Speciation of heavy metals in untreated sewage sludge by using microwave assisted sequential extraction procedure

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ABSTRACT

A fast microwave assisted extraction procedure was developed and optimized for their eventual exploitation in the three-stage sequential extraction procedure proposed by modified BCR protocol (the community Bureau of Reference now the European Union "Measurement and Testing Programme"). The effects of the microwave treatment on the extraction of Cd, Cr, Cu, Ni, Pb and Zn from untreated sewage sludge collected from Hyderabad city (Pakistan) were compared with those obtained from sequential BCR extraction procedure. In sequential BCR method, each extraction step takes 16 h, where as with the use of compromised microwave conditions, extraction steps could be completed in about 120 s, for each step, respectively. Extractable Cd, Cr, Pb and Ni obtained by both comparable methodologies were measured by electrothermal atomic absorption spectrometry (ETAAS), while for Cu and Zn flame atomic absorption spectrometry (FAAS) was used. The validations of both extraction techniques were compared by the analysis of certified reference material of soil amended with sewage sludge (BCR 483). The results of the partitioning study of untreated waste water sewage sludge, indicate that more easily mobilized forms (step 1) were predominant for Cd, Ni and Zn (28.3, 28.4 and 43.7%), in contrast, the largest amount of Cd and Pb (66.4 and 72.8%) was associated with the iron/manganese oxide while Cr and Ni (71.2 and 38.7%) in organic matter/sulphide fractions. The overall metal recoveries in steps 1–3 (excluding residual step) were 95.3–104% of those obtained with the sequential BCR protocol. The accuracy of the proposed microwave extraction method (expressed as %R.S.D.) was lower than 10% for all metals.

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1. Introduction

The increasing quantity of sewage sludge due to rise in population concentration in cities, constitutes a serious problem of pollution when they are discharged on the environment. The reusing of these devastate for agricultural apply is a useful alternative to burning, since they can perform as a resource of nutrients for crops due to their organic matter and inorganic nutrient contents. Unlike organic contaminants, heavy metals are determined environmental impurities, which cannot be destroyed [1]. In low amount, several heavy metals (HMs) such as Cu, Fe, Mn, Mo, Ni and Zn are important micronutrients for plants [2,3]. Elevated amount

of HMs in the soil surface creates environmental troubles, including toxicity to plants, animals and humans. In developing countries, municipalities of large cities dispose solid wastes on nearby unused lands. Such practices lead to increase the HMs contents of the biosphere [4,5].

The distribution of metals in sewage sludge can provide researchers with evidence of the anthropogenic impact on ecosystems and, therefore, aid in assessing the risks associated with discharged human waste. The accumulation of trace and toxic elements in environmental samples (sediments, soils and sewage sludges, etc.) causes a potential risk to human health due to the transfer of these elements in aquatic media, their uptake by plants and their subsequent introduction into the food chain [6].

The speciation studies of HMs go on to be a large value in environmental monitoring, because not the total amount of the HMs, but more critically their forms offered in environment will decide their toxicity, mobility and bioavailability [7]. Several sequential extraction methods have been widely applied to differentiate the chemical forms in which trace metals are present in soils, sediments and sludges. In all sequential extraction schemes, extractants are

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applied in order of increasing reactivity so that the successive fractions obtained correspond to metal association forms with lesser mobility. In sequential BCR extraction (SE), environmental samples (soil, sediment, sewage sludge) are treated with a chain of reagents and determined the elemental concentration into fractions, linked with different mineralogical phases (e.g. carbonates, sulfides and organic matter bound phases) [8].

Sahuquillo et al. [9] and Rauret et al. [10] have been revised the original BCR procedure due to irreproducibility of, in particular reducing extraction (NH$_2$OH·HCl) fraction of step 2. This procedure is very popular during recent years and their application has increased lately, during the certification of reference materials reported [11–14]. This method is broadly accepted and applied to elemental fractionation in different environmental samples. But one of the main limits of SE methods is that they are extremely time-consuming. As far as we identify, the use of microwave power for acceleration of a SE method with the plan of metal speciation in sewage sludge has not been reported extensively. This power could be introduced to change the magnetic shaking and conventional warming in order to shorten the treatment time.

Microwave power is an extremely useful auxiliary factor, which has lately been exploited for increasing the rate of different chemical processes [15,16]. A rapid solvent chemical extraction [17,18] and metals fractionation in different solid samples [19–20] are some of the most known applications. Various microwave oven designs [21] have been used as energy sources and their mechanical parameters have been specifically optimized in order to obtain the best positive operation conditions in each case.

We investigated that if microwave heating techniques were applicable to the dissolution of total metals from different solid matrixes within very low time period as compared to conventional methods, the microwave techniques could also be used to stimulate the rapid release of heavy metals in each of the different chemical binding fractions of soils, solid waste and sediments. So the main objective of this work was to apply and compare time-saving extraction device (microwave oven) in the BCR three-step sequential extraction procedure, with the principal aim of reducing the 51 h treatment time, whilst at the same time maintaining, from an environmental point of view, the same recovery values as provided by the conventional BCR sequential procedure. Speeding up of the SE method has been previously approved out with the use of ultrasound power [22]. The use of simple and widely available extraction device, such as a domestic microwave oven, was investigated. The microwave oven procedure was optimized using a soil amended with sewage sludge reference material, with target values in each step being those obtained by the application of the conventional BCR procedure using mechanical shaker.

For the quality control of the analytical performance and the validation of the newly developed method, the conventional and optimized alternative extraction methods were compared using the analysis of BCR 483. Microwave operation parameters (heating time and power) were optimized for Cu, Cr, Ni, Pb and Zn and in each case the most positive conditions were selected for them. Flame atomic absorption spectrometry (FAAS)/electrothermal atomic absorption spectrometer (ETAAS) were used for the measurement of metals in the extracts.

2. Materials and methods

2.1. Reagents and glassware

Ultrapure water obtained form ELGA labwater system (Bucks, UK), was used throughout the work. The extracting reagents as listed in Fig. 1, was prepared from analytical-grade reagents and also checked for possible trace metal contamination. CH$_3$COONH$_4$

was purchased from Sigma (Aldrich, Milwaukee, USA). Acetic acid, hydrochloric acid, nitric acid, and hydrogen peroxide were analytical-reagent grade from Merck (Darmstadt, Germany). Standard solutions of Cd, Cr, Cu, Ni, Pb and Zn were prepared by dilution of 1000 ppm certified standard solutions, Fluka Kamica (Buchs, Switzerland) of corresponding metal ions. The hydroxylammonium chloride reagents were prepared prior to use. Mg(NO$_3$)$_2$ stock standard solution, $5.0\,g\,l^{-1}$, used as a chemical modifier, was prepared from Mg(NO$_3$)$_2$ (Merck Ltd., Poole, Dorset, UK). Ammonium dihydrogen phosphate ($2.0\,g\,100\,ml^{-1}$ NH$_4$H$_2$PO$_4$) was prepared from (Sigma), Pd stock standard solution, $3.0\,g\,l^{-1}$ used as a chemical modifier, was prepared from Pd 99.999% (Aldrich, Milwaukee, WI, USA). Portion of standard and sample (sample volume 10 μl + 10 μl modifier in each case), for Cd, Cr, Ni, and Pb with appropriate volume of palladium, magnesium nitrate and ammonium dihydrogen phosphate were transferred into auto-sampler cups and stirred magnetically before measurement. Magnesium nitrate and palladium: 5 μg Pd + 3 μg Mg(NO$_3$)$_2$ (10 ml + 10 ml from stock solution in 100 ml) used for, Cd. Ammonium dihydrogen phosphate: 50 μg NH$_4$H$_2$PO$_4$ (25 ml from stock solution in 100 ml) for Pb; magnesium nitrate: 15 μg Mg(NO$_3$)$_2$ (50 ml from stock solution in 100 ml) for Cr and Ni.

The certified reference material BCR 483 was purchased from the bureau of references of European communities. All glassware and plastic materials used were previously treated for 24 h in 2 M nitric acid and rinsed with double distilled water and then with ultrapure water. We used 50 ml of acid washed polyethylene centrifuge tubes for extraction, while 50 ml polyethylene vessels Bibby (Sterilin Ltd., UK) were used for storage of extractants.

2.2. Instrumentation

A horizontal flask shaker, electric shaker (Gallenkamp) was used for shaking the samples. WIROWKA Laboratoryjna type WE-1, nr-6933 centrifuge; speeds range 0–6000 rpm, timer 0–60 min (Mechanika Phecyzjna, Poland) used for centrifugation. A WTW pH meter was used for pH adjustments of the reagents. PM023 domestic programmable microwave (MW) oven PEL (Japan) used for MW extraction and digestion. The determination of metals in extracts and digests was carried out by means of a double beam PerkinElmer atomic absorption spectrometer model AA700 (Norwalk, CT, USA) equipped with a graphite furnace HGA-400, pyrocoated graphite tube with integrated platform, an auto sampler AS-800. The intensity and bandpass width of energy source, as recommended by manufacturer. The Cu and Zn were measured under optimized operating conditions by FAAS with air–acetylene flame, while the Cd, Cr, Ni and Pb were determined by ETAAS.

2.3. Sampling of sewage sludge and its preparations

The sewage sludge or biosolid samples were collected from domestic catchments areas of Hyderabad city, Pakistan, at sites where the wastewater was separated from solid waste. This solid waste is mostly used on agricultural land near by the city, where vegetables and grain crops are grown. The sampling was done after each 15 days randomly i.e. from October to December in 2006, so far six individual samples of biosolid sample were collected. To ensure samples were as representative as feasible, each biosolid sample was collected by taking subsamples from various points in same site (between 2 and 5 kg boisolid). Samples were collected using a polypropylene shovel, and subsequently transferred to clean polypropylene bags. Sampling place temperature was measured. On back to the laboratory, the wet sewage sludge samples were spread onto plastic sheet in fume cupboards and allowed to dry at room temperature. Big pieces of stones or plastic were seen in
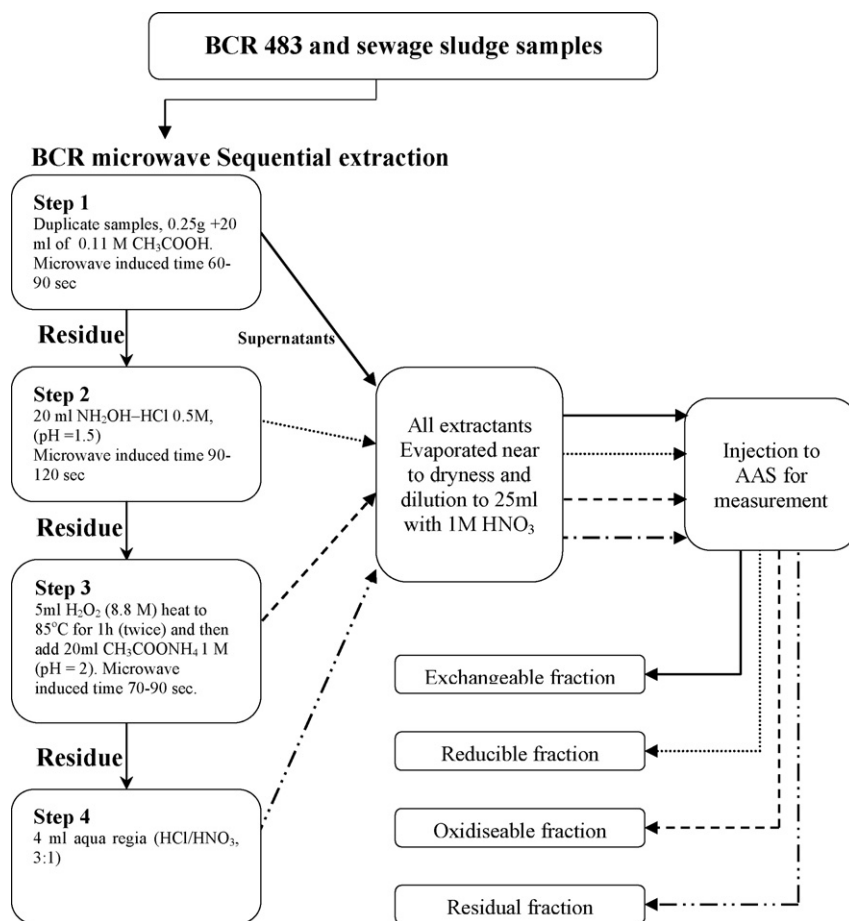
Fig. 1. Flow diagram of the modified BCR microwave sequential extraction procedure.

the samples and these were picked out manually before the samples were further homogenized to make a representative sample for that sampling day. Each mixed samples after air drying for 8 days, grind with a mortar and pestle, after initial grinding the samples were passed through a 3 mm nylon fiber sieve. It was extra ground to pass a 1 mm sieve size and finally kept in labeled with collected date in polypropylene containers at room temperature before analysis.

2.4. Physicochemical studies

The physicochemical parameters for the sewage sludge samples (pH, conductively, organic matter (OM), organic carbon (OC), total nitrogen (TKN), sulfur, phosphate and silica content) were assessed by standard methods. pH and conductivity values were determined for each batch, using a ratio of wastewater sludge to ultrapure water of 1:2.5 (w/v) [23,24]. The OC was measured by the Walkeley-Black method [25]. The OM content was obtained by ashing duplicate samples of each batch in muffle furnace at 540 °C for 6 h. The change in the dry weight of sewage sludge before and after ashing was used to calculate the organic matter content [26]. Total sulfate/sulfur was determined by the turbidity method [27]. The phosphate content was determined by the ascorbic acid standard method and the total nitrogen level by the Kjeldahl method [28,29] (Table 1).

2.5. Sequential extraction procedure (SE)

The extractants used, the extraction conditions, and the supposed forms of elemental measured to be extracted are outlined in Fig. 1, and were performed as described elsewhere [30–33].

2.6. Microwave sequential extraction (MSE)

Microwave power has long been used successfully for the acceleration of sample preparation process; its efficiency is significantly expressed by the increase in temperature of the sample [34]. The possibility of exploitation of a MW oven in the sequential extraction steps of BCR procedure was investigated. As the SE procedure requires, those extractions are performed at ambient temperature, the optimization of the MW power and extraction time was carried out by carefully controlling the temperature of the extracting solutions. For sequential extraction steps of BCR method, to maintain power of MW and time, where the temperature did not exceed

Table 1
Physicochemical parameters of domestic wastewater sludge

Parameters	$\bar{x} \pm s^*$
pH	7.70 ± 0.452
Conductivity (mS cm^{-1})	1519 ± 168
Dry matter (%)	87.6 ± 3.13
Silica (%)	54.6 ± 1.81
Organic matter (%)	35.6 ± 5.43
Organic carbon (%)	20.8 ± 1.82
Total nitrogen (µg g^{-1})	8050 ± 2220
Total sulphur (µg g^{-1})	849 ± 29.2
Total phosphate (µg g^{-1})	1080 ± 113

Key = *Mean ± standard deviation.

Table 2
Comparison of heavy metals contents extracted by sequential (SE) and microwave sequential extraction method (MSE) in CRM 483 ($\mu g\,g^{-1}$)

		Cd	Cr	Cu	Ni	Pb	Zn
1 step							
MSE	Mean	10.5 ± 1.02	10.1 ± 1.03	16.0 ± 1.21	17.9 ± 1.51	0.812 ± 0.045	438 ± 18.9
	%R.S.D.	9.71	10.2	7.56	8.44	5.54	4.32
SE	Mean	11.0 ± 0.398	10.2 ± 2.99	16.2 ± 1.3	18.2 ± 1.6	0.84 ± 0.28	438 ± 40.0
	%R.S.D.	3.62	29.3	8.32	8.82	33.3	9.11
Indicative values [36]	Mean	10.0 ± 0.8	9.4 ± 3.5	16.8 ± 1.5	17.9 ± 2.0	0.76 ± 0.7	441 ± 39.0
	%R.S.D.	8.00	37.2	8.93	11.2	92.0	8.80
2 step							
MSE	Mean	24.6 ± 2.12	656 ± 47.9	145 ± 11.2	24.6 ± 1.61	378 ± 13.8	443 ± 25.9
	%R.S.D.	8.62	7.30	7.72	6.55	3.65	5.85
SE	Mean	24.5 ± 2.0	660 ± 85.0	148 ± 16.5	25.0 ± 2.5	381 ± 20.0	452 ± 56.0
	%R.S.D.	8.22	12.9	11.1	10.1	5.22	12.4
Indicative value	Mean	24.8 ± 2.3	654 ± 108	141 ± 20.0	24.4 ± 3.3	379 ± 21.0	438 ± 56.0
	%R.S.D.	9.32	16.5	14.2	13.6	5.51	12.8
3 step							
MSE	Mean	1.58 ± 0.11	2220 ± 141	130 ± 11.8	5.51 ± 0.23	65.3 ± 4.81	38.2 ± 2.51
	%R.S.D.	6.33	6.34	9.08	4.18	7.35	6.54
SE	Mean	1.62 ± 0.2	2230 ± 280	128.0 ± 22.5	5.3 ± 0.8	65.2 ± 6.0	38.4 ± 6.80
	%R.S.D.	12.3	12.6	15.1	9.20	17.5	17.7
Indicative value	Mean	1.22 ± 0.48	2215.0 ± 494	132.0 ± 29.0	5.9 ± 1.4	66.5 ± 22.0	37.1 ± 9.9
	%R.S.D.	39.3	22.3	22.0	23.7	33.1	26.7
Residue							
MSE	Mean	0.401 ± 0.04	231 ± 22.6	44.5 ± 4.31	15.0 ± 1.41	75.6 ± 7.30	82.7 ± 8.10
	%R.S.D.	9.98	9.80	9.69	9.33	9.66	9.79
SE	Mean	0.38 ± 0.14	286 ± 28.0	45.5 ± 3.2	14.9 ± 2.2	75.3 ± 13.5	83.4 ± 7.4
	%R.S.D.	36.8	9.80	7.20	14.8	17.9	8.91
Indicative values	Mean	0.423 ± 0.16	183.0 ± 40.0	43.3 ± 3.8	15.2 ± 4.3	76.9 ± 17	82.1 ± 9.6
	%R.S.D.	37.8	21.9	8.82	28.3	22.1	11.7
Aqua regia (pseudo-total)		36.3 ± 2.5	3230 ± 375	341 ± 15.8	64.8 ± 6.1	510 ± 47.0	997 ± 78.5
SE sum 3 steps + residual		37.5 ± 2.1	3187 ± 293.9	338 ± 28.1	63.4 ± 3.8	522 ± 24.9	1012 ± 69.6
Relative error (%)		3.33	$^-1.31$	−0.721	−2.21	2.42	1.61
MSE sum 3 steps + residual		37.1 ± 2.38	3114 ± 150	336 ± 16.9	63.0 ± 2.61	520 ± 16.3	1002 ± 33.2
Relative error (%)		2.10	−3.60	−1.44	−2.73	2.00	0.502

50 °C, and the solutions were never brought to boiling. Experiment details are given in Fig. 1.

The optimization was carried out in six experiments with six different sets of parameters (power and time) in the range of 20–80% of nominal power 900 W, and 20–120 s, respectively. In MSE, lower amount of sewage sludge samples and extracting reagents were used, as compared to the SE. Exactly 0.25 g of certified material (BCR 483) and sewage sludge samples were placed in 25 ml Polytetrafluoroethylene (PTFE) tubes, added 10 ml of 0.11 M acetic acid. The quantity of sewage sludge was lower than in the certified procedure, while the ratio of sewage sludge to the volume of extraction solution was the same [35]. The H_2O_2 treatment for the digestion of organic matter was carried out as in the modified BCR sequential extraction procedure, the obtained residue was extracted by ammonium acetate at pH 2 using microwave at six different sets of parameters. It is important to emphasize that prior to subjecting microwave energy the extraction mixture (extractant-sample) was mixed for 10 s by means of magnetic stirrer in order to obtain homogenous suspension. The experimental parameters were chosen from a combination of the lowest, highest and mean values within the ranges already exploited by sequential extraction values of metals under study. Blanks (containing reagent but no samples) were also taken through each step.

Before extraction 0.25 g of certified BCR 483 and sewage sludge samples were precisely weighed in weighing bottles, and placed in an oven (105 ± 2 °C) until constant weight. This treatment produced a 3.1% loss of weight in BCR 483 and different ranged values for sludge samples. All subsequent measurements were approved to take account of these losses.

2.7. Statistical analysis

All reagents used were of analytical-reagent grade or better. Statistical analysis was performed by use of Microsoft office (Excel 2003®).

2.8. Quality control

The linear range of the calibration curve reached from the detection limit up to 0.0–0.025, 0.0–0.2, 0.0–1.0, 0.0–0.2, 0.0–0.1, 0.0–1.0 $\mu g\,ml^{-1}$ for Cd, Cr, Cu, Ni, Pb and Zn, respectively. The limits of detection (LOD) for elements were calculated as under, LOD = $3 \times (S/m)$, respectively, where "S" is the standard deviation of 10 measurements of the blank and "m" is the slope of the calibration graph obtained for each case, the LODs; 0.327, 4.7, 17.3, 6.67, 3.38, 10.0 $\mu g\,L^{-1}$ for Cd, Cr, Cu, Ni, Pb and Zn, respectively. Measurements of HMs in environmental samples require quality control of the analytical methodology employed. For this purpose, certified reference material, Soil amended with sewage sludge BCR 483, and matches as closely as possible the soil matrix were analyzed. Recoveries of metals from BCR 483 are listed in Table 2. The experimental values are in agreement (BCR 483, 96.4–102%) with the indicative values reported in literature [36] (Table 2).

3. Results and discussion

3.1. Sludge characteristics

As can be seen in Table 1, the pH values of 12 batches of wastewater sludge were found to lie in the range 6.5–7.6. At low pH (at 6.5 for instance), the mobility and leaching of HMs increases and their mobility and availability decreases as the pH approaches neutral or rises above pH 7. The considerable amount of total nitrogen, sulfur and phosphate highlights the benefits of using sewage sludge as an agricultural fertilizer. The OM is an important component because it tends to either form soluble or insoluble complexes with the HMs, to migrate, or to be retained in the soil. The results show that the sewage sludge contains $32.0 \pm 5.7\%$ OM.

3.2. Microwave sequential extraction (MSE)

The results obtained from heating the extraction mixture (extractants – sample) in a MW oven at 20–80% of total power (900 W), at different heating time intervals (20–120 s) were compared with those values obtained by SE method. It was observed that in first step of BCR protocol the maximum recovery of all heavy metals (HMs) was observed at 60 s heating time except when Cu and Pb achieved high recovery after 90 s, longer time up to 120 s, not enhance the recovery of Cd, Cu, Cr, Ni, Pb and Zn.

In reducible fraction, the maximum recovery of Cd, Cr, Ni and Zn was observed at 90 s while Cu and Pb required 120 s for optimum values. In oxidizable fraction Cd, Cr and Ni recover at 60 s, Zn recover at 70 s, while Cu and Pb recovery were obtained at 90 s. So, it was observed that the Cu and Pb required longer duration for optimum recovery from matrixes. In summary, the most favorable extraction conditions for all three steps of MSE were found at 60–120 s and 60% of total power 900 W for BCR 483 and sewage sludge samples (Fig. 2b). The power values of MW at 20% of nominal power did not release the quantitative amount of HMs (Fig. 2a). It was observed that at 80% of total power the abrupt change in temperature causes significant changes on metal extraction and fractionation pattern, but when using 60% of total power, the temperature did not change significantly, so we select 60% of total power of MW oven for all experiments to release all HMs at optimum levels as compared to those obtained from SE procedure.

3.3. Comparison between extractable heavy metals from the microwave and sequential extraction procedures

The obtained data against the proposed MSE could be compared to the SE procedure, extractable metal contents in BCR 483 (Table 2) and sewage sludge samples applying the MSE extractions as shown in Table 3. The values of HMs obtained from SE, were used as reference values for calculating the percentage of HMs recovered by
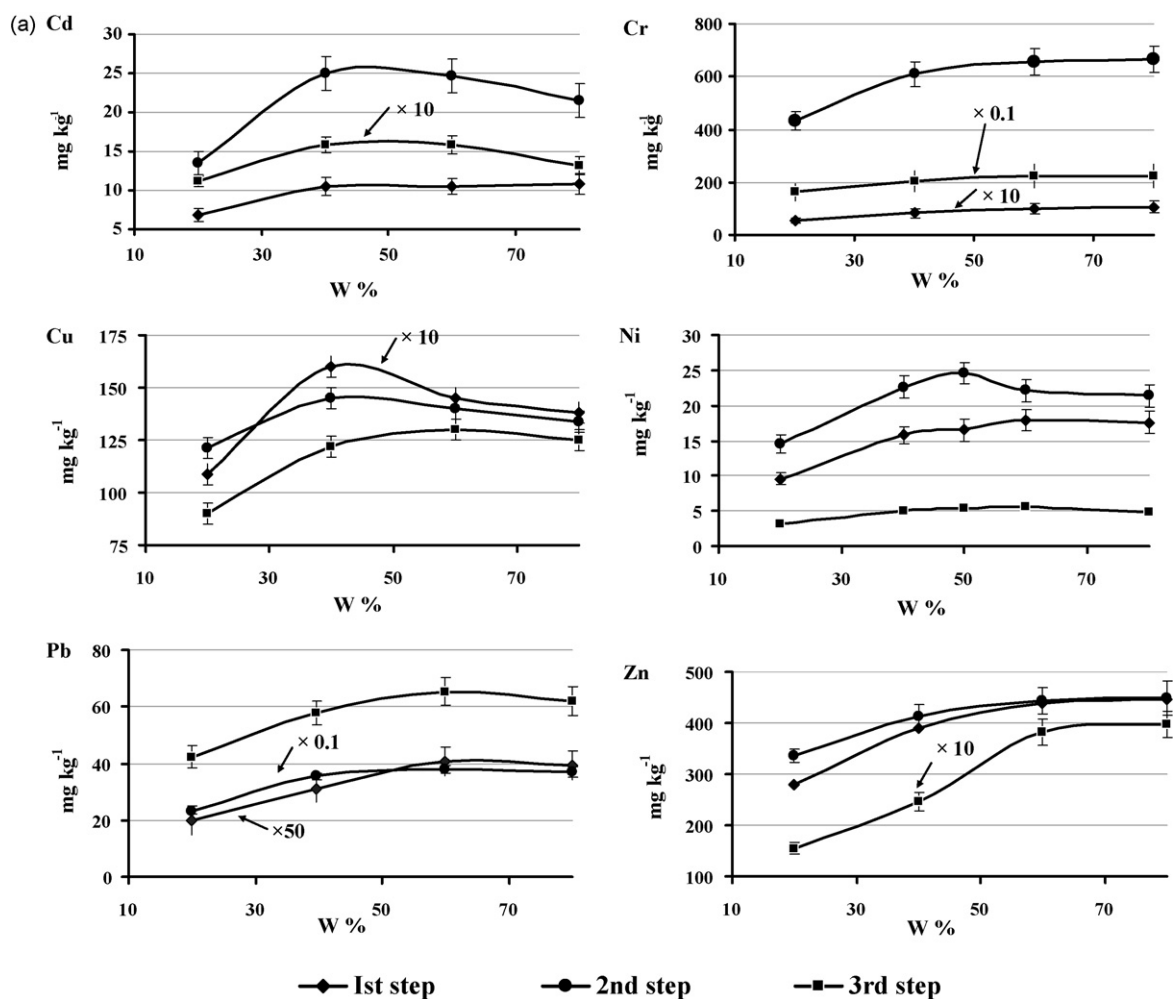

Fig. 2. (a) Recovery of heavy metals in the fractions BCR 483 with the use of different microwave power (W% of 900 W). (b) Recovery of heavy metals in the fractions BCR 483 with the use of fixed microwave energy (60% of 900 W) at different time intervals.
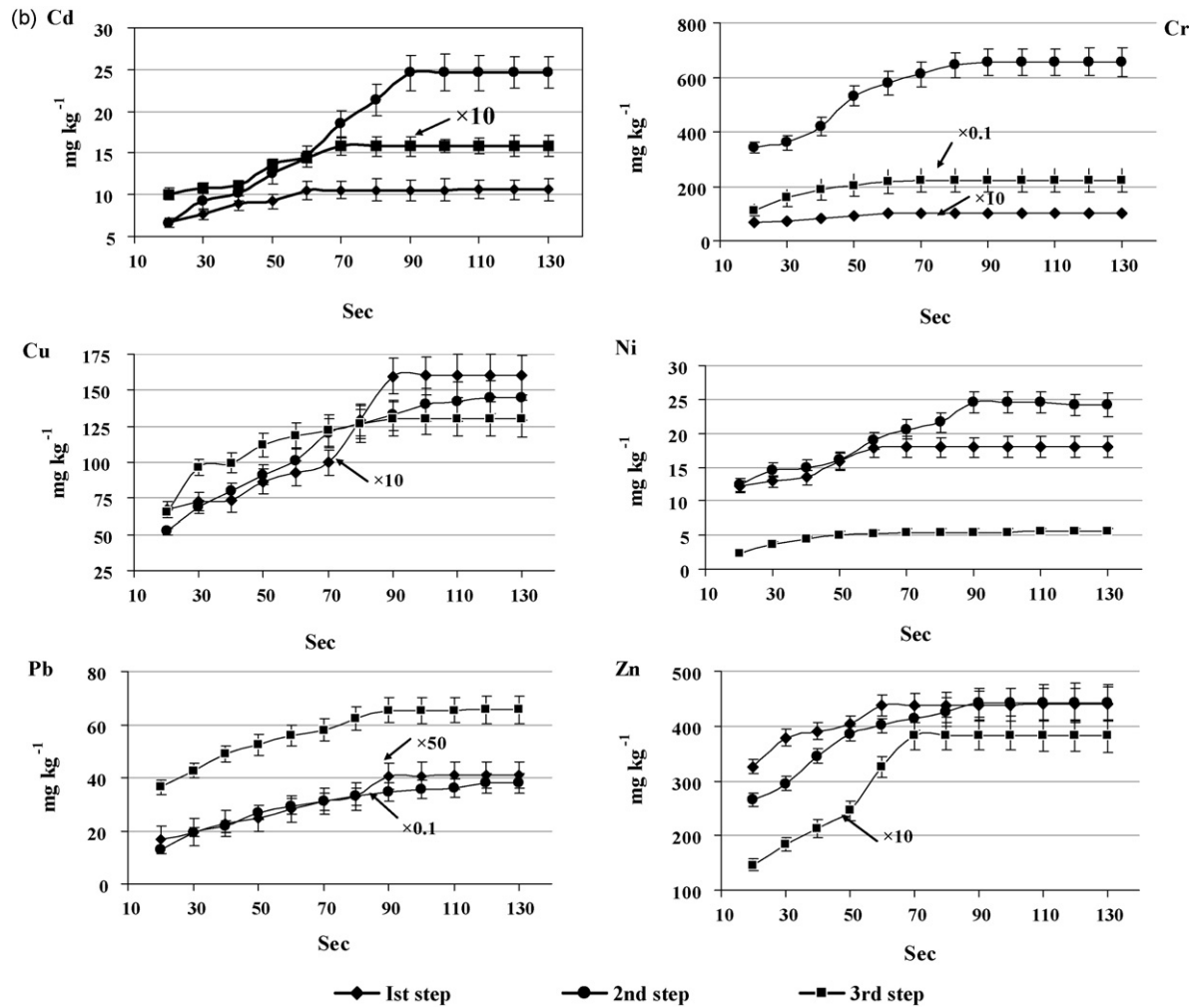
Fig. 2. (*Continued*).

Table 3
Speciation of toxic metals and Comparison of results ($\bar{x} \pm s$) obtained from MSE and aqua regia (pseudo-total) metals in domestic wastewater sludge ($\mu g\,g^{-1}$)

Elements		Cd	Cr	Cu	Ni	Pb	Zn
1 step	Mean	6.56	1.92	2.21	2.98	3.12	23.5
	S.D.	0.523	0.124	0.163	0.232	0.246	1.98
	%R.S.D.	7.97	6.46	7.38	7.79	7.88	8.43
2 step	Mean	9.92	4.12	9.56	6.32	10.6	62.6
	S.D.	0.752	0.364	0.768	0.423	0.916	5.24
	%R.S.D.	7.58	8.83	8.03	6.69	8.64	8.37
3 step	Mean	2.76	22.3	31.2	16.8	37.6	121.9
	S.D.	0.212	1.98	2.13	1.85	2.98	10.5
	%R.S.D.	7.68	8.88	6.83	11.01	7.93	8.61
Residue	Mean	1.8	36.1	48.5	17.9	66.3	49.8
	S.D.	0.128	2.56	3.26	1.15	4.34	3.69
	%R.S.D.	7.11	7.09	6.72	6.42	6.55	7.41
Aqua regia (pseudo-total)		20.8 ± 1.59	66.9 ± 3.98	92.8 ± 5.69	43.5 ± 2.48	119 ± 6.97	256 ± 14.9
Sum 3 steps + residual		21.0 ± 0.949	64.4 ± 3.26	91.5 ± 3.97	44.0 ± 2.23	118 ± 5.35	258 ± 12.5
Relative error (%)*		1.15	−3.68	−1.43	1.15	−1.16	0.782

Key = * Relative error % = $\left[\dfrac{(\text{Sum of 3 steps + Residual}) - \text{Pseudo-total}}{\text{Pseudo-total}} \right] \times 100.$

MSE. The data are mentioned as mean values $(mg\,kg^{-1}) \pm$ standard deviation. No major differences were examined for $p = 0.05$ when comparing the values obtained by the proposed MSE and SE. The R.S.D.% values obtained by SE procedures were generally comparable. The precision, usually in the range 4.34–9.71% was achieved for most of the elements analyzed but in some cases the high R.S.D. percentage was observed in MSE, Cr (10.2) in steps 1.

3.3.1. Exchangeable fraction

The exchangeable fraction constitutes the step 1 of the SE method and consequently it is always directly extracted, result shown in Table 2. The results obtained by MSE show that the %recovery of all HMs Cd, Cr, Cu, Ni, Pb and Zn (95.3, 99.0, 98.7, 98.4, 96.7 and 99.6%), respectively. The amount of elements extracted by MSE is 0.1–4% lower than the values obtained by SE; however. This could be attributed to the long shaking time used in the sequential method, which is responsible for this difference. It should be noted that the objective of this study was not only to improve the extraction efficiency of the SE, but also to find out that MSE yields identically extractable metal contents using shorter treatment time. The previous work by Canepari et al. [37] compares the quantities of metals extracted from certified sediment samples (BCR 601 and 701) using Ultrasonic bath and MSE, while they only perform first step of SE. They declared less metal extracted by SE and MSE extraction in the range of 53.98–77.6% and 68.3–126.3% in 701 while 40–67.6% and 65.7–103.32% in BCR 601, respectively as compared to certified values.

3.3.2. Reducible fraction

The reducible fraction estimated from MSE displayed variability in values of HMs as compared to those obtained by SE, the recoveries of HMs obtained by MSE are varied between 97.8 and 100% for Cd, Cr, Cu, Ni, Pb and Zn as compared to the SE (Table 2). Moreover, negative concentration values of Cd (98.8%) was found in BCR 483 using the SE, which means that these elements were poorly extracted when the treatment with $NH_2OH \cdot HCl$ was carried out on an individual sub-samples. The MSE of reducible fraction assisted by SE released Cu (105%), Ni and Cu (103%). The order of extraction of all these heavy metals is found, Cd > Cr > Pb > Ni > Zn > Cu as compared to SE.

The reducible fractions of HMs obtained by MSE, show %recoveries varied between 99.2 and 103% for all metals, the reproducibility of the MSE was also comparable to that of SE. The results indicate that in relation to reducible fraction of Cu in CRM 483, the extraction efficiency of $NH_2OH \cdot HCl$ was increased when MW energy were applied.

3.3.3. Oxidizable fraction

The oxidizable fraction evaluated by MSE method showed some discrepancies in extraction efficiency of HMs, the metals extracted by SE procedure were considerably higher. To compare the values of HMs released by MSE with those obtained by SE procedure was varied between 93.4 and 103%, except Cd, the considerably higher amount of Cd (130%) was released than those obtained by MSE procedure. In the case of MSE, the recovery of all HMs except Cd, was found to be lower as compared with SE (Table 2).

The total HMs contents obtained by aqua regia digestion of the BCR 483 were compared with the sum of the extracted metals forms the three steps plus residual (Table 2). No significant difference was observed between the total metal extracted following the aqua regia protocol and the sum of extracted metals following both sequential extraction procedures (SE and MSE). The overall recoveries of Cd, Cr, Cu, Ni, Pb and Zn by using the MSE at 120 s are 102%, 96.4%, 98.7%, 97.3%, 102% and 101%, respectively of the corresponding values obtained by SE procedure.

3.4. Application of proposed microwave extraction methods to sewage sludge

The optimization of MW and SE by modified BCR procedure based on the use of MW oven was performed on sewage sludge samples. The amount of metals extracted in each step of the SE procedures is given in Table 3. Among the metals studied the Cd, Ni, and Zn were extracted predominantly in the first step of MSE, which represents the metal bound to carbonates or sorbed/exchangeable phases [38]. On the other hand, only very small fractions of Pb, and Cr were present as acid exchangeable species in all sewage sludge samples. The proportion of the metals in the acetic acid extractable fraction followed the order Cd > Zn > Ni > Cr > Pb > Cu. In reducible Cd > Zn > Ni > Cu > Pb > Cr, while Zn > Ni > Cr > Cu > Pb > Cd in oxidizable fractions. The surfaces of Fe and Mn oxides have special affinity with the cations to natural pH. Perez Cid et al. [21,19] found that Fe and Mn hydroxides are important scavengers of these elements in sewage sludge. The range of relative percentage of Cu in oxidizable fraction was observed 860.6% [21], 1317.9% in urban sewage sludge and 102.4% [19] in olive oil sludge, and shows the increase in incoming of this element in the sewage sludge, probably due to the input of organic matter from domestic sewage and other human activities. According to the results, it is possible to say that Cd and Zn are the most mobilizable metals in sewage sludge samples.

4. Conclusions

The purpose of sequential extraction methods to environmental samples provides relevant information about possible toxicity when they are discharged into the environment. The proposed method (MSE) significantly reduced the time required for the SE extraction to 6 min without losing accuracy and precision of the fractionation analysis of HMs in untreated sewage sludge, whereas the SE requires 51 h although there was some variation in the extraction time from metals to metals. In the SE method, the use of MSE provides similar results to the sequential treatment for Cd, Cr, Cu, Ni, Pb and Zn. The variances associated with the results of MSE were statistically equivalent to those obtained by the SE procedure.

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