

# Metal fractionation in olive oil and urban sewage sludges using the three-stage BCR sequential extraction method and microwave single extractions

B. Pérez Cid,\* A. Fernández Alborés, E. Fernández Gómez and E. Falqué López

Departamento de Química Analítica y Alimentaria, Universidad de Vigo, Facultad de Ciencias, As Lagoas s/n, 32004 Ourense, Spain. E-mail: benita@uvigo.es;  
Fax: +34-986-812382; Tel: +34-986-814083

Received 26th March 2001, Accepted 1st June 2001  
First published as an Advance Article on the web 27th July 2001

The conventional three-stage BCR sequential extraction method was employed for the fractionation of heavy metals in sewage sludge samples from an urban wastewater treatment plant and from an olive oil factory. The results obtained for Cu, Cr, Ni, Pb and Zn in these samples were compared with those attained by a simplified extraction procedure based on microwave single extractions and using the same reagents as employed in each individual BCR fraction. The microwave operating conditions in the single extractions (heating time and power) were optimized for all the metals studied in order to achieve an extraction efficiency similar to that of the conventional BCR procedure. The measurement of metals in the extracts was carried out by flame atomic absorption spectrometry. The results obtained in the first and third fractions by the proposed procedure were, for all metals, in good agreement with those obtained using the BCR sequential method. Although in the reducible fraction the extraction efficiency of the accelerated procedure was inferior to that of the conventional method, the overall metals leached by both microwave single and sequential extractions were basically the same (recoveries between 90.09 and 103.7%), except for Zn in urban sewage sludges where an extraction efficiency of 87% was achieved. Chemometric analysis showed a good correlation between the results given by the two extraction methodologies compared. The application of the proposed approach to a certified reference material (CRM-601) also provided satisfactory results in the first and third fractions, as it was observed for the sludge samples analysed.

## Introduction

Heavy metals are common pollutants in environmental samples, such as solid wastes, sewage sludges, industrial soils and contaminated sediments. These elements are usually associated with the principal components of the samples (carbonates, organic matter, Fe–Mn oxides and minerals) and they are available to living organisms when they are dissolved in environmental materials.

Determination of the total metal content in these samples does not provide sufficient criteria for estimating their ecological and biological effects. Chemical fractionation using sequential extraction methods is particularly useful in this way,<sup>1,2</sup> but their application is still subject to some limitations and is controversial.<sup>3–5</sup> In order to harmonize and validate various fractionation schemes found in the literature, the Community Bureau of Reference (BCR), now called the Standards, Measurements and Testing Programme (M&T), developed a three-stage sequential extraction method,<sup>6</sup> which has been widely accepted for metal partitioning in soil<sup>7–9</sup> and sediment samples.<sup>9–13</sup>

The BCR method, although apparently faster and simpler (consisting of only three stages) than other previous methods (Tessier *et al.*'s method<sup>14</sup> or its modifications<sup>15</sup>), the overall operation time is over twice as long. This means that it could be considered as a time-consuming procedure, excessively tedious for routine analysis. This problem could be partially solved by leaching the sequential fractions in single extractions, using operating conditions similar to those in the conventional methods and employing an individual subsample for each reagent. This experimental approach was proposed by Tack

*et al.*<sup>16</sup> for the Tessier method and our previous work on the application of the Tessier and BCR methods to sewage sludge samples gave satisfactory results with both simplified approaches.<sup>17</sup> Subsequently, the use of microwave energy was introduced in our recent application<sup>18</sup> in order to replace the conventional treatment, and consequently to reduce the long operational time of the sequential procedure. In this case, it is important to emphasize that, for most of the elements studied, good agreement was found between the results obtained from the conventional and the accelerated procedures and, in our opinion, the latter should be tested with the BCR method, given that no work on this aspect was found in the literature.

The main purpose of this work was to compare, for sewage sludge samples, the results obtained using the sequential BCR method and those estimated from microwave single extractions but employing the same reagents as in the BCR extractions. In this case, the conventional treatment (magnetic shaking and conventional heating) was replaced by microwave heating and the operating parameters (heating time and power) were optimized for Cu, Cr, Ni, Pb and Zn in each stage. Flame atomic absorption spectrometry (FAAS) was used for the measurement of metals in the extracts. The results obtained by both extraction procedures (conventional and microwave single extractions) were compared for sewage sludge samples from an urban wastewater treatment plant and a domestic olive oil factory. A Certified Reference Material (CRM-601) with extractable contents of various metals in the three fractions of the BCR method<sup>19</sup> was used to validate the proposed procedure. Finally, the results obtained for the samples studied by both applied methodologies were analysed by principal component analysis in order to display the data trends and corroborate the similarity between the compared extraction procedures.

## Experimental

### Instrumentation

A Perkin-Elmer atomic absorption spectrometer (Model 2380) was used for metal determinations. Hollow cathode lamps (Cathodeon) were used as the radiation source. The resonance lines employed were 324.8, 357.9, 230.0, 217.0 and 213.9 nm for Cu, Cr, Ni, Pb and Zn, respectively. The lamp intensity and slit width were used according to the manufacturer's recommendations. The air-acetylene flow rate was 11–1 dm<sup>3</sup> min<sup>-1</sup> for all the elements except Cr, where a more reducing flame was needed (11–2 dm<sup>3</sup> min<sup>-1</sup>). A domestic microwave oven (Moulinex, 900 W power) was used for microwave heating. A domestic Nevir cutter was used for particle size reduction. A centrifuge (Kubota Model 5100) was used for complete separation of the extracts. A Crison pH meter was employed for pH adjustments of the reagents.

### Reagents

The extractant solutions for the BCR method were prepared from analytical-reagent grade reagents (Merck). Stock standard solutions of metals (1000 µg cm<sup>-3</sup>) were obtained by dissolving the pure metal (in the case of Cu and Zn) or the appropriate salts (in the case of Pb, Ni and Cr) and diluting to the appropriate volume with ultra-pure water. Standard calibration solutions were prepared daily by dilution of the stock standard solutions. Hydroxylammonium chloride solution was prepared just prior to use. A Certified Reference Material (CRM-601) from the BCR was used to validate the microwave single extractions proposed in this work.

### Sample collection and pre-treatment

Urban sewage sludge (USS) was collected in polyethylene containers from a wastewater treatment plant next to the town of Ourense (Spain). Olive oil sludge (OOS) was collected in a similar way from an olive oil factory located in the province of Jaén (Spain). In the laboratory, the samples were dried to constant weight at 110 °C in a heater.<sup>13,17,20,21</sup> Subsequently, they were ground with a Nevir cutter since it was proved in previous work<sup>17</sup> that the metallic blades of the cutter do not affect the extractable metal contents in the sample. The samples were then sieved using a nylon fibre sieve and the sludge with

particle size < 70 µm was separated, homogenized and stored in polyethylene vessels at room temperature in a desiccator.

### Extraction procedures

The BCR sequential extraction method was applied, in triplicate, to 2 g of pre-treated sewage sludge (< 70 µm), employing the reagents and experimental conditions shown in Table 1. The extraction was carried out in polyethylene tubes (50 cm<sup>3</sup> capacity) also employed for centrifugation in order to minimize the possible loss of sample in the successive extraction steps of the sequential procedure. The extracts were separated from the solid phase by centrifugation at 2500 rpm for 5 min and decanted into polyethylene vessels and stored at 4 °C before analysis. The remaining residue was washed by shaking for 15 min, with 10 cm<sup>3</sup> of ultra-pure water and the washings were discarded after centrifugation.

The microwave single extractions were carried out on 2 g of pre-treated sample, but using a separate subsample for each individual reagent. In this case, the weighed sample was also placed in 50 cm<sup>3</sup> polyethylene tubes and the corresponding extracting agents were added. The extraction was performed using the most favourable extraction conditions for each fraction, which are given in Table 1.

The results for the acid soluble fraction were directly displayed because it is the first fraction in the BCR method. In contrast, metals bound to reducible and oxidizable fractions were calculated by subtracting the amount of metals leached in the acid soluble and reducible fractions (stages 1 and 2) from those leached in the reducible and oxidizable fractions (stages 2 and 3), respectively. Moreover, as shown in Table 1, the oxidizable fraction was leached using a simplified procedure consisting of the elimination of an intermediate evaporation step required in the sequential procedure. This extraction procedure is also explained in the scheme in Fig. 1.

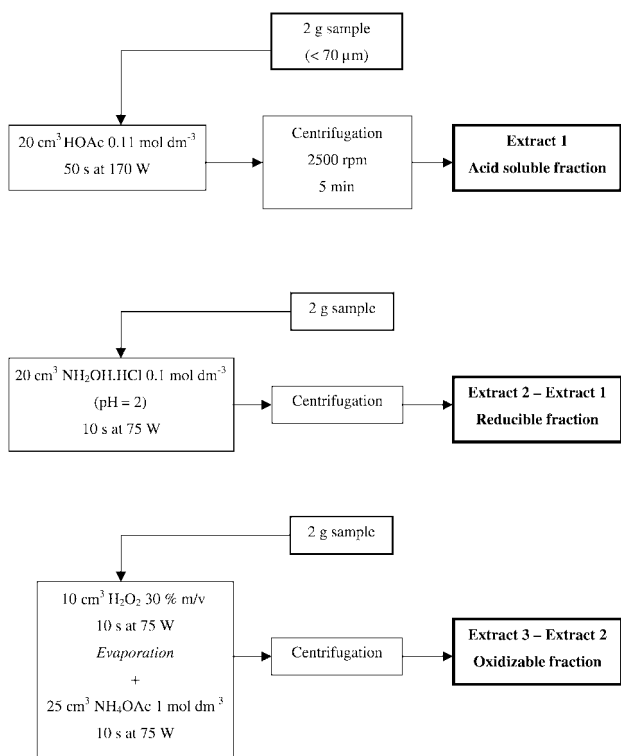
In all cases, the percentage recovery (or relative extraction) corresponding to each fraction was calculated as [metal leached using the microwave single extraction/metal leached using the conventional BCR method] × 100.

### Statistical analysis

Statistica<sup>22</sup> was used to carry out the principal component analysis (PCA) of the results obtained by the conventional BCR

**Table 1** Operating conditions required in the conventional BCR sequential extraction method and microwave single extractions

| Stage                                | Fraction                          | Reagent   | Experimental conditions                      |
|--------------------------------------|-----------------------------------|---|--|
| <i>BCR Method—</i>                   |                                   |   |  |
| 1                                    | Acid soluble (e.g., carbonates)   | 20 cm <sup>3</sup> HOAc 0.11 mol dm <sup>-3</sup>   | 16 h at 25 °C                                |
| 2                                    | Reducible (e.g., Fe–Mn oxides)    | 20 cm <sup>3</sup> NH <sub>2</sub> OH·HCl 0.1 mol dm <sup>-3</sup> (pH 2)   | 16 h at 25 °C                                |
| 3                                    | Oxidizable (e.g., organic matter) | 5 cm <sup>3</sup> H <sub>2</sub> O <sub>2</sub> 30 % m/v<br>(evaporation)<br>+<br>5 cm <sup>3</sup> H <sub>2</sub> O <sub>2</sub> 30% m/v<br>(evaporation)<br>+ | 1 h at 25 °C<br>1 h at 85 °C<br>1 h at 85 °C |
| 4                                    | Residual                          | 25 cm <sup>3</sup> NH <sub>4</sub> OAc 1 mol dm <sup>-3</sup><br>HNO <sub>3</sub> –HCl–HF   | 16 h at 25 °C<br>26 min                      |
| <i>Microwave single extractions—</i> |                                   |   |  |
| 1                                    | Acid soluble (e.g., carbonates)   | 20 cm <sup>3</sup> HOAc 0.11 mol dm <sup>-3</sup>   | 50 s at 170 W                                |
| 2                                    | Reducible (e.g., Fe–Mn oxides)    | 20 cm <sup>3</sup> NH <sub>2</sub> OH·HCl 0.1 mol dm <sup>-3</sup> (pH 2)   | 10 s at 75 W                                 |
| 3                                    | Oxidizable (e.g., organic matter) | 10 cm <sup>3</sup> H <sub>2</sub> O <sub>2</sub> 30% m/v<br>(evaporation)<br>+  | 10 s at 75 W                                 |
| 4                                    | Residual                          | 25 cm <sup>3</sup> NH <sub>4</sub> OAc 1 mol dm <sup>-3</sup><br>HNO <sub>3</sub> –HCl–HF   | 10 s at 75 W<br>26 min                       |



**Fig. 1** Schematic diagram of the procedure for microwave single extractions using BCR reagents.

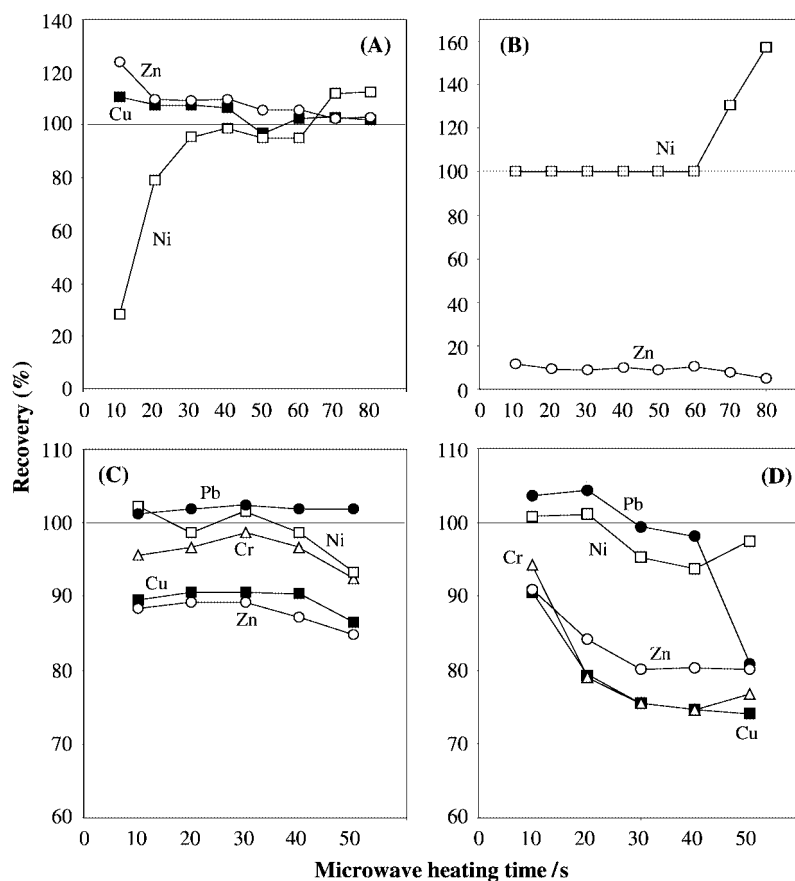
method and the microwave single extractions from the sewage sludge samples analysed.

## Results and discussion

### Optimization of microwave heating time and power

The influence of the microwave heating time on the extraction efficiency was evaluated by comparing the results obtained using the conventional procedure with those obtained using the microwave single extractions. The latter procedure was applied at 75 W of power and for 10–80 s for the two first fractions and 10–50 s for the third fraction. The results of this study are shown in Fig. 2.

According to Fig. 2, the proposed extraction procedure provides, for all the elements studied in the first fraction [Fig. 2(A)], similar results to the conventional BCR method when using heating times between 50 and 60 s. In this case, the recoveries obtained ranged from 95 to 105%. In the second fraction [Fig. 2(B)], Cr and Pb did not appear at quantifiable levels and Ni was leached according to the conventional procedure (recovery 100%) using heating times between 10 and 60 s. In contrast, Cu and Zn were poorly leached in this fraction using the proposed approach. In fact, the extraction efficiency of Zn was too low (around 9%) and even a negative recovery was found for Cu [this element is not represented in [Fig. 2(B)]. This means that the extraction efficiency of these metals in the reducible fraction was considerably reduced when the microwave single extraction was employed. Particularly in the case of Cu the extraction of the reducible fraction was even decreased



**Fig. 2** Influence of microwave heating time on the extraction efficiency. (A) first stage; (B) second stage; (C) third stage (procedure I); (D) third modified stage (procedure II).

when the microwave heating time was prolonged. This could be attributed to readsorption and redistribution of metals among phases during the extraction,<sup>23–26</sup> which was enhanced when the microwave treatment was extended. This same effect was also found for Cu in other work<sup>27,28</sup> where the conventional treatment was replaced by microwave heating.

The third fraction was extracted using two different methodologies. Procedure I corresponds to Fig. 2(C) and was carried out in a similar way to the conventional method, but replacing the heating and shaking systems. Procedure II corresponds to Fig. 2(D) and it could be considered as a simplified approach of the first procedure, where the treatment with H<sub>2</sub>O<sub>2</sub> takes place in a unique step with the consequent elimination of an intermediate evaporation phase. The microwave heating times employed in each step of the procedure I ranged from 5 to 25 s, whereas in procedure II the overall heating times varied between 10 and 50 s. The final treatment with NH<sub>4</sub>OAc was fixed at 10 s of microwave heating since longer treatment times caused excessive foam in the extract with the consequent risk of sample losses.

As can be seen in Fig. 2(C) and (D), the results obtained by both extraction procedures I and II were very similar for all metals when using the shortest microwave heating time (*i.e.*, 10 s). In this case, the recoveries obtained ranged from 88 to 103% for all the elements studied. For heating times longer than 10 s, Ni and Pb show a similar behaviour in both extraction methodologies. In contrast, the extraction efficiency of Cu, Cr and Zn was lower when the simplified procedure was used [Fig. 2(D)]. According to the above, the application of procedure II during 10 s provides satisfactory results for the oxidizable fraction.

As a general conclusion, it is possible to say that using the proposed microwave single extraction at 75 W for 50 s in the first fraction and for 10 s in the second and third fractions, quantitative recoveries can be obtained for all the elements studied, except for Cu and Zn in the reducible fraction where they were poorly leached.

Once the most favourable heating times had been selected in each stage, the influence of the microwave power was evaluated in order to enhance the relative extraction of metals in those cases where this is required. The power values studied ranged from 75 to 170 W; values higher than 170 W were not used in order to avoid boiling of the solution.

The results of this study are shown in Fig. 3(A)–(C). As can be seen, variation of the power within the considered interval hardly affected the extraction efficiency of the metals in the three fractions studied. This means that the extraction efficiency of Cu and Zn in the second fraction was not improved when the microwave power was increased from 75 to 170 W. Hence the most favourable microwave extraction conditions were achieved when using 75 W of power in the reducible [Fig. 3(B)] and oxidizable [Fig. 3(C)] fractions. In contrast, in the acid soluble fraction [Fig. 3(A)] the application of 170 W power provides a slight increase (around 8%) in the extraction efficiency of Ni and it was employed in subsequent work.

### Analysis of sewage sludges

The conventional BCR method and the microwave single extractions proposed in this work were both applied, in triplicate, for metal fractionation in two urban sewage sludges

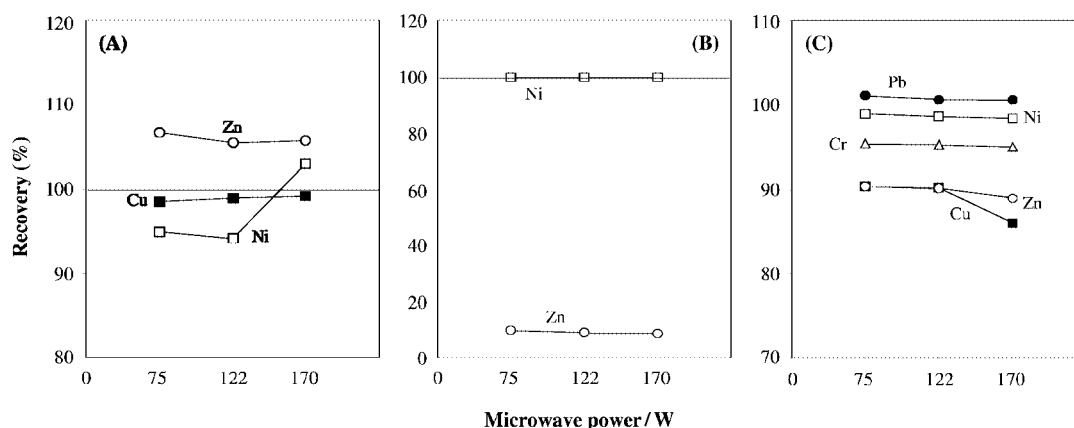


Fig. 3 Influence of microwave power on the extraction efficiency. (A) first stage; (B) second stage; (C) third stage.

Table 2 Analytical results obtained for urban sewage sludges (expressed in mg kg<sup>-1</sup>) using the conventional BCR method and microwave single extractions

|                  | Acid soluble fraction |                             |              | Reducible fraction    |                             |                | Oxidizable fraction   |                             |              |
|------------------|-----------------------|-----------------------------|--------------|-----------------------|-----------------------------|----------------|-----------------------|-----------------------------|--------------|
|                  | Sequential extraction | Microwave single extraction | Recovery (%) | Sequential extraction | Microwave single extraction | Recovery (%)   | Sequential extraction | Microwave single extraction | Recovery (%) |
| <i>Sample A—</i> |                       |                             |              |                       |                             |                |                       |                             |              |
| Cu               | 23.70 ± 0.49          | 23.27 ± 0.65                | 98.20        | nd <sup>a</sup>       | -4.06 ± 0.31                | — <sup>b</sup> | 275.5 ± 7.3           | 250.8 ± 6.1                 | 91.08        |
| Cr               | nd                    | nd                          | —            | nd                    | nd                          | —              | 9.98 ± 0.25           | 9.43 ± 0.19                 | 94.49        |
| Ni               | 3.56 ± 0.21           | 3.51 ± 0.13                 | 98.59        | nd                    | 0.02 ± 0.11                 | —              | 8.66 ± 0.51           | 8.40 ± 0.67                 | 96.99        |
| Pb               | nd                    | nd                          | —            | nd                    | nd                          | —              | 5.29 ± 0.42           | 5.24 ± 0.38                 | 99.05        |
| Zn               | 50.05 ± 1.97          | 52.38 ± 2.11                | 104.6        | 69.84 ± 3.01          | 8.72 ± 0.41                 | 12.48          | 571.6 ± 12.2          | 541.8 ± 14.6                | 94.27        |
| <i>Sample B—</i> |                       |                             |              |                       |                             |                |                       |                             |              |
| Cu               | 25.77 ± 0.32          | 25.70 ± 0.19                | 99.73        | nd                    | -5.89 ± 0.55                | — <sup>b</sup> | 366.8 ± 8.74          | 336.9 ± 4.9                 | 91.86        |
| Cr               | nd                    | nd                          | —            | nd                    | nd                          | —              | 9.38 ± 0.74           | 8.95 ± 0.17                 | 95.42        |
| Ni               | 2.90 ± 0.21           | 3.04 ± 0.01                 | 104.8        | nd                    | 0.04 ± 0.13                 | —              | 7.86 ± 0.40           | 7.85 ± 0.42                 | 99.87        |
| Pb               | nd                    | nd                          | —            | nd                    | nd                          | —              | 1.62 ± 0.13           | 1.68 ± 0.15                 | 103.7        |
| Zn               | 40.33 ± 1.04          | 42.74 ± 1.66                | 105.9        | 47.43 ± 1.17          | 4.61 ± 0.29                 | 9.72           | 617.5 ± 14.9          | 560.5 ± 13.7                | 90.77        |

<sup>a</sup> Not determined by FAAS. <sup>b</sup> Negative recovery value was obtained.

(USS) and two olive oil sludges (OOS), which could be considered as typical samples with high and low metal contents, respectively. The results obtained for Cu, Cr, Ni, Pb and Zn in these samples are reported in Table 2 for USS and Table 3 for OOS and they allow us to compare the behaviour of the samples studied.

As can be observed, the results obtained from microwave single extractions in the first and third fractions were, for all metals, in good agreement with those obtained using the sequential procedure. The recoveries achieved in these fractions ranged from 90.77 to 105.9% in the two studied USS (Table 2) and from 91.36 to 102.9% in the OOS samples (Table 3).

In the second fraction, Cr, Ni and Pb were not extracted at quantifiable levels from any of the samples studied, which could be attributed to a low extraction efficiency of the BCR method in relation to other sequential extraction procedures such as those of Tessier *et al.*<sup>17,20,29</sup> and Förstner *et al.*<sup>10</sup> As expected, Cu and Zn were hardly leached in the samples studied when the proposed approach was employed, except in OOS sample B where they were not extracted at a detectable level (Table 3). The recoveries were always lower than 13% in the case of Zn and even negative values were found for Cu.

In Table 4 are shown the total extractable metals (sum of the three extractable fractions) obtained in the sewage sludges analysed by the conventional BCR method and the microwave single extractions proposed in this work. The results indicate that, although some significant differences were found in the

metal fractionation of the two extraction methods, the overall amounts of metals extracted were in good agreement in most cases. The total amounts of metals leached with the two methods were similar for all the elements studied in the OOS (recoveries between 90.09 and 93.72%) and for Cu, Cr, Ni and Pb in the USS (recoveries between 90.22 and 103.7%). In contrast, Zn was more extracted in the USS when using the conventional procedure, although the extraction efficiency achieved for this metal (around 87%) was also considerably elevated and close to 90%. This means that the overall environmental information provided by the two fractionation methodologies could be considered to be basically the same, and consequently the proposed method could be used as a screening methodology to evaluate the potential mobility and bioavailability of heavy metals in the environment. However, it is necessary to apply the conventional sequential extraction procedure when complete information about the metal fractionation in the samples is required.

A certified reference material (CRM-601) was used in order to validate the extraction efficiency of the proposed microwave single extractions. This is a sediment sample with certified extractable contents of Cd, Cr, Ni, Pb and Zn in the three fractions of the BCR sequential extraction method and the results of this study for the elements determined in this work (Cr, Ni, Pb and Zn) are given in Table 5. As can be seen, the results obtained by the accelerated procedure in the first and third fractions were in good agreement with the certified values

**Table 3** Analytical results obtained for olive oil sludges (expressed in mg kg<sup>-1</sup>) using the conventional BCR method and microwave single extractions

|                  | Acid soluble fraction |                             |              | Reducible fraction    |                             |                | Oxidizable fraction   |                             |              |
|------------------|-----------------------|-----------------------------|--------------|-----------------------|-----------------------------|----------------|-----------------------|-----------------------------|--------------|
|                  | Sequential extraction | Microwave single extraction | Recovery (%) | Sequential extraction | Microwave single extraction | Recovery (%)   | Sequential extraction | Microwave single extraction | Recovery (%) |
| <i>Sample A—</i> |                       |                             |              |                       |                             |                |                       |                             |              |
| Cu               | 4.37 ± 0.01           | 4.05 ± 0.01                 | 92.68        | 3.11 ± 0.01           | -0.77 ± 0.06                | — <sup>b</sup> | 27.35 ± 0.04          | 28.10 ± 0.20                | 102.7        |
| Cr               | nd <sup>a</sup>       | nd                          | —            | nd                    | nd                          | —              | nd                    | nd                          | —            |
| Ni               | nd                    | nd                          | —            | nd                    | nd                          | —              | 3.77 ± 0.01           | 3.53 ± 0.21                 | 93.63        |
| Pb               | nd                    | nd                          | —            | nd                    | nd                          | —              | nd                    | nd                          | —            |
| Zn               | 6.79 ± 0.10           | 6.99 ± 0.08                 | 102.9        | 1.51 ± 0.00           | -1.16 ± 0.09                | — <sup>b</sup> | 24.12 ± 0.51          | 24.55 ± 1.09                | 101.8        |
| <i>Sample B—</i> |                       |                             |              |                       |                             |                |                       |                             |              |
| Cu               | 0.39 ± 0.01           | 0.39 ± 0.02                 | 100.0        | nd                    | nd                          | —              | 28.72 ± 0.22          | 26.24 ± 1.84                | 91.36        |
| Cr               | nd                    | nd                          | —            | nd                    | nd                          | —              | nd                    | nd                          | —            |
| Ni               | nd                    | nd                          | —            | nd                    | nd                          | —              | 12.60 ± 0.36          | 11.71 ± 1.09                | 92.94        |
| Pb               | nd                    | nd                          | —            | nd                    | nd                          | —              | nd                    | nd                          | —            |
| Zn               | 1.76 ± 0.03           | 1.68 ± 0.09                 | 95.45        | nd                    | nd                          | —              | 39.62 ± 0.99          | 37.10 ± 1.51                | 93.64        |

<sup>a</sup> Not determined by FAAS. <sup>b</sup> Negative recovery value was obtained.

**Table 4** Total extractable metal contents obtained in the USS and OOS by the conventional BCR method and using microwave single extractions

|                  | USS/mg kg <sup>-1</sup>            |  |                           | OOS/mg kg <sup>-1</sup>            |  |                           |
|------------------|------------------------------------|--|---------------------------|------------------------------------|--|---------------------------|
|                  | Sequential extraction <sup>a</sup> | Microwave single extraction <sup>b</sup> | Recovery (%) <sup>c</sup> | Sequential extraction <sup>a</sup> | Microwave single extraction <sup>b</sup> | Recovery (%) <sup>c</sup> |
| <i>Sample A—</i> |                                    |  |                           |                                    |  |                           |
| Cu               | 299.2 ± 7.3                        | 269.9 ± 6.2                              | 90.22                     | 34.83 ± 0.04                       | 31.38 ± 0.21                             | 90.09                     |
| Cr               | 9.98 ± 0.25                        | 9.43 ± 0.19                              | 94.49                     | nd <sup>d</sup>                    | nd                                       | —                         |
| Ni               | 12.22 ± 0.55                       | 11.93 ± 0.69                             | 97.63                     | 3.77 ± 0.01                        | 3.53 ± 0.21                              | 93.63                     |
| Pb               | 5.29 ± 0.42                        | 5.24 ± 0.38                              | 99.05                     | nd                                 | nd                                       | —                         |
| Zn               | 691.4 ± 12.7                       | 602.9 ± 14.8                             | 87.20                     | 32.42 ± 0.52                       | 30.38 ± 1.09                             | 93.71                     |
| <i>Sample B—</i> |                                    |  |                           |                                    |  |                           |
| Cu               | 392.6 ± 8.7                        | 356.8 ± 5.0                              | 90.88                     | 29.11 ± 0.22                       | 26.63 ± 1.84                             | 91.48                     |
| Cr               | 9.38 ± 0.74                        | 8.95 ± 0.17                              | 95.42                     | nd                                 | nd                                       | —                         |
| Ni               | 10.76 ± 0.45                       | 10.93 ± 0.48                             | 101.2                     | 12.60 ± 0.36                       | 11.71 ± 1.09                             | 92.94                     |
| Pb               | 1.62 ± 0.13                        | 1.68 ± 0.15                              | 103.7                     | nd                                 | nd                                       | —                         |
| Zn               | 705.3 ± 15.0                       | 607.8 ± 13.8                             | 86.19                     | 41.38 ± 0.99                       | 38.78 ± 1.51                             | 93.72                     |

<sup>a</sup> Sum of metals extracted in the three stages of the conventional BCR method (expressed in mg kg<sup>-1</sup>). <sup>b</sup> Sum of metals extracted in the three stages of the BCR method using microwave single extractions (expressed in mg kg<sup>-1</sup>). <sup>c</sup> Recovery is calculated as [total metal content extracted by the proposed microwave single extractions/ total metal content extracted by the sequential procedure] × 100. <sup>d</sup> Not determined.

(recoveries between 94 and 105%). Moreover, they were compared statistically and no significant differences were found between them ( $p = 95\%$ ). In contrast, in the second fraction the extraction efficiency of the accelerated procedure was considerably inferior to that of the conventional method (certified values) for the two metals determined, which was also previously observed in sewage sludge samples analysed in this work (USS and OOS). This means that the accelerated procedure shows a similar behaviour when it is applied for metal fractionation of samples with different matrices, such as sludges and sediments.

The total metal content in the samples was determined using a microwave digestion method optimized in previous work.<sup>30</sup> The values obtained for USS and OOS samples A and B are given in Table 6, where all the results are expressed as mean values ( $\text{mg kg}^{-1}$ )  $\pm$  the standard deviation of three separate determinations.

### Principal component analysis

In order to obtain more complete information about the extraction efficiency of the two extraction methodologies, PCA was applied. The analysis of the data matrix containing the overall extractable metals obtained with the two methods, for all samples studied, allow the original information to be reduced to only three variables (principal components or factors), which retained 97.43% of the initial variance of the data. The highest variance (92.69%) corresponds to the first factor, named factor 1, whereas factors 2 and 3 achieved values of only 2.98 and

1.76%, respectively. A representation of the first two principal components (factors 1 and 2) is shown in Fig. 4, where the USS samples are positioned on the positive side of the second principal component and the distribution of the OOS samples was mainly influenced by the negative part of the second factor, independently of the extraction method employed (conventional and microwave accelerated).

As a result of the previous exploratory analysis and for a better appreciation of the similarities between the two extraction methods, the correlations between paired methods for each sample were also evaluated. As shown in Fig. 5, excellent correlations ( $p < 0.01$ ) with satisfactory correlation coefficients ( $r > 0.9997$ ) were found in all cases. The intercepts of the regressions were not significantly different from zero for any of the equations, but the slope ranged from 0.87 (USS samples) to 0.92 (OOS samples), which means that the total metals extracted with the two methods pointed to a slight systematic bias towards lower values when microwave single extractions were used.

### Conclusions

The results reported have demonstrated that microwave single extractions provide, for most of the elements studied, equivalent results to the conventional BCR sequential extraction method. This means that the proposed method could be considered as a promising approach to a conventional method for obtaining fast screening of the mobility and bioavailability of toxic heavy metals in the environment, in order to evaluate their ecological and biological effects on living organisms. Given the operational character of the sequential extraction procedure, it is clear that the developed method cannot be considered as a substitute for the conventional procedure but rather as a supporting method to provide comparable results in a shorter treatment time. Despite some extraction differences, the proposed method gives satisfactory results for Cu, Cr, Ni and Pb with overall recoveries close to 100%. In the case of Zn, the extraction efficiency of the conventional method was slightly higher than that of the microwave single extraction procedure. Moreover, chemometric analysis of the results obtained by the two extraction methods gave a good correlation.

Although the results obtained by the two extraction methodologies were not identical, it is important to emphasize that in those cases where is possible to replace the sequential treatment of the BCR method by microwave single extractions, the total treatment time is greatly reduced. First, the use of single extractions provides an important simplification of the experimental procedure, since all fractions can be leached simultaneously. Second, when microwave heating was introduced the treatment time was considerably reduced, *i.e.*, the 16 h required

**Table 5** Analytical results obtained for the Certified Reference Material (CRM 601) using the microwave single extractions

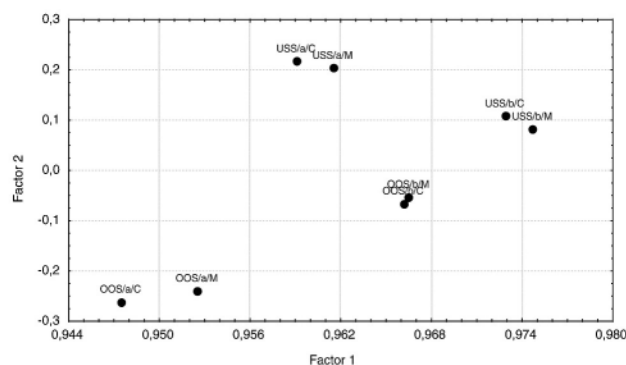
|                               | Certified value/<br>$\text{mg kg}^{-1}$ | Found/ $\text{mg kg}^{-1}$ | Recovery (%)   |
|-------------------------------|---|----------------------------|----------------|
| <i>Acid soluble fraction—</i> |   |                            |                |
| Cr                            | $0.36 \pm 0.04$                         | nd                         | —              |
| Ni                            | $8.01 \pm 0.73$                         | $7.98 \pm 0.19$            | 99.62          |
| Pb                            | $2.68 \pm 0.35$                         | $2.83 \pm 0.41$            | 105.6          |
| Zn                            | $264 \pm 5$                             | $257.9 \pm 4.9$            | 97.70          |
| <i>Reducible fraction—</i>    |   |                            |                |
| Ni                            | $6.05 \pm 1.09$                         | $-4.20 \pm 0.25$           | — <sup>a</sup> |
| Zn                            | $182 \pm 11$                            | $-232.6 \pm 4.9$           | — <sup>a</sup> |
| <i>Oxidizable fraction—</i>   |   |                            |                |
| Ni                            | $8.55 \pm 1.04$                         | $8.11 \pm 0.47$            | 94.85          |
| Pb                            | $109 \pm 13$                            | $107.8 \pm 0.9$            | 98.94          |

<sup>a</sup> Negative recovery value was obtained.

**Table 6** Total metal contents obtained for the USS and OOS by total digestion

|             | Sample A/<br>$\text{mg kg}^{-1a}$ | Sample B/<br>$\text{mg kg}^{-1a}$ |
|-------------|-----------------------------------|-----------------------------------|
| <i>USS—</i> |                                   |                                   |
| Cu          | $374.3 \pm 7.1$                   | $497.9 \pm 11.5$                  |
| Cr          | $71.32 \pm 1.1$                   | $66.25 \pm 0.48$                  |
| Ni          | $48.66 \pm 2.21$                  | $44.00 \pm 1.19$                  |
| Pb          | $435.3 \pm 3.8$                   | $490.2 \pm 1.82$                  |
| Zn          | $1097 \pm 15.9$                   | $1066 \pm 20$                     |
| <i>OOS—</i> |                                   |                                   |
| Cu          | $50.91 \pm 1.25$                  | $39.30 \pm 0.00$                  |
| Cr          | $59.04 \pm 3.45$                  | $3.63 \pm 0.37$                   |
| Ni          | $45.32 \pm 5.31$                  | $17.41 \pm 1.75$                  |
| Pb          | $23.10 \pm 1.00$                  | nd <sup>b</sup>                   |
| Zn          | $70.10 \pm 1.27$                  | $47.84 \pm 0.00$                  |

<sup>a</sup> All concentrations are expressed as average values ( $\text{mg kg}^{-1}$ )  $\pm$  the standard deviation of three determinations. <sup>b</sup> Not determined by FAAS.



**Fig. 4** Sample loading plot by principal component analysis (PCA). Symbols: a and b mean samples A and B, respectively; M = microwave method; C = conventional method.

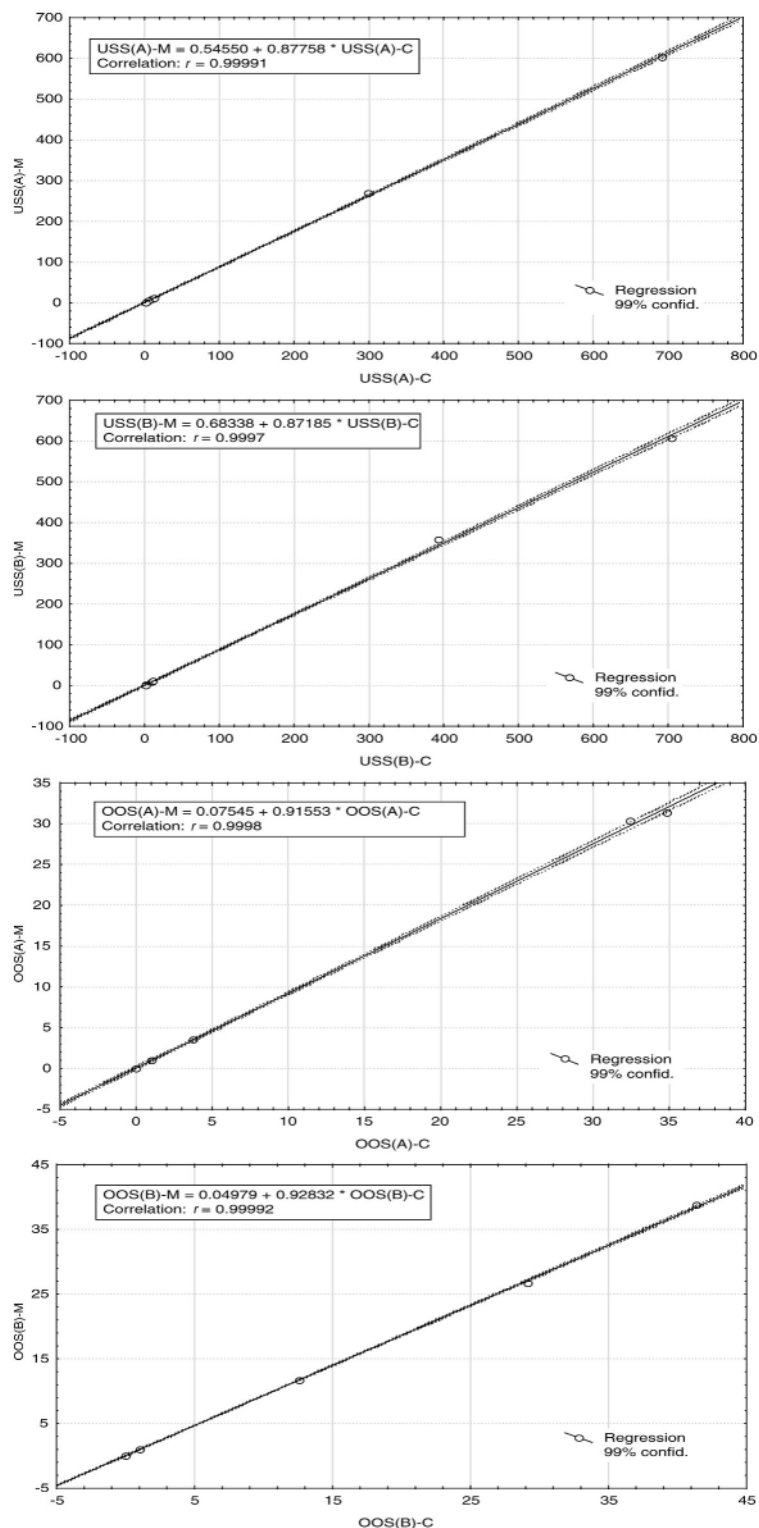
for the two first fractions was reduced to only 50 and 10 s of microwave heating, respectively. For the third fraction, the oxidation with H<sub>2</sub>O<sub>2</sub> requires only 10 s, with the additional elimination of an intermediate evaporation step and the tedious control of the temperature at 85 °C. Moreover, the treatment with NH<sub>4</sub>OAc was also shortened from 16 h to only 10 s, which also provides an important acceleration of this stage.

Finally, it could be said that microwave single extractions might be considered as a useful tool for the rapid evaluation of the extractable metals in sewage sludge samples, in order to estimate their potential toxicity to the environment. From the

results for the certified reference material (CRM-601), it is possible to conclude that the accelerated procedure could also be employed to predict the mobility of heavy metals in different matrix samples.

## References

- 1 A. M. Ure and C. M. Davidson, *Chemical Speciation in the Environment*, Blackie, Glasgow, 1995.



**Fig. 5** Correlation between the results of the BCR sequential extraction method and the microwave single extractions for all the samples studies. Symbols: (A) and (B) mean samples A and B, respectively; M = microwave method; C = conventional method.

- 2 G. Rauret, *Talanta*, 1998, **46**, 449.
- 3 J. R. Lead, J. Hamilton Taylor and W. Davidson, *Sci. Total Environ.*, 1998, **209**, 193.
- 4 J. L. Gómez Ariza, I. Giraldez, D. Sánchez Rodas and E. Morales, *Anal. Chim. Acta*, 1999, **399**, 295.
- 5 J. L. Gómez Ariza, I. Giraldez, D. Sánchez Rodas and E. Morales, *Talanta*, 2000, **52**, 545.
- 6 Ph. Quevauviller, G. Rauret and B. Griepink, *Int. J. Environ. Anal. Chem.*, 1993, **51**, 231.
- 7 M. D. Ho and G. J. Evans, *Anal. Commun.*, 1997, **34**, 363.
- 8 C. M. Davidson, A. L. Duncan, D. Littlejohn, A. M. Ure and L. M. Garden, *Anal. Chim. Acta*, 1998, **363**, 45.
- 9 M. D. Petit and M. I. Rucandio, *Anal. Chim. Acta*, 1999, **401**, 283.
- 10 Z. Mester, C. Cremisini, E. Ghiara and R. Morabito, *Anal. Chim. Acta*, 1998, **359**, 133.
- 11 R. Martín, D. M. Sánchez and A. M. Gutierrez, *Talanta*, 1998, **46**, 1115.
- 12 J. L. Gómez Ariza, I. Giraldez, D. Sánchez Rodas and E. Morales, *Sci. Total Environ.*, 2000, **246**, 271.
- 13 Ş. Tokalioglu, Ş. Kartal and L. Elçi, *Anal. Chim. Acta*, 2000, **413**, 33.
- 14 A. Tessier, P. G. C. Campbell and M. Bisson, *Anal. Chem.*, 1979, **51**, 844.
- 15 H. A. Elliot, B. A. Dempsey and P. S. Maille, *J. Environ. Qual.*, 1990, **19**, 330.
- 16 F. M. G. Tack, H. A. H. Vossius and M. G. Verloo, *Int. J. Environ. Anal. Chem.*, 1996, **63**, 61.
- 17 A. Fernández Alborés, B. Pérez Cid, E. Fernández Gómez and E. Falqué López, *Analyst*, 2000, **125**, 1353.
- 18 B. Pérez Cid, A. Fernández Alborés, E. Fernández Gómez and E. Falqué López, *Anal. Chim. Acta*, 2001, **431**, 209.
- 19 J. F. López Sánchez, A. Sahuquillo, H. D. Friedler, R. Rubio, G. Rauret, H. Muntau and Ph. Quevauviller, *Analyst*, 1998, **123**, 1675.
- 20 B. Pérez Cid, I. Lavilla and C. Bendicho, *Analyst*, 1996, **121**, 1479.
- 21 R. Zufiaurre, A. Olivar, P. Chamorro, C. Nerín and A. Callizo, *Analyst*, 1998, **125**, 255.
- 22 *Statistica for Windows, Release 5.0*, Statsoft, Tulsa, 1995.
- 23 P. S. Rendell, G. E. Batley and A. J. Cameron, *Environ. Sci. Technol.*, 1980, **14**, 314.
- 24 P. Nirel, A. J. Thomas and J. M. Martin, in *Speciation of Fission and Activation Products in the Environment*, ed. R. Bulman and J. Cooper, Elsevier Applied Science, London, 1985.
- 25 N. Belzie, P. Lecomte and A. Tessier, *Environ. Sci. Technol.*, 1989, **23**, 1015.
- 26 J. L. Gómez Ariza, I. Giraldez, D. Sánchez Rodas and E. Morales, *Anal. Chim. Acta*, 1999, **399**, 295.
- 27 M. Gulmini, G. Ostacoli, V. Zelano and A. Torazzo, *Analyst*, 1994, **119**, 2075.
- 28 B. Pérez Cid, I. Lavilla and C. Bendicho, *Anal. Chim. Acta*, 1999, **378**, 201.
- 29 M. Raksasataya, A. G. Langdon and N. D. Kim, *Anal. Chim. Acta*, 1996, **332**, 1.
- 30 I. Lavilla, B. Pérez Cid and C. Bendicho, *Fresenius' J Anal. Chem.*, 1998, **361**, 164.