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Comparison between conventional and ultrasound accelerated Tessier sequential extraction schemes for metal fractionation in sewage sludge

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Abstract Chemical sequential extraction for metal fractionation in sewage sludge using the Tessier method has been accelerated by ultrasound. The two sequential extraction schemes (conventional and ultrasound accelerated) were compared in terms of extraction efficiency, precision, treatment time and partitioning patterns of metals. Extractable contents of Cu, Cr, Ni, Pb and Zn were measured by flame atomic absorption spectrometry, and the analytical results obtained by the two procedures were statistically compared ($P = 0.95$). No significant differences were found in the two first fractions (i.e. exchangeable and carbonate-bound), however, in the third and fourth extracts (i.e. Fe-Mn oxides-bound and organic matter-bound) the extraction capability of the two methods differed significantly.

Introduction

The increasing accumulation of sewage sludge obtained in the residual wastewater recycling process has been recognised as a pollution hazard for the environment [1]. The determination of total heavy metal content of such samples is not sufficient to evaluate the possible mobility and, consequently, the bioavailability of toxic metals to living organisms. The application of sequential extraction procedures, despite some limitations, provides relevant information [2]. They allow the determination of the chemical “forms”, operationally defined, in which the elements appear associated in the sample [3].

Many sequential extraction procedures have been proposed [3, 4], and some of them have been applied to determine the chemical association of trace elements in various environmental samples. For instance, the Tessier [5]

scheme has been widely employed for metal fractionation in soil [6–9] and sediments [10, 11], but it has rarely been applied to sewage sludge samples [12]. This scheme allows metal distribution in four different stages: exchangeable, associated to carbonates, associated to Fe and Mn oxides and associated to organic matter and sulphides. The authors made a comparison between the Tessier and the BCR (proposed by the European Community Bureau of Reference) sequential extraction methods for metal partitioning in sewage sludge and found similar extractable metal contents, except for lead, with overall operation times of several hours [12].

Some attempts have been made to shorten and simplify the Tessier sequential extraction procedure. Thus, the use of microwave heating instead of the traditional treatment (i.e. conductive heating and magnetic shaking) in all the stages [13, 14], or in a particular one [15], has been attempted. The extraction efficiency of single extractions in comparison with sequential ones was also investigated [16]. Ultrasound energy, widely employed as auxiliary energy in numerous chemical processes [17–22], was identified as an attractive alternative [23].

The aim of this work is to apply ultrasound by means of a probe sonicator in order to accelerate each of the stages in the conventional Tessier extraction method. The best extraction conditions (optimised amplitude and sonication time) were specifically selected in each fraction for copper, chromium, nickel, lead and zinc. Metal determination in the extracts was carried out by flame atomic absorption spectrometry (FAAS). Finally, both conventional and ultrasonic accelerated Tessier extraction methods were compared for all metals studied in terms of efficiency, precision, treatment time and partitioning patterns.

Experimental

Instrumentation

Metal determination in the extracts was carried out by means of a double beam Perkin-Elmer Atomic Absorption Spectrophotometer model 2380. Hollow cathode lamps were used as radiation sources.

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Resonance lines at 324.8, 357.9, 232.0, 217.0 and 213.9 nm were employed for Cu, Cr, Ni, Pb and Zn, respectively. Lamp intensity and bandpass were used according to the manufacturer's recommendations. Air/acetylene flow rates were 11/1 L min⁻¹ for all elements, except for Cr, where a more reducing flame was required (11/2 L min⁻¹). A 100 W, 20 kHz ultrasonic processor (Sonic and Materials, model VC 100), equipped with probes made of a Ti-Al-V alloy, was employed as ultrasound energy source. A microwave oven (CEM MDS-2000), equipped with controlled pressure reactors, was used for a complete dissolution of the sample. A centrifuge (Alresa) operated at 3000 rpm was used for complete separation of the extracts. A ball-mill (Retsh model MM 2000), equipped with 10 mL capacity agate cups, was employed for a fast reduction of the sample particle size. A Crison 2000 pH-meter was used for adjustments of extraction pH.

Reagents

Extracting solutions (see Table 1) were prepared from analytical grade reagents (Merck) by suitable dissolution in ultrapure water. Stock metal solutions (1000 µg mL⁻¹) for atomic absorption spectrometry were obtained from the pure metal (in the case of Cu and Zn) or from the appropriate salts (in the case of Pb, Ni and Cr) and were made up to a corresponding volume with ultrapure water. Standard calibration solutions were prepared daily by appropriate dilution of the stock solutions. The hydroxylammonium chloride solution was prepared prior to use. Diluted nitric acid and ammonia solutions were used for pH adjustments.

Sample preparation

The sewage sludge sample (about 1 kg) was collected in polyethylene containers from an urban residual water treatment plant located near Orense (Spain). Samples collected on different days in

the same treatment plant showed similar extractable metal contents [12]. Once in the laboratory, the sample was dried at 110 °C in an air-forced heater until a constant weight was achieved, then ground, firstly, with an agate mortar and, secondly, with a ball-mill. Then, the sample was passed through a nylon fibre sieve to separate the sewage sludge fraction of particle size < 100 µm. The selected sludge sample was homogenised and stored in polyethylene vessels at room temperature in a desiccator.

Optimisation of sonication conditions

2 g of the pretreated sample (particle size < 100 µm) were placed into a clean 50 mL polyethylene tube, and the appropriate extractant solutions were added. The effect of sonication time and ultrasound amplitude was investigated in each fraction of the four-step Tessier procedure.

The influence of sonication time on the exchangeable metals was evaluated by extracting this fraction on the one hand following the conventional procedure (Table 1) and on the other hand by shaking with the ultrasonic probe for between 30 s to 15 min at 50% ultrasound amplitude. The remaining fractions (metals bound to carbonates, to Fe-Mn oxides and to organic matter) were extracted in a similar way. Only for optimisation purposes, each fraction was ultrasonically extracted by appropriate treatment of the resulting solid residue after the elimination of the previous stages using the conventional method. Desorption with NH₄OAc (after the oxidation step) was limited to 2 min of ultrasonic shaking, since higher treatment times caused excessive foaming. In both methods, after each extraction step the supernatant solution was separated from the solid phase by centrifugation for 12 min at 1600 rpm. After this, and prior to the addition of the next extractant solution, the solid residue was washed with approx. 10 mL ultrapure water, and the washings were decanted.

In order to study the influence of the ultrasound amplitude on the extraction efficiency, the ultrasonic extraction procedure was

Table 1 Operating conditions used in both the conventional and ultrasound accelerated Tessier sequential extraction procedures

Stage	Fraction	Reagent	CT/UT ^a conditions
1	Exchangeable	8 mL MgCl ₂ 1 mol L ⁻¹	1 h, 25 °C (pH = 7) or 3 min sonication time 50% amplitude (pH = 7)
2	Carbonate-bound	8 mL NaOAc 1 mol L ⁻¹	5 h, 25 °C (pH = 5) or 1 min sonication time 50% amplitude (pH = 5)
3	Fe-Mn oxides-bound (reducible)	20 mL NH ₂ OH · HCl 0.04 mol L ⁻¹ /HOAc 25% w/w	6 h, 96 °C or 7 min sonication time 50% amplitude
4	Organic matter-bound (oxidisable)	3 mL HNO ₃ 0.02 mol L ⁻¹ /5 mL H ₂ O ₂ 30% w/v + 3 mL H ₂ O ₂ 30% w/v + 5 mL NH ₄ OAc 3.2 mol L ⁻¹	2 h, 85 °C 3 h, 85 °C or 7 min sonication time 50% amplitude 30 min, 25 °C or 2 min sonication time 50% amplitude

^aCT: conventional treatment;
UT: ultrasound treatment

carried out using the optimised sonication time and operating under sonication amplitude values in the range 10–100%.

Metal determination in the extracts was carried out by FAAS under conditions described in detail elsewhere [12].

Results and discussion

Optimisation of sonication conditions

The results are reported in Figs. 1 and 2 (A–D). The recovery (in %) was calculated as the ratio between the metal extracted using the ultrasound accelerated Tessier method and the metal extracted applying the conventional method. It is important to emphasise that the aim of this work was to find ultrasound extraction conditions for each stage that provided a similar extraction capability as the original Tessier method with a shorter treatment time.

Influence of sonication time

Exchangeable metals. The only detectable metals in this fraction were Cu, Ni, Pb and Zn. With an ultrasonic treatment of 3 min the recoveries ranged from 96.84 to 100.0% for all the elements (Fig. 1 A). When the sonication time was increased, the extraction efficiency was considerably improved in the cases of Pb and Zn and slightly changed for Cu and Ni.

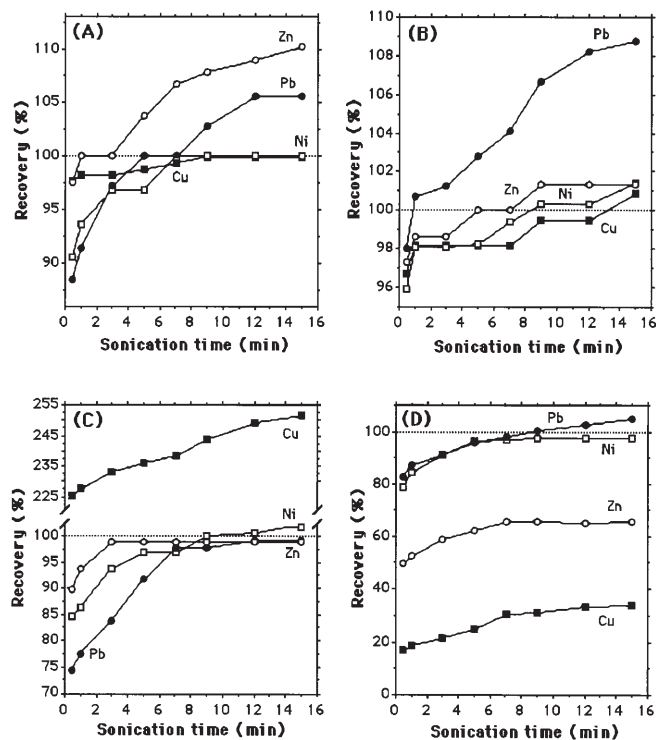


Fig. 1 A–D Influence of sonication time on metal recovery. First fraction (A); second fraction (B); third fraction (C); fourth fraction (D). Cu ■; Ni □; Pb ●; Zn ○. Metal recovery was calculated as the following ratio: [metal content using ultrasonic extraction / metal content using conventional treatment] × 100

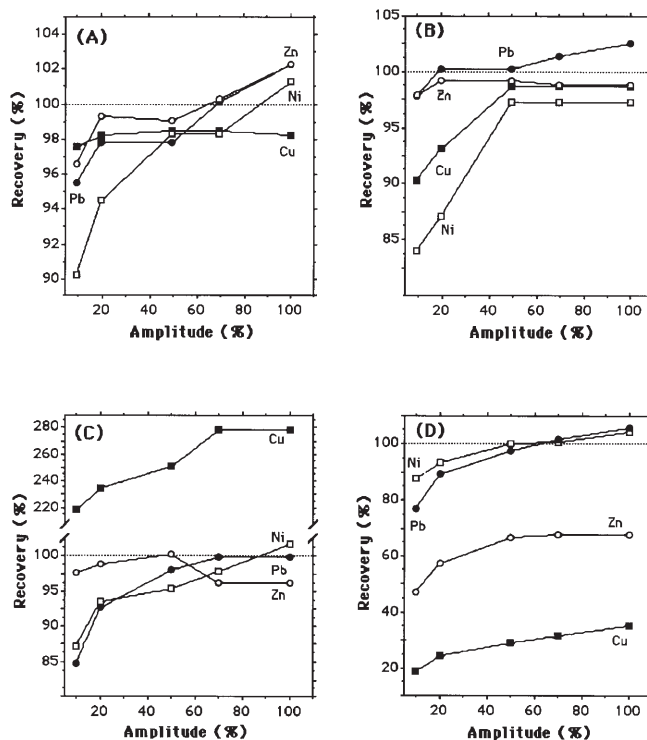


Fig. 2 A–D Influence of ultrasound amplitude on recovery. First fraction (A); second fraction (B); third fraction (C); fourth fraction (D). Cu ■; Ni □; Pb ●; Zn ○. Metal recovery was calculated as the following ratio: [metal content using ultrasonic extraction / metal content using conventional treatment] × 100

Carbonate bound metals. As above, the only elements detected by FAAS were Cu, Ni, Pb and Zn. With an ultrasonic treatment of only 1 min all the elements were quantitatively extracted, reaching recoveries in the range 98.11–100.7% (Fig. 1 B). The extraction efficiency of Pb increased when the sonication time was prolonged, as in the previous stage. However, the extraction efficiencies of Cu, Ni and Zn remained unchanged when the sonication time was changed from 3 to 15 min.

Fe-Mn oxides bound metals. All the elements, except Cr, could be quantified in this fraction. Extraction efficiencies were satisfactory for Ni, Pb and Zn (recoveries between 96.95% and 99.11%) with sonication times of 7 min. In contrast, Cu was strongly leached (recovery about 240%), as compared with the conventional Tessier method, operating under the previous sonication conditions. Cu recovery slightly increased with increasing sonication time (Fig. 1 C).

Organic matter bound metals. All studied elements could be detected by FAAS in this fraction. Recoveries of around 97% for Ni and 98% for Pb were obtained by applying ultrasonic extraction for 7 min. Cu and Zn were weakly leached in this fraction, the extraction efficiency being slightly improved for sonication times longer than 7 min (Fig. 1 D). Recovery for both elements was not satisfactory (around 65% for Zn and 30% for Cu) when the

ultrasound accelerated method was employed. In addition, Cr was not extracted at any studied ultrasonic shaking time.

We can conclude that with the use of an ultrasound amplitude of 50%, quantitative recoveries can be obtained for all the studied elements in the first and second fractions with a shaking time of 3 and 1 min, respectively. For the remaining fractions, the use of optimised sonication conditions (50% amplitude and 7 min of sonication time in both cases), provide satisfactory results for Ni, Pb and Zn in the third fraction and for Ni and Pb in the fourth fraction. Cu displays a different behaviour, since it was strongly released in the third fraction and scarcely leached in the fourth one. Moreover, Cr and Zn were also weakly leached in the fourth fraction as compared with the conventional Tessier method.

Influence of ultrasound amplitude

Once the sonication time was selected in each fraction, the effect of different amplitude values was evaluated. The results of this study are shown in Fig. 2 (A–D).

As can be observed in Figs. 2A and 2B corresponding to exchangeable and carbonate bound metals, respectively, recoveries higher than 95% can be obtained for all metals. In the third fraction (i.e. Fe-Mn oxide bound metals), recovery was satisfactory for Ni, Pb and Zn, but Cu was excessively leached (Fig. 2C). The recovery for Ni and Pb increased from 95 to 100% with increasing ultrasound amplitude. Zn extraction was successful, but recovery decreased slightly with increasing ultrasound amplitude. In the fourth fraction (i.e. organic matter bound metals), recovery increased with increasing ultrasound amplitude for all metals. In this last fraction, it was possible to achieve quantitative recovery only for Ni and Pb. It is thought that Zn was not sufficiently leached in the fourth stage, but the low recovery obtained for Cu may be due to the excessive leaching undergone by this metal in the third fraction, hence changing its partitioning pattern as compared with the conventional Tessier method.

Metal partitioning in a sewage sludge sample by both conventional and ultrasound accelerated Tessier sequential extraction methods

The experimental conditions corresponding to the conventional and ultrasound accelerated Tessier procedures are given in Table 1. Unlike the optimisation procedure described above, where previous fractions were removed by using heating and shaking conditions according to the conventional Tessier extraction method, before the ultrasound treatment was applied to the required fraction, for metal partitioning all fractions were successively obtained by ultrasound treatment.

The analytical results obtained using the two methods are listed in Table 2. Figure 3 shows the partitioning patterns corresponding to the conventional and ultrasound

Table 2 Analytical results obtained applying the conventional and ultrasound accelerated Tessier sequential extraction procedures in a sewage sludge sample

	Ultrasound method (X ± s) ^a	Conventional method (X ± s) ^a	Metal recovery ^b (%)
<i>Exchangeable</i>			
Cu	18.15 ± 0.12	18.38 ± 0.18	98.91
Cr	nd ^c	nd ^c	–
Ni	9.24 ± 0.23	9.51 ± 0.18	97.16
Pb	10.65 ± 0.26	10.91 ± 0.26	97.62
Zn	96.20 ± 3.68	96.66 ± 2.09	99.52
<i>Carbonate-bound</i>			
Cu	8.05 ± 0.12	8.16 ± 0.11	98.65
Cr	nd ^c	nd ^c	–
Ni	6.16 ± 0.25	6.35 ± 0.09	97.00
Pb	13.57 ± 0.19	13.67 ± 0.24	99.77
Zn	78.55 ± 1.64	79.98 ± 1.07	98.21
<i>Fe-Mn oxides bound</i>			
Cu	26.07 ± 0.27	10.29 ± 0.16	253.35
Cr	nd ^c	nd ^c	–
Ni	4.42 ± 0.14	4.58 ± 0.30	96.29
Pb	19.23 ± 0.16	19.71 ± 0.71	97.65
Zn	392.8 ± 3.27	396.6 ± 3.27	99.05
<i>Organic matter-bound</i>			
Cu	46.34 ± 0.43	165.4 ± 2.85	28.01
Cr	nd ^c	8.31 ± 0.23	0.00
Ni	5.97 ± 0.32	6.00 ± 0.12	99.50
Pb	15.61 ± 0.47	16.02 ± 0.46	97.44
Zn	58.42 ± 1.98	90.04 ± 1.98	64.88

^a Average of three determinations (expressed in µg g⁻¹) ± standard deviation

^b Metal recovery (%) was calculated as the ratio between extracted metal using ultrasound and extracted metal using conventional treatment

^c nd = not detected

accelerated Tessier methods. A statistical comparison ($P = 0.95$) of the results, for each element and fraction, allowed us to ensure that no significant differences were found between them, except for Cu in the third and fourth fractions and for Cr and Zn in the final fraction.

As can be seen in Table 2, the ultrasonic extraction method provided quantitative recoveries for all metals in the two first fractions, for Ni, Pb and Zn in the third fraction and for Ni and Pb in the fourth fraction. The overall quantitative recoveries attained ranged from 96.29% to 99.77%. Cu was highly leached in the reducible fraction, probably due to the fact that this element was weakly extracted in the oxidisable fraction (recovery about 28%). In addition, low recoveries were also found for Cr (not detected) and Zn (recovery about 64%) in the oxidisable fraction. This common problem may be attributed to a poor extraction capability of the oxidant reagent (H₂O₂) when the ultrasonic treatment was used as compared with the conventional treatment in the original Tessier method.

The behaviour of Cu in the third and fourth fractions was also observed previously [13] when the Tessier pro-

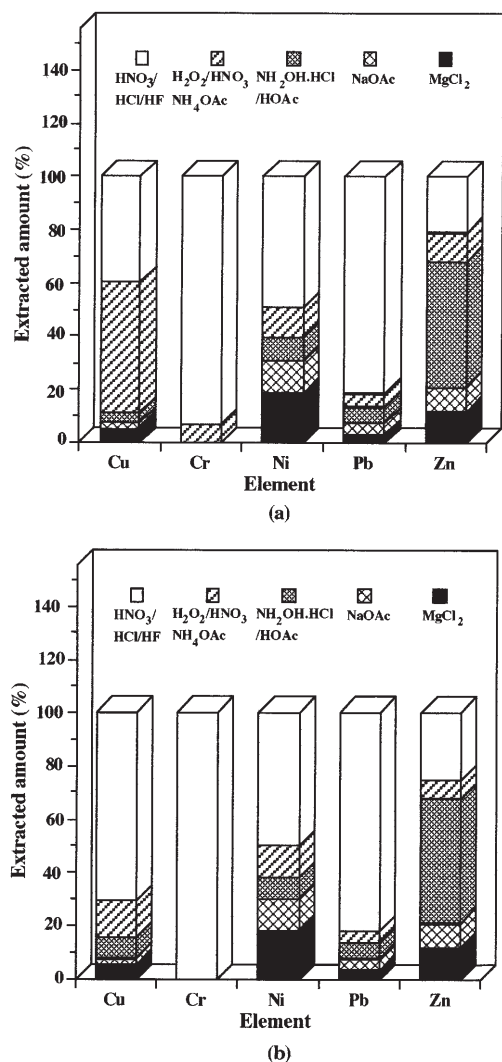


Fig.3 a, b Partitioning patterns corresponding to the conventional (a) and the ultrasound accelerated (b) Tessier sequential extraction methods

cedure was accelerated by means of microwave energy for metal partitioning in a sediment sample. In addition, other elements (e.g. Fe and Mn) were also scarcely leached in the fourth fraction, like Cr and Zn in the present work. Another study [20] reported that Cu and Zn were not quantitatively leached from biological tissues by means of ultrasonic probe extraction using the $\text{H}_2\text{O}_2/\text{H}_2\text{SO}_4$ mixture as extractant solution.

Total extractable metal contents obtained by the two extraction methods for the sewage sludge sample are summarised in Table 3. Good agreement can be observed for Ni, Pb and Zn (overall recoveries from 94.38% to 97.93%), however, in the case of Cu and Cr the extraction efficiency of the conventional procedure was considerably higher than that obtained using the accelerated method. On the other hand, both methods show similar precision, since the relative standard deviation (RSD) corresponding to between-batch measurements was always lower than 5%, except for Ni determination in the third (conventional

Table 3 Total metal content and total extractable metal contents obtained in a sludge sample using the conventional and the ultrasound accelerated metal partitioning schemes

	Total metal content Microwave digestion ($\mu\text{g g}^{-1}$)	Total extractable metals		Recovery (%)
		Ultrasound ^a ($\mu\text{g g}^{-1}$)	Conventional ^a ($\mu\text{g g}^{-1}$)	
Cu	335.63 ± 2.33	98.61	202.21	48.77
Cr	122.14 ± 8.50	nd ^b	8.31	–
Ni	51.61 ± 3.10	25.79	26.45	97.50
Pb	322.87 ± 5.29	59.06	60.31	97.93
Zn	840.98 ± 21.87	625.97	663.25	94.38

^aSum of metals extracted in the four fractions (in $\mu\text{g g}^{-1}$)

^bnd = not detected

method) and fourth (ultrasound accelerated method) fractions with RSD's of 6.65% and 5.39%, respectively.

The distribution of heavy metals in the sample allow us to predict their mobility when the sludge is discharged to the environment. As can be seen in Table 2 and Fig. 3, Zn is the most mobilisable element since it is mainly distributed (about 79% of the total content) among the non-residual fractions, being mainly bound to Fe-Mn oxides. A great amount of Cu (40% of the total content) was found in the residual fraction, and the non-residual element was mostly located in the oxidisable fraction. Like Cu, around 50% of the total Ni content is associated with the residual fraction. The rest of this element is distributed among the extractable stages, particularly associated with exchangeable and carbonate fractions, which could be easily leached under usual environmental conditions. Cr and Pb can be considered almost immobile, given that elevated percentages of these elements (93% of total Cr and 81% of total Pb) are located in the residual fraction.

In order to evaluate the residual fraction, the total metal content in the sewage sludge sample was determined using the microwave digestion procedure optimised elsewhere [24], and the results are given in Table 3. In all cases the results are expressed as mean values ($\mu\text{g g}^{-1}$) \pm standard deviation of three separate sample preparations.

Conclusions

Conventional and ultrasound accelerated Tessier sequential extraction procedures provide similar fractionations for Ni and Pb in sewage sludge. For Zn, although significant differences are found in the oxidisable fraction, the two methods provide identical information about the environmental mobility of this element. For Cu, the extraction capability achieved with the proposed method was close to that obtained with the conventional one in the two first fractions, but a different partitioning pattern was observed in the latter two. The worst case corresponds to Cr, which was not extracted at all in the oxidisable fraction when the accelerated procedure was used.

When the two procedures are compared, the following conclusions can be drawn: i) when conventional shaking and heating are replaced by the ultrasound extraction method, the overall treatment time is considerably reduced; ii) the most mobilisable fractions (i.e. first and second fractions) can be correctly evaluated applying the suggested sonication procedure during a short time of only 4 min; iii) when applying ultrasonic extraction in the third and fourth fractions, the experimental task is considerably simplified because tedious heating is not necessary; iv) whereas in the conventional procedure several samples can be simultaneously processed, in the proposed method each sample is individually treated; however, the complete extraction of a particular sample (in triplicate) using the ultrasound procedure requires an operation time of only 1 h.

Finally, the partitioning pattern corresponding to this sample confirms that Cr and Pb are mainly associated with the oxidisable and residual fractions and, consequently, a low mobility is expected for these two elements.

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References

- Smith SR (1996) *Agricultural Recycling of Sewage Sludge and the Environment*, Cab International
- Rauret G, Rubio R, López-Sánchez JF, Casassas E (1989) *Int J Environ Anal Chem* 35: 89–100
- Ure AM, Davidson CM (1995) *Chemical Speciation in the Environment*, Blackie, Glasgow
- Batley GE (1991) *Trace Element Speciation: Analytical Methods and Problems*, 3rd edn, CRC Press, Boca Raton, Florida
- Tessier A, Campbell PGC, Bisson M (1979) *Anal Chem* 51: 844–851
- McGrath D (1996) *Sci Total Environ* 178: 37–44
- Li X, Coles BJ, Ramsey MH, Thornton I (1995) *Analyst* 120: 1415–1419
- Krishnamurti GSR, Huang PM, Van Rees KCJ, Kozak LM, Rostad HPW (1995) *Analyst* 120: 659–665
- Raksasataya M, Langdon AG, Kim ND (1996) *Anal Chim Acta* 332: 1–14
- López-Sánchez JF, Rubio R, Rauret G (1993) *Int J Environ Anal Chem* 51: 113–121
- Accomasso GM, Zelano V, Daniele PG, Gastaldi D, Ginepro M, Ostacoli G (1993) *Spectrochim Acta Part A* 49A: 1205–1212
- Perez-Cid B, Lavilla I, Bendicho C (1996) *Analyst* 121: 1479–1484
- Gulmini M, Ostacoli G, Zelano V, Torazzo A (1994) *Analyst* 119: 2075–2080
- Mahan KI, Foderaro TA, Garza TL, Martinez RM, Maroney GA, Trevisonno MR, Willging EM (1987) *Anal Chem* 59: 938–945
- Ginepro M, Gulmini M, Ostacoli G, Zelano V (1996) *Int J Environ Anal Chem* 63: 147–152
- Tack FMG, Vossius HAH, Verloo MG (1996) *Int J Environ Anal Chem* 63: 61–66
- Luque de Castro MD, da Silva MP (1997) *Trends Anal Chem* 16: 16–24
- Majors RE (1996) *LC-GC* 14: 88, 90, 93, 96
- Sánchez J, García R, Millán E (1994) *Analisis* 22: 222–225
- Mamba S, Kratochvil B (1995) *Int J Environ Anal Chem* 60: 295–302
- Klemm W, Bombach G (1995) *Fresenius J Anal Chem* 353:12–15
- Lavilla I, Perez-Cid B, Bendicho C (1998) *Int J Environ Anal Chem* 67: 1–11
- Perez-Cid B, Lavilla I, Bendicho C (1998) *Anal Chim Acta* 360: 35–41
- Lavilla I, Perez-Cid B, Bendicho C (1998) *Fresenius J Anal Chem* 361: 164–167