Application of a Sequential Extraction Procedure to Calcareous Soil Samples: Preliminary Studies

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APPLICATION OF A SEQUENTIAL EXTRACTION PROCEDURE TO CALCAREOUS SOIL SAMPLES: PRELIMINARY STUDIES

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The localization of trace metals in soils is usually performed by sequential extraction procedures. Our purpose was to study the fractioning of copper in calcareous soils, in order to predict its mobility, using the extraction procedure proposed by Tessier et al. This preliminary study is devoted to the improvements of this method applied to calcareous soil samples.

First, it was necessary to assess the experimental conditions allowing the complete solubilisation of the different compartments involved in the extraction procedure with their appropriate reagent (acetic acid-sodium acetate and carbonates, iron hydroxide and hydroxylamine, organic matter and hydrogen peroxide, fluorhydric-perchloric acid and residual fraction). The complete solubilisation of each compartment was tested by measuring the residual phase.

Secondly, it was necessary to study the analytical protocol for the determination of copper with electrothermal atomic absorption; an improvement of this determination was particularly necessary for the reagent used for the exchangeable fraction (sodium acetate at pH = 8.5).

According to the experimental results, the quality (repeatability, concordance of the sum of fractions with the total content of copper determined with independent measurement) of the proposed protocol seems to be quite good.

Some results of the fractioning of copper in calcareous soil samples are given; these samples are characterised by a high value of copper in the residual fraction corresponding to a poor availability of this element.

KEY WORDS: Copper, calcareous soils, sequential extraction.

INTRODUCTION

A sequential extraction procedure consists in submitting a soil or sediment sample to successive attacks with reagents of different chemical properties (acidity, redox potential, complexing properties) which extract each a part of the trace metals associated to the sample. The most well-known sequential extraction scheme is the one of Tessier [1] but, since then, several other protocols have been described in the literature [2-4].

These protocols have been studied and designed for the speciation of trace metals in soils and sediments (assessment of the components responsible for the binding of these elements)

and then to predict the mobility and bioavailability of these elements [5-8]. However, the selectivity of the chemical reagents has been criticized [9-17] and back-adsorption phenomena of trace metals during the extraction step(s) have been mentioned.

The interest of the use of extraction protocols for predicting the bioavailability of trace metals in soils has not been clearly demonstrated. We propose to carry out thorough investigations on copper in calcareous soils using the Tessier protocol [1]. The samples studied are characterised by high calcium carbonate and organic matter contents, low total copper content, and pH values up to 8.0. A study has been necessary to validate the extraction protocol, not much used on this type of soil, and to optimise the analytical methods for copper; this paper describes this preliminary study.

EXPERIMENTAL

The soil samples were collected in two soil parcels located in the East of France (chalky Champagne) where copper deficiencies were observed for wheat. The soils were air–dried, sieved at 2 mm mesh and ground.

The main parameters related to the chemical composition of the samples (fine-grained fraction <2 mm after air-drying and sieving) are reported in Table 1.

The different extractions were performed in the dark in tubes containing the appropriate reagent and the soil sample; the tubes were placed in an aluminium block maintained at the desired temperature. For treatments requiring a temperature up to 50 °C, important losses by evaporation may occur if the tube is badly closed; consequently, a cap with a needle insert was adapted to limit these losses. This allowed the reaction to be carried out at a volume almost constant.

At the end of each extraction the extract was separated from the solid residue by centrifuging at 5000 rpm during 45 min; the supernatant was collected with a pipette and stored at 6 °C in the dark prior to analysis.

Each residue was washed with two additions of 2.5 ml of sodium acetate (1 mol/l. pH = 8.5) by homogenising after each reagent addition. After agitation during 60 min at 20 °C followed by centrifugation at 5000 rpm during 45 min, the supernatant obtained was collected with a pipette and stored at 6 °C in the dark prior to analysis.

The determinations of organic carbon, $CaCO_3$ and iron hydroxides were performed using classical methods [22]:

Table 1 Physico-chemical parameters of the soil samples (extreme values).

Organic matter (%)	3 – 5.4
$CaCO_3$ (%)	55 – 88
pH	8.2 – 8.5
Total Cu (HF) in µg/g	2 – 5.2
EDTA extract. Cu in µg/g	0.64 – 1.40

Table 2 Temperature programme of the furnace.

Step	1	2	3	4	5
Temp. (°C)	20	600	2300	2600	20
Gradient (s)	15	2	0	1	10
Duration (s)	10	15	6	2	2
Gas flow rate (ml)	300	300	50	300	300

- the determination of organic carbon was carried out with the Ann method (detection by colorimetry of the chromate);
- the determination of $CaCO_3$ was performed with a Bernard calcimeter (volumetric measurement of the CO_2;
- the determination of iron hydroxides was carried out by atomic absorption spectrophotometry after HF mineralisation.

The copper determination was performed by flameless atomic absorption spectrometry with a Perkin-Elmer equipment (spectrophotometer 2380 equipped with a graphite furnace HGA400, sampler AS40, recorder R100 and micro-computer for the treatment of data). The furnace was equipped with a L'vov platform pyrolytically treated [20] to increase the sensitivity. In addition, a background noise correction was systematically applied [21]. The electrothermal programme used for copper determination is given in Table 2; the step at 2600 °C corresponds to a cleaning step of the graphite tube.

All the reagents used in this study were of very high purity.

Optimisation of the copper fractioning

The fractioning protocol of metallic cations used by Tessier [1] is defined by the five following fractions:

- exchangeable cations;
- cations bound to carbonates;
- cations bound to iron and manganses hydroxides;
- cations bound to organic matter;
- cations bound to the residual fraction.

Considering the controversies underlined above and particularly the non-selectivity of chemical reagents used in this procedure, these fractions will be referred to as F1, F2, F3, F4 and F5 throughout the text.

In the Tessier scheme [1], the F1 fraction is obtained with a magnesium chloride solution (1 mol/l, pH = 7.0) or a sodium acetate solution (1 mol/l, pH = 8.2). According to Chapman [18] the solubility of $CaCO_3$ in sodium acetate is low. The determination of calcium in the extracts allows to assess the amount of calcium dissolved by this reagent. The Figure 1 shows the calcium carbonate fraction solubilised in relation to the total $CaCO_3$ content of the samples
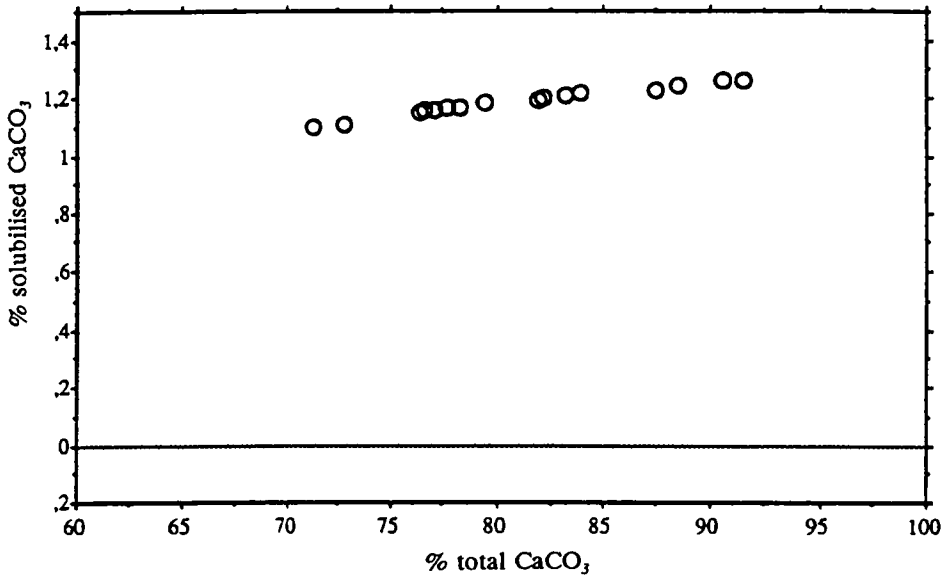
Figure 1   Amount of solubilised CaCO₃ (in %) in relation to the calcium carbonate content of the soil sample.

studied; in these conditions, the amount of CaCO₃ solubilised is low and may be neglected in comparison to the total CaCO₃ content of the samples. Consequently, a sodium acetate solution (1 mol/l) adjusted to pH = 8.5 and a sample intake of 0.5 g have been chosen for the F1 fraction.

Figure 2   Study of the acetic acid amount necessary to solubilise the carbonates present in the soil samples.

Figure 3 Calcium carbonate solubilised in function of the reaction time (F2 fraction, acetic acid + sodium acetate).

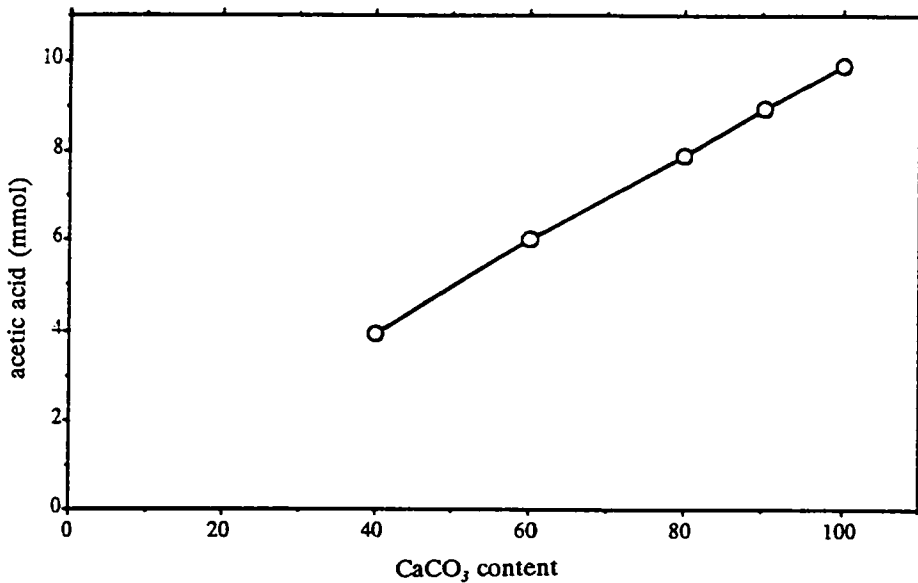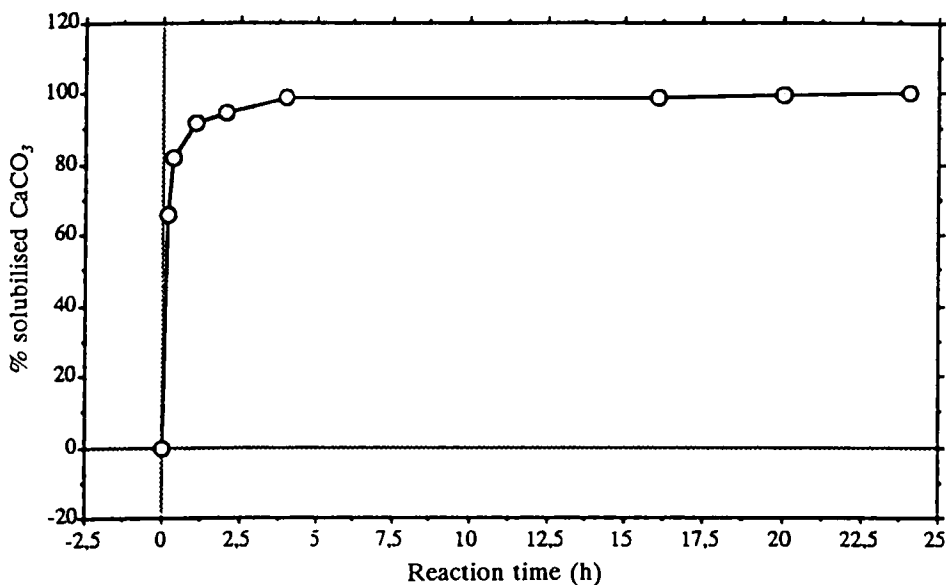For the F2 fraction, corresponding to the carbonate-bound cations according to Tessier[1], a complete dissolution of $CaCO_3$ has to be achieved. The solubilisation of $CaCO_3$ can be limited by its solubility in the extract and by the dissolution kinetic. Loring [19] mentioned that the dissolution time depends upon several parameters related to the sample: the grain size, the $CaCO_3$ content, the nature of the carbonates and the sample intake. A sodium acetate solution (1 mol/l) adjusted at pH = 5 with acetic acid has been chosen; the amount of acetic acid has to be sufficient to completely dissolve the calcium carbonate without partially dissolving the iron and manganese oxides. The Figure 2 indicates the amount of acetic acid necessary in relation to the $CaCO_3$ content of the soil samples whereas the Figure 3 shows that the complete dissolution of $CaCO_3$ is achieved after five hours. During this reaction, the pH increases and the $CaCO_3$ dissolution rapidity decreases to become very slow up to pH = 6.2. The necessary acetic acid additions should not, however, lower the pH in a too large extent. The study of the acidity trend of the extracts allows to verify that additions of 0.5 ml of acetic acid (5 mol/l) after 1 h, 1 h and 30 min, 2 h and 30 min maintain the pH at a value around 5.0.

The sodium acetate solution (1 mol/l) previously adjusted to pH = 5 with acetic acid (volume of 5 ml) and to which acetic acid is added during the reaction is therefore adopted as second step (S2) of the Tessier protocol for the determination of the soil F2 fraction of copper.

For the F3 fraction, corresponding to the cations bound to iron and manganese hydroxides, Tessier [1] recommends the use of a reducing solution of hydroxilamnine 0.04 mol/l in acetic acid (ca. 4.4 mol/l) for solubilising the iron and manganese oxides. A reaction temperature of 96 °C is necessary for this treatment; the time necessary to dissolve the reducible iron and manganese oxides is assessed by determining the iron content of the
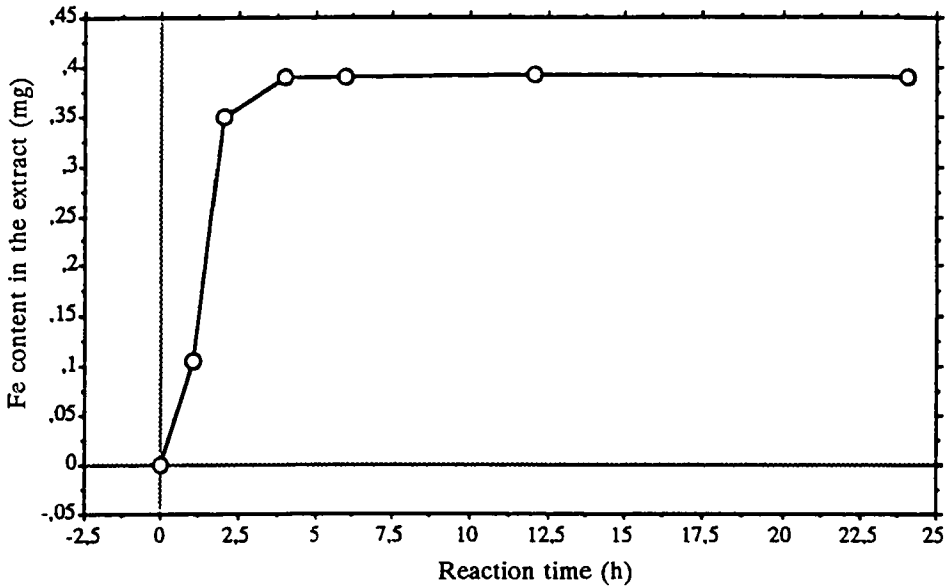
Figure 4　Amount of Fe extracted in function of the reaction time (F3 fraction, hydroxylamine).

extracts in function of time. The results reported in Figure 4 show that this reaction is complete after 4 hours.

The hydroxilamine 0.04 mol/l solution in acetic acid 4.4 mol/l (V = 5 ml) is adopted for the determination of the F3 fraction.

The fraction F4 corresponds to the cations bound to organic matter. The reagent used for this fraction is a hydrogen peroxide solution (ca. 8.8 mol/l) in nitric acid 0.02 mol/l at a temperature of 85 °C [1]. Considering the high organic matter content, we have observed that a digestion during 24 h at ambient temperature is more suited to avoid the formation of foam; this digestion is followed by an attack at 85 °C during 3 h. The kinetic of the destruction of the organic matter is followed by determining the organic matter content in the solid residue in function of time. The results obtained are presented in the Figure 5 and show that the reaction of organic matter destruction is achieved after 24 h. The hydrogen peroxide solution (8.8 mol/l) in presence of nitric acid 0.02 mol/l is therefore adopted for the determination of the soil F4 fraction of copper.

For the last fraction (F5), corresponding to the cations bound to the residual fraction, Tessier [1] recommends to perform the solubilisation of the residue in a platinum crucible in the presence of fluorhydric acid and addition of perchloric acid. For commodity reasons, the use of polypropylene tubes has been preferred instead of platinum crucible. The digestion is carried out at 50 °C by adding a reagent composed of 2 ml of concentrated nitric acid and 1 ml concentrated fluorhydric acid. The Figure 6 shows the copper amount solubilised in function of reaction time; it is noted that a duration of 24 h is sufficient to achieve a complete solubilisation. This duration has been adopted for this study.

For the F2, F3 and F4 fractions, a washing of the residue completes the treatment; sodium acetate at pH = 8.5 has been chosen as washing solution. This reagent is preferred to distilled
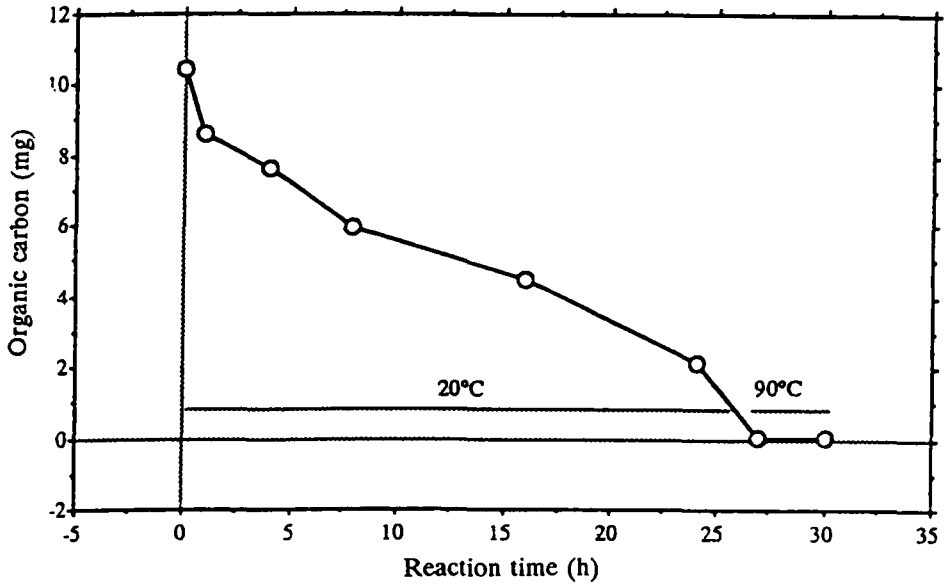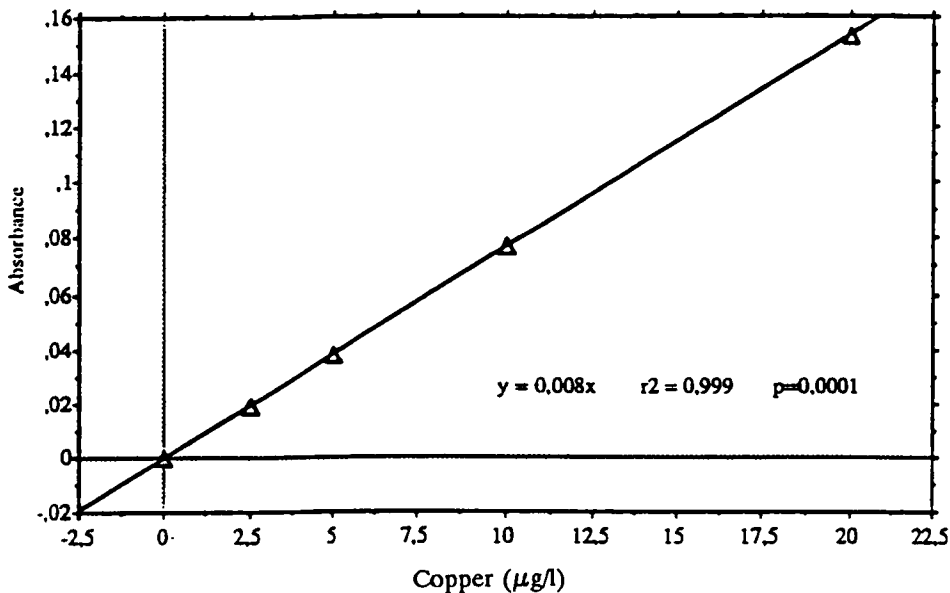
Figure 5 Amount of residual organic carbon in function of the time and temperature of reaction (F4 fraction, hydrogene peroxide).
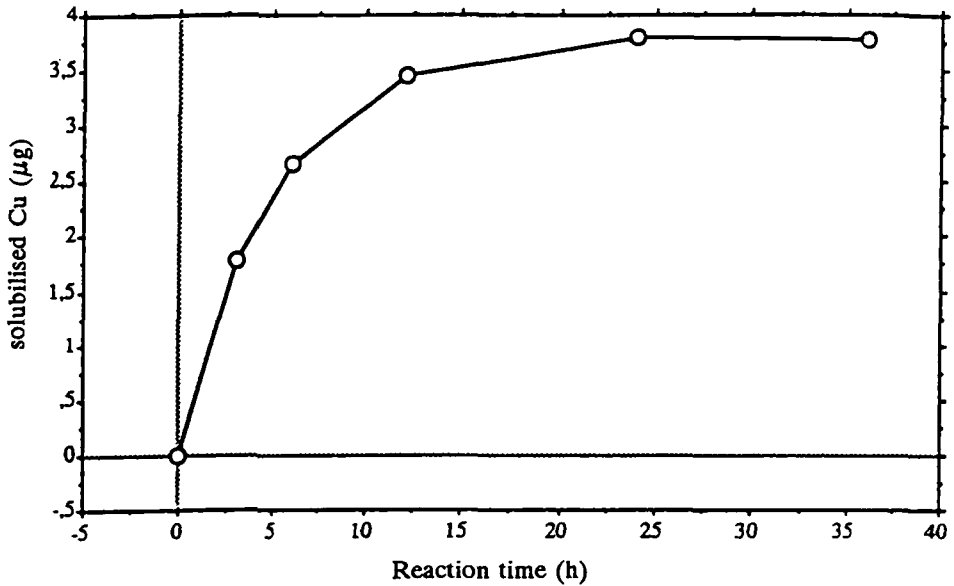
Figure 6 Amount of copper solubilised in function of the reaction time (F5 fraction, fluorhydric acid + nitric acid).
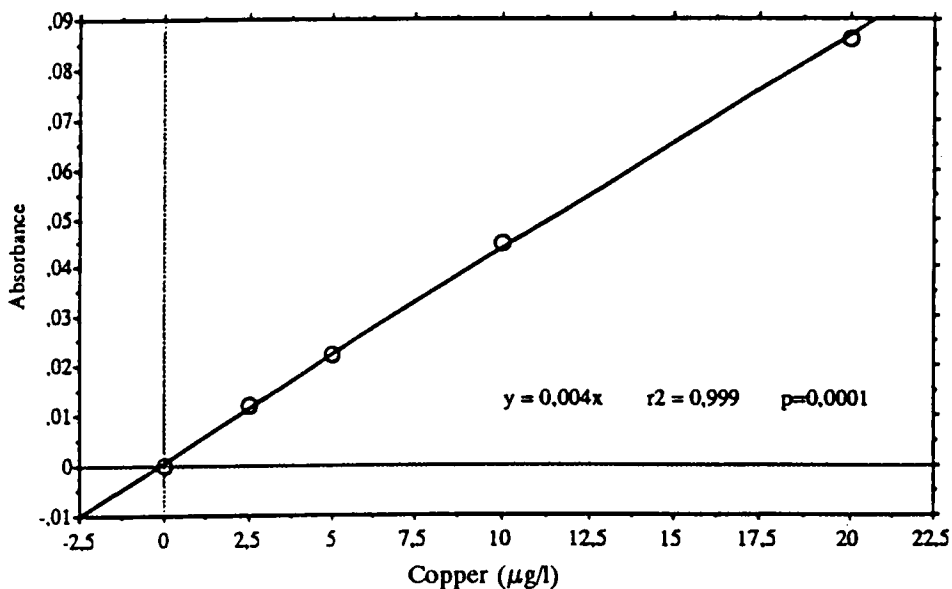
Figure 7 Calibration curve of copper in solution (hydroxylamine 0.08 mol/l and acetic acid 4.4 mol/l) by electrothermal atomic absorption spectrometry.

water recommended by Tessier [1] owing to its cation exchange and copper complexation properties. The results of copper determination in these washing solutions are added to the corresponding fractions.

Optimisation of the analytical conditions

In order to take into account the influence of the different reagents on the measurements, the calibrant solution were prepared in the different extraction media. Two calibration curves obtained (with at least three repetitions per measurement) are presented as an example in the Figures 7 and 8. The comparison of the two curves shows that the two slopes are significantly different: this demonstrates that the matrix effects are only taken into account for each reagent if the calibration is done in the proper medium. In all the cases, for the range of copper concentration concerned, the calibration curves are linear and the high correlation coefficients (>0.99) demonstrate a good measurement quality.

However, preliminary experiments have shown that, in the case of the sodium acetate solution used for the extraction of the F1 fraction and for each of the washing step of the other fractions, it was not possible to determine copper in the same conditions than the ones used for the other reagents. Due to explosions occurring in the graphite furnace during the atomisation step, measurement errors were observed as well as a rapid deterioration of the graphite tubes and contact cylinders; the most probable cause may be attributed to the presence of sodium brought by this reagent. An optimisation of the electrothermal pro-gramme have been necessary to overcome these problems and determine copper in these solutions in the conditions described below.

Figure 8 Calibration curve of copper in solution (sodium acetate 1 mol/l) by electrothermal atomic absorption spectrometry.

Owing to the particular conditions of this optimisation, the quality of the results during the experiments is verified by controlling the variations of the two following indices:

- Repeatability index (Ri) = W/M; where W is absorbance wavelength of a series of at least 3 measurements and M is the corresponding mean value of the absorbances.
- Sensitivity index (Si) = M*10/C; where M is the mean value of the absorbance measured for a copper solution with a given concentration C; C is taken as 20μg/l of copper.

First, the solutions are diluted with a matrix modifier [23] containing 50 g/l of ammonium nitrate and 50 ml of concentrated nitric acid (d = 1.19). The optimisation of the electrothermal programme has focussed on the following parameters:

- drying temperature;
- ashing temperature;
- atomisation temperature;
- carrier gas flow rate.

An example of results obtained (Table 3) shows the influence of the atomisation temperature on the two indices Ri and Si. In the case of the Si indice, the optimum is achieved at about 2600–2700 °C but the repeatability of measurements is then less good. A compromise has eventually been adopted (atomisation temperature at 2300 °C) corresponding to both a better repeatability and adequate conditions for the determination of low copper contents in these solutions.

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Table 3   Influence of the atomisation temperature on the
absorbance of a sodium acetate solution (1 mol/l) at pH =
8.5 (20 g/l of copper).

T °C	Abs.	Si	Ri
2000	0.043	0.022	0.024
2100	0.076	0.039	0.025
2200	0.076	0.039	0.026
2300	0.080	0.041	0.035
2400	0.080	0.043	0.038
2500	0.092	0.047	0.049
2600	0.098	0.050	0.061
2700	0.096	0.049	0.075

The study of the different parameters has finally led to perform, for the determination of copper in sodium acetate solutions, a thermal treatment of the injected solutions in several steps; the corresponding electrothermal programme is presented in the Table 4.

RESULTS AND DISCUSSION

Assessment of the quality of copper fractionning

The quality of the different optimisations may be tested in various ways; first, the repeatibility of the copper fractionning has been studied in a soil sample. The results corresponding to five replicate analyses of the same sample are presented in the Table 5 and may considered as satisfactory as the coefficients of variations related to the different fractions were less than 10%.

The values obtained with the classical method used for the determination of total copper[22] have also been compared with the values corresponding to the sum of the five fractions. This comparison enables generally to ensure that no bias occurred in one of the fractionning steps. The results are presented in Table 6; it is noted that the two values are comparable within the analytical uncertainties.

Table 4   Steps of the electrothermal programme for the determination of copper.

Steps	1	2	3	4	5	6	7
T °C	130	210	380	1200	2300	2600	20
Gradient (s)	10	10	20	60	0	1	1
Maintenance (s)	25	10	15	15	6	3	10
Gas flow rate (ml/min)	300	300	300	300	50	300	300

Table 5 Determination of the repeatibility of copper fractionnation.

	F1	F2	F3	F4	F5
1	0.06	0.17	0.06	0.06	3.3
2	0.09	0.16	0.07	0.07	3.25
3	0.09	0.18	0.05	0.09	3.2
4	0.10	0.16	0.09	0.06	3.2
5	0.09	0.16	0.07	0.08	3.2
Mean	0.087	0.17	0.066	0.07	3.25
S.D.	0.007	0.004	0.006	0.005	0.013

Copper distribution in the different fractions

The results of the application of the extraction protocol for copper in some soil samples collected in two parcels (eight samples per parcel) are given in Table 7.

Results obtained using the same type of procedure for copper in calcareous soils are not available. To our knowledge, the single work carried out on this type of sample[24] cannot be compared to this study as the reagents and the extraction sequence were different.

It is noted that the two parcels present different characteristics with regard to copper; the second parcel is poorer in total copper and the fractions defined in this study contain less copper than the corresponding fractions of the parcel 1, with the exception of the F4 fraction (organic matter).

The residual F5 fraction is generally very important in all the soil samples: for the parcel 1, the residual fraction represents ca. 90% of the total copper whereas for the parcel 2 it represents more than 70% of the total copper. It is assumed that the copper of this fraction is likely to be not bioavailable; this observation is to be linked to the carence phenomena of copper observed on the plants cultivated on these parcels.

For the two parcels, the copper contents in the F1 fraction (sodium acetate at pH = 8.5) and F3 fraction (hydroxylamine) are very low.

Finally, the importance of both the F2 fraction (sodium acetate - acetic acid) in the parcel 1 and the F4 fraction (hydrogen peroxide) in the parcel 2 are noted. It is hardly possible to firmly attribute this result to a difference of copper location in the two parcels and it would be necessary, in particular, to verify that transfer phenomena of copper do not occur during the successive extractions.

Table 6 Comparison of the total copper contents (HF) and the sum $^{m}F_i$ of the fractions (F1 to F5)(g of copper per g of soil sample).

Total Cu	mF_i
5.28	5.43
5.09	5.12
3.07	3.10
4.53	4.66
4.20	4.09

Table 7   Copper fractionnation in the two soil parcels (extreme values).

	F1	F2	F3	F4	F5
Parcel 1	0.10–0.14	0.20–0.42	0.06–0.09	0.02–0.04	4.57–5.54
Parcel 2	0.05–0.12	0.10–0.15	0.02–0.06	0.39–0.76	2.40–3.62

CONCLUSIONS

This preliminary study shows that it is possible to apply a sequential extraction procedure to calcareous soils, defining similar fractions as those described by Tessier [1]. The aim of this investigation is to assess the influence of each copper fraction on the bioavailability of this element but it will not likely be possible to compare the results obtained with those of other sequential extraction procedures. A common extraction scheme should therefore be adopted to compare the data throughout the European Communities, which is one of the objectives of the BCR.

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