tr>
<tr>
<td>H₂O₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaClO₄</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complexing reagents—</td>
<td>Organic matter, Fe oxides, etc.</td>
<td></td>
</tr>
<tr>
<td>Na₂PO₄</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDTA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2 Some physico-chemical characteristics of the soil samples

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Couhins</th>
<th>Evin</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.1</td>
<td>7.9</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td>2.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>2.9</td>
<td>16.5</td>
</tr>
<tr>
<td>Cd (total)/µg g⁻¹</td>
<td>94.9</td>
<td>19.4</td>
</tr>
<tr>
<td>Zn (total)/µg g⁻¹</td>
<td>151</td>
<td>1415</td>
</tr>
<tr>
<td>Cu (total)/µg g⁻¹</td>
<td>45.3</td>
<td>43.5</td>
</tr>
<tr>
<td>Pb (total)/µg g⁻¹</td>
<td>44.8</td>
<td>1120</td>
</tr>
</tbody>
</table>

---

1 Presented at The Third International Symposium on Speciation of Elements in Toxicology and in Environmental and Biological Sciences, Port Douglas, Australia, September 15–19, 1997.
The study of the extraction of trace metals from soils by EDTA

Extractions with EDTA

The isolated effect of the pH at equilibration time was also studied: when using dilute solutions of nitric acid for trace metal extraction, the effect of decreasing the redox potential alone can be deduced from comparison with trace metal concentrations extracted in the more or less acidified hydroxyamine medium (NH₂OH + H⁺).

A study of the amounts extracted at equilibrium as a function of pH was carried out using 1 g of soil and 50 ml of solution (nitric acid and/or hydroxylamine hydrochloride) at various pH values, with stirring as above for 24 h; the same protocol as described above was then used and the pH range studied was 2–6.

To study the possible sorption of metal cations extracted during the reaction with hydroxyamine, we carried out an additional extraction after the extraction with this reducing reagent and using barium perchlorate, a reagent used by some workers to extract exchangeable trace metals. The sample reagent and using barium perchlorate, a reagent used by some workers to extract exchangeable trace metals. The sample reagent (0.0001–0.05 mol l⁻¹) over a wide range of pH values. Therefore, extraction solutions were acidified with nitric acid or neutralized with sodium hydroxide. In order to determine the influence of acidity on the amounts of trace metals extracted, we also carried out extractions without EDTA at different pH values (calibration curves). For all extractions, 1 g of the soil sample was mixed with the extracting solution (50 ml) and stirred for 24 h. Solutions were filtered (filter pore diameter 0.45 μm) after the reaction to permit analysis of the extracted trace metals.

A kinetic study of the extraction of metal cations by EDTA was made on a 19 g test sample and an initial volume of 950 ml of the extracting solution (0.05 n EDTA at pH 6.5), which corresponds to a soil/solution ratio of 1 : 50. Samples of 25 ml were processed for the determination of trace elements, as in the study at equilibrium.

The various reagents used for this part of the study were of analytical-reagent grade.

Determination of extracted cations

All solutions were prepared from analytical-reagent grade salts or Titrisol solutions (Merck, Darmstadt, Germany) and Milli-Q-purified water. Cations in solution (Cd, Zn, Pb, Cu, Ni) and a few major cations (Fe, Mn, Mg, Ca) were measured with a Varian (Palo Alto, CA, USA) Spectra 250 Plus atomic absorption spectrometer using an air-acetylene flame and external standards. The analytical performances (sensitivity, detection thresholds) for the various extraction environments studied were not different from those obtained with simple matrices. Furthermore, we used the standard addition method to verify that there were no interferences and in all cases we made extraction blanks. Under these conditions, the relative standard deviations obtained for the extraction results with the reagents used, including the variability of the soil sample, were satisfactory and ranged between 2 and 6% (n = 4) depending on the metal.

Estimate of kinetic parameters

We adjusted the experimental curves (concentration of extracted cations as a function of time) using a non-linear regression program based on Marquardt’s algorithm and developed by the INA chemical laboratory. The calculation of the coefficients used in a given model was accompanied by the calculation of their confidence intervals and the various statistical parameters that evaluate the quality of the estimate.

Results and discussion

Studies at equilibrium

Hydroxyamine

Hydroxylamine is a reducing reagent that makes some of the Fe (and Mn) hydroxides in soils pass into solution, based on the following reaction:

\[
\text{Fe}_2\text{O}_3 (s) + 4\text{H}^+ + 2\text{NH}_2\text{OH} \rightleftharpoons 2\text{Fe}^{2+} + 5\text{H}_2\text{O} + \text{N}_2
\]

Fig. 1 shows that equilibria are reached for most metal cations after about 24 h. However, equilibrium seems slower for iron.

The amounts of trace elements extracted after 24 h of reaction decrease when the pHₐ (the solution pH at the end of the experiment) increases. This is a classical result that involves the extracting power of H⁺ ions based on the following reaction:

\[
\text{S}_i\text{M} + 2\text{H}^+ \rightleftharpoons \text{S}_i\text{H}_2 + \text{M}^{2+}
\]

where \( S_i \) represents an adsorption site of a constituent of the solid phase of the soil and M⁡²⁺ is a metal ion that can be fixed to it. However, the effect of acidity is more complex since, as indicated by reaction (1), the protons also take part in the oxidation–reduction reaction and are therefore liable to modify the amounts of Fe dissolved.

Monitoring the extracted cations as a function of time can give asymptotic curves for the two samples in this study, and therefore there is no reason to suppose that there are any refixation phenomena, as some workers have demonstrated using an almost identical reagent. The use of an additional extraction with barium perchlorate allowed us to establish that refixation phenomena occur during hydroxyamine reaction. As an example, it can be seen that the magnitude of the refixation phenomena depends on the final acidity (Fig. 2).

This metal cation refixation phenomenon has already been demonstrated by a number of workers for other reagents, e.g., hydrogen peroxide, which are assumed to destroy a
compartment of the soil. This phenomenon can be explained from a thermodynamic point of view by considering the soil as a multi-ligand system and by displacement of all equilibria towards the fixation of trace elements on the remaining compartments when one of them is destroyed.EDTA is a well known complexing reagent. It is capable of directly extracting metal cations from several soil compartments by a competitive classic complexation reaction, but also of dissolving some other compartments with which trace elements are associated, e.g., iron hydroxides or carbonates. In other words, EDTA is a non-specific reagent that cannot a priori lead to localization of trace elements in a particular soil compartment. It is nevertheless used in soil science in some sequential extraction protocols or as a reagent capable of forecasting bioavailability. Therefore, it is useful to make a complete study of the reactivity of trace metals in soils in the presence of this molecule. Results obtained when this reagent is used with two soil samples that we have already described have been presented and discussed elsewhere. As an example, the amounts of Pb (Evin sample) extracted by EDTA at different concentrations as a function of the pH of the solution are shown in Fig. 3.

Finally, it can be seen that EDTA at a high concentration is a powerful means of extracting trace elements. Table 3 gives the amounts extracted: with all the studied cations included, this complexing reagent extracts between 40 and 90% of the total contents.

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When the various coefficients are known, the variation of the concentration of A can be calculated for the same example as a function of time. This curve represents the concentration of cations related to the iron hydroxide compartment in the sample. According to this result, at time \( t = 0 \), the concentration \( A_0 \) (the hydroxyamine-extractable amount of trace metals) is approximately 0.9 mg g\(^{-1}\), in other words, about twice the value of the concentration of B when equilibrium is reached. The difference between these two values clearly corresponds to the refixed cations. Therefore, this kinetic approach is sufficient to determine the amounts of cations actually associated with the iron hydroxide compartment. In fact, considering the relatively high acidity of the reagent and the resulting non-selectivity (other compartments attacked by protons), it would be more accurate to state that this kinetic approach gives a better estimate of the amounts of metals in the soil sample concerned by attack with the acidified hydroxylamine reagent than can be obtained by measurement.

**EDTA**

The use of kinetic data for the extraction of trace metals from soils by EDTA cannot give the speciation (localization) of these elements in terms of soil compartment, as we have already seen. However, we studied the feasibility of a strictly operational speciation in which trace metals extracted by EDTA would be classified into labile metals (quickly extracted) and non-labile metals (less quickly extracted).

This objective, together with simplifying assumptions about first-order reactions, requires that the constants of an equation of the following type should be estimated by non-linear regression:

\[
Q = C_1 \exp(-k_1 t) + C_2 \exp(-k_2 t)
\]

(5)

where \( Q \) represents the amount of metals extracted at time \( t \). \( C_1 \) and \( C_2 \) are the math.; labile and non-labile amounts, respectively, for a given metal and \( k_1 \) and \( k_2 \) are the kinetic constants associated with them.

An example of the results obtained using this procedure and operational conditions is presented in Fig. 5. It can be seen that there is good agreement between the calculated and experimental curves; furthermore, the various statistical tests (correlation coefficient, confidence interval, residual variance) are also satisfactory. Identical results were found for other metals and other samples.

Table 4 presents simple statistical parameters obtained during four repetitions (four independent samples) of this kinetic speciation method. In this case, \( C_1 \) and \( C_2 \) were calculated with respect to total metal concentrations. It can be seen that the proportion of the labile element is between 40 and 55% for the four metals studied. The values of the relative standard deviations (< 10%) show that the repeatability of this method is satisfactory considering the variability of the samples.

Therefore it appears feasible to classify the extraction curves for these metals in soils by EDTA into two metal categories, labile and non-labile. The benefits of doing so in terms of predicting the bioavailability and/or mobility of trace elements in the environment still have to be demonstrated. We shall simply present the initial results of this study that is now in progress. We have applied this method to freshly contaminated samples in the laboratory for which it is known that the added metals are very mobile, i.e., they should be classified in the labile category for the kinetic speciation method. Table 5 shows an example of the results obtained in which it is seen that added copper and cadmium are mostly found in the labile partition. Therefore, the various results appear to show that this operational method is useful and a more detailed study is under way.

**Conclusion**

Although chemical reagents are frequently used nowadays to extract and determine the localization or speciation of trace metals in soils, the two studies presented here on the extraction of trace metals at equilibrium show the limitations of this approach.

The hydroxylamine example shows that trace metal refixation phenomena take place during the attack on the soil sample and consequently the measurements made at equilibrium underestimate the amount of metal cations related to the ‘reducible’ partition of the soil. Furthermore, a second problem occurs following the use of this reagent due to acidification of the hydroxylamine and the specific reactivity of protons concerning the extraction of trace metals.

EDTA is necessarily a non-specific reagent since it can extract trace elements from several soil partitions. Therefore, measurements of trace elements extracted at equilibrium cannot be related to their speciation.

A kinetic approach to the speciation of trace elements in soils (or sediments) may be an alternative way. The few examples shown in this work establish the advantage of this approach. Considering the results obtained with hydroxyamine, the use of kinetic data gives a more precise estimate of the amount of extracted trace metals than can be obtained by conventional measurements made when equilibria are reached, owing to refixation phenomena.

![Fig. 5 Monitoring of EDTA-extracted zinc as a function of time and comparison with the calculated curve (Evin sample; EDTA concentration = 0.05 M; pH = 6.5).](image)

---

**Table 4** Statistical parameters used to characterize the repeatability of the ‘kinetic speciation’ of trace metals in soils

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cd</th>
<th>Zn</th>
<th>Cu</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calculated values of ( C_1 ) and ( C_2 ) (mg g(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( C_1 )</td>
<td>55–56</td>
<td>41–41</td>
<td>41–41</td>
<td>45–41</td>
</tr>
<tr>
<td>( C_2 )</td>
<td>51–49</td>
<td>16–19</td>
<td>23–21</td>
<td>28–29</td>
</tr>
<tr>
<td>Mean (mg g(^{-1}))</td>
<td>5.2</td>
<td>9.5</td>
<td>4.3</td>
<td>5.4</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>9.5</td>
<td>17</td>
<td>21.5</td>
<td>28.5</td>
</tr>
</tbody>
</table>
Kinetic data can be used differently when EDTA is used as a trace metal extraction reagent, to define the amounts of labile and non-labile cations for a given sample. If the results of this study (which still needs further work and validation) are satisfactory, it could contribute towards the creation of a tool for predicting risks to the environment due to the presence of trace metals in soil.

Table 5 Labile ($C_1$) and not labile ($C_2$) cadmium and copper in freshly spiked samples

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Concentration/μg g⁻¹ (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labile fraction ($C_1$)</td>
<td>Cd 35.8 (97.3) Cu 50 (92.5)</td>
</tr>
<tr>
<td>Non-labile fraction ($C_2$)</td>
<td>Cd 1.0 (2.7) Cu 4.0 (7.5)</td>
</tr>
</tbody>
</table>

References