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Chromium available fractions in arousa sediments using a modified microwave BCR protocol based on microwave assisted extraction

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Abstract

In a research of chromium availability, the three-stage sequential extraction procedure, proposed by European Community Bureau of Reference (BCR), has been applied for the metal fractionation in marine sediment samples. The procedure has been modified, evaluating the effect of microwave energy to perform the sequential method. Results achieved a substantial reduction in time in comparison to the traditional shaking technique. The time of the first and the second extractions were reduced to 30 s in both steps using microwave heating and working at 66 W of power. To the third extraction, higher power was necessary so it was chosen to work at 198 W. In the last step investigated, time was reduced to 1 min, being a great improvement respect to the conventional BCR sequential extraction protocol.

Chromium determinations in these extracts were carried out by Electrothermal Atomic Absorption Spectrometry (ETAAS). The developed method was applied for chromium determination in marine sediment samples from Ria de Arousa (Galicia, NW of Spain). The values obtained by our laboratory ranged from 0.10 to 1.02 $\mu g\,g^{-1}$ for soluble and reducible fractions, and from 5.5 to 60.0 $\mu g\,g^{-1}$ for the oxidisable fraction. The higher concentrations obtained were the chromium associated with the organic fraction of the marine sediments.

Keywords: Chromium; Marine sediments; Microwave extraction; Fractionation; Electrothermal atomic absorption spectrometry; Metal sequential extraction; Chromium availability

1. Introduction

Chromium is a frequent estuarine contaminant due to its industrial applications such as leather tanning, dyeing or chromium plating, as well as other anthropogenic activities. Metals bioavailability in aquatic systems is difficult to assess due to the complexity of the matrix and many other environmental factors. A full detailed study of chromium bioavailability should consider among other sources, the chromium available fraction contained in the sediments direct in contact with the water column [1–4]. Marine sediments in estuaries and coasts are a common sink of pollutants. In fact, theories developed by earlier studies have demonstrated that chromium is liberated to the aquatic media depending on parameters such as pH, salinity or redox conditions [5,6].

The determination of chemical species is often difficult due to the composition and heterogeneity of the samples. Environmental studies developed methods to determine which metals are extracted using operationally defined procedures that employed several extractant reagents. The distribution and associated phase of a metal in a sediment usually involves the application of chemical extraction procedures. Different extraction techniques have been used to study the distribution of heavy metals throughout the different fractions in soils and sediments, including both single and sequential extraction procedures [7,8]. The determination of specific chemical species or binding forms using extraction procedures can be a good compromise to give information on environmental contamination risk and therefore to give chromium availability. The extracted fractions allow the study of bioavailability and behaviour of metals fixed to the sediments. Many different sequential extraction procedures have been recommended [9–11]. Amongst the most widely used are those of

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Tessier et al. [12] and that developed under the auspices of the Commission of the European Communities, Community Bureau of Reference (BCR) [13]. In the BCR, sequential extraction procedure three fractions are obtained using CH_3COOH, $NH_2OH{\cdot}HCl$ and $H_2O_2–CH_3COONH_4$ for the first, second and third steps, respectively. Each extraction step does not differ in time consumed and they required 16 h shaking the sediment in contact with the extractant. The exchangeable, reducible and oxidisable fractions are obtained after each step. Therefore, one of the main limitations of sequential extraction procedures is that they are extremely time-consuming.

In the last years, different studies have been developed with the aim of reducing the time of these procedures. In the literature, different works have been published with this purpose, using ultrasound or microwave energy.

There are previous accelerated BCR procedures using an ultrasonic bath [14] for Cu, Fe, Mn and Zn determination in soils and sediments. The same ultrasound technique has been applied to accelerate the Tessier and BCR protocols [15–17]. A great decrease of the extraction time for Cu, Cr, Ni, Pb and Zn was obtained. The main problem is the poor sensitivity obtained with FAAS. It was not possible the determination of chromium in the first and the second fractions with the selected technique. The recovery obtained for the oxidisable and residual fractions are very low. Later, there were analyses of chromium by Electrothermal Atomic Absorption Spectrometry (ETAAS) in a certified sediment CRM BCR 601 [18] that only gave the certificated content of the first step of the BCR extraction procedure. Results are very interesting for chromium, despite of the problems with certification of the content in two of the three extracts of the protocol.

The second way to accelerate the procedure is by using microwave energy. Sequential extraction techniques with application of microwave heating were initially investigated [19] to improve the release of metals in each step of the sequential extraction procedure. Other works [20] used microwave conditions to accelerate the Tessier procedure for Fe, Mn, Cu, Cr, Ni and Zn. Great reduction of time was attained. Other workers [21–24] also used a microwave technique to analyse Cu, Cr, Ni, Pd and Zn and times obtained in Tessier procedure were less than 1 min. Again, the problem was the poor sensitivity of the FAAS that made impossible the chromium determination.

To avoid problems with matrix interferences in chromium determination by ETAAS, chemical modification is recommended. Different matrix modifiers have been used for chromium determination in samples with high saline matrix. Thus, researchers used NH_4VO_3, $NaWO_4{\cdot}2H_2O$ and $Pd(NO_3)_2$ [25] or introduced a mixture of $Mg(NO_3)_2$ and $Pd(NO_3)_2$ [26]. There are publications that applied a mixture of $Pd(NO_3)_2$ and $NaVO_3$ or $Pd(NO_3)_2$ for digested sediments [27].

In this work, a modified BCR procedure was developed, shortening each step using microwave heating to accelerate the action of the reagents.

The method developed was applied to the chromium determination in different marine sediment samples from the Ria de Arousa (Galicia, Spain). Each fraction reflects the availability of the metal along the estuary.

2. Experimental

2.1. Apparatus

Measurements were carried out using a Perkin-Elemer Model 1100B atomic absorption spectrometer equipped with an HGA-400 graphite furnace atomizer and an AS-40 autosampler. The source of radiation was a chromium hollow cathode lamp, operating at 12 mA, which provided a 357.9 nm line, with a spectral bandwidth of 0.7 nm. HGA Pyrocoated L'Vov Graphite Tubes were used. For all measurements made during this study, integrated absorbance with an integration time of 4 s was used. The temperature and time programmes for the atomiser are shown in Table 1. The volume injected was 20 μl.

A shaker (Vibromatic) and a magnetic stirrer (Agimatic) from Selecta (Barcelona, Spain) were used in the extraction procedures.

A microwave oven (Panasonic NN-5256) was used to accelerate the extractions, and solid residues were subsequently separated using a centrifuge Centromix, Selecta, Model 540.

2.2. Reagents

Chromium standard solution. A 1000 mg l^{-1} stock solution of chromium (Merk, Darmstadt, Germany) was used. Each test solution was prepared with ultrapure water immediately before use.
Magnesium nitrate Suprapur (Merck, Darmstadt, Germany).
Acetic acid HPLC grade 99.8% (Scharlau, Barcelona, Spain).
Hydrogen peroxide p.a. (Panreac, Barcelona, Spain).
Ammonium acetate p.a. (Panreac,. Barcelona, Spain).
Hydroxylammonium chloride Suprapur (Merck, Darmstadt, Germany).
Certified Reference Material CRM 601. Lake sediment, European Commission (Brussels).
Certified Reference Material CRM 701. Lake sediment, European Commission (Brussels).
Argon N50 99.999% purity, used as sheath gas for the atomiser and for internal purge.
Ultrapure water. Resistivity 18 MΩ cm obtained using a Milli-Q water purification system (Millipore).

All glassware was kept in 10% nitric acid at least 48 h and washed three times with ultrapure water before use.

2.3. Sample collection and pre-treatment

Surface sediment samples were collected by triplicate, from the Ria de Arousa (North West of Spain), with a grab,

Table 1
Optimized graphite furnace programme for chromium determination

Step	Temperature (°C)	Ramp time(s)	Hold time (s)	Argon flow (ml min^{-1})
Dry	120	10	20	300
Mineralization	1500	10	20	300
Atomisation	2400	0	4	0 (read)
Clean	2650	1	2	300

Table 2
BCR sequential extraction procedure for sediments

Step	Extraction	Fraction obtained
1	CH_3COOH 0.11 M, 16 h	Exchangeable, acid- and water-soluble fraction
2	$NH_2OH.HCl$ 0.1 M, pH = 2 (with HNO_3), 16 h	Reducible fraction
3	H_2O_2 8.8 M, 3 h; extraction with ammonium acetate1 M, pH = 2 (with nitric acid), 16 h	Oxidisable fraction

on board of the R/V Mytilus. The samples were removed from the surface layer with a polyethylene spoon to avoid contamination, and stored in a hermetic polyethylene bottle at 4 °C, previously cleaned with 10% nitric acid for 48 h and rinsed with Milli-Q water. The samples were lyophilised and sieved through a nylon mesh to separate the fraction with particle size less than 63 μm and stored in polyethylene bottles. Two sub-samples taken from each sediment sampling point were prepared for the BCR extraction procedure for chromium fractionation.

2.4. Accelerated sequential extraction procedure

In the first step, 10 ml of 0.11 mol l^{-1} acetic acid was added to 0.25 g of marine sediment sample in a centrifuge tube, and introduced in a microwave oven 30 s at 66 W. The tube was then removed, centrifuged (3000 rpm) and the supernatant was taken for the analysis. In the second step, 10 ml of 0.1 mol l^{-1} hydroxylammonium chloride adjusted to pH 2 with nitric acid was added to the residue from the first step, and the extraction was performed as described for the step 1. In the third step, peroxide digestion was performed according to the conventional BCR procedure. Finally, 12.5 ml of 1.0 mol l^{-1} ammonium acetate adjusted to pH 2 with nitric acid were added and the extraction was performed heating 1 min at 198 W in the microwave oven. The accelerated extraction procedure sequence is shown in Fig. 1. Chromium concentration in the three extracts was determined by ETAAS using the optimised graphite furnace programme.

3. Results and discussion

3.1. Chromium in the modified BCR fraction extracted

3.1.1. Optimisation of the graphite furnace programme

To optimise the graphite furnace programmes, a lyophilised marine sediment sample with a particle size less than 63 μm was used. The BCR standard extraction procedure (Table 2) was applied at this sample and the three extracts obtained were used to optimise the temperatures and times of the graphite furnace programme. All the extracts showed similar electrothermal results and the same programme was used for all the analysis. Optimum mineralization temperature was 1500 °C for each extract, and optimum atomisation temperature was 2400 °C. The influence of the addition of

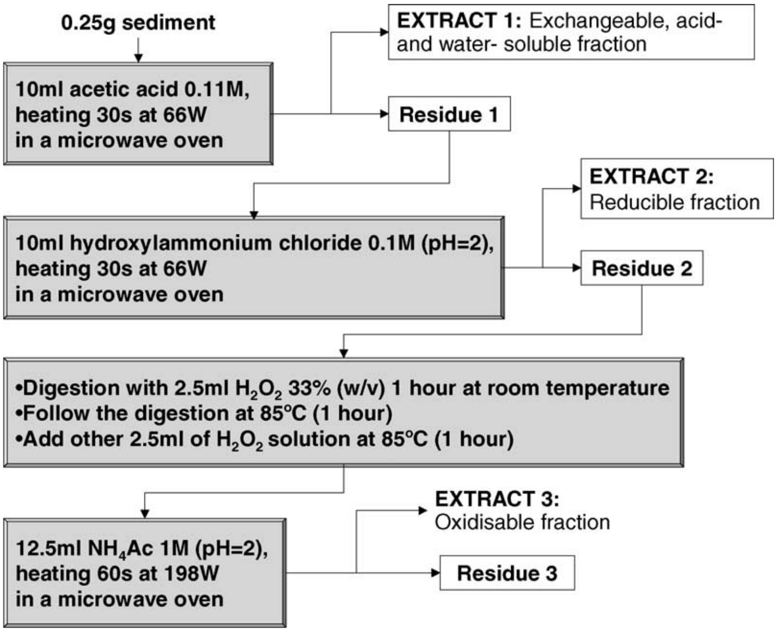

Fig. 1. Accelerated BCR sequential extraction procedure.

magnesium nitrate as chemical modifier was also studied and no improvement in the atomic absorption signal was obtained. For this reason, the ETAAS measurements were carried out in absence of matrix modifier.

3.1.2. Use of microwave energy to accelerate the extraction process

Since the BCR standard sequential extraction requires a long time in each extraction (16 h), it is no attractive to use in routine analysis of environmental samples. To accelerate the sequential extraction procedure, a microwave oven has been used. The experiments were carried out using centrifuge tubes because strong degrading conditions, such as powerful microwave digestions, were not employed. At these conditions, no extractant looses were detected and final volume was not modified through the microwave step. A conventional microwave oven has been used in other works. Neither problems have been observed nor the use of a conventional microwave has represented a danger to the analyst. No losses of analyte have been either observed, as long as the microwave power was not extremely high and the heating time was not too long [22,23]. The microwave power was recalculated to verify whether the experimental power corresponded with the one indicated by the manufacturer. The experiments were carried out using a fix volume of water and several apparatus powers were selected. The results obtained shown that the experimental power was 66% of the theoretical one. The experimentally determined power is shown through all this paper rather than the manufacturer indicative power in the apparatus.

The variables studied in this work were microwave power and time of exposition. Times studied were between 30 and 180 s for the first extraction, 30–120 s for the second extraction and between 60 and 180 s for the third extraction. Experimental microwave power studied were 66 W for the first and second extraction and 66 and 198 W for the third extraction.

Results obtained with one of our marine sediment samples in each acceleration conditions were compared with results obtained with the conventional BCR sequential extraction (Fig. 2). Chromium concentration obtained (with six subsamples of the same sediment) for the first and second extraction at 66 W was compared with the conventional BCR using a *t*-test and no differences were found at 95% of confidence level. For this reason, 30 s at 66 W were chosen as optimum conditions for the first and second extraction. For the third extraction, 66 W power was not enough to extract chromium. Therefore, experiments were done at 198 W working at different times. Chromium concentrations obtained were comparable with the value obtained for the conventional BCR extraction procedure. Thus, heating 1 min at 198 W was selected as optimum condition time for the third extraction.

In the conventional BCR sequential extraction, a digestion step with H_2O_2 was done for 3 h, heated at 85 °C previously to the third extraction. This step has not been accelerated in this work due to the foam formed during the first 2 h that made necessary stir manually the sample.

3.1.3. Calibration and standard addition graphs

The linear relationship between peak absorbance measurements (integrated absorbance) and chromium concentration ($\mu g\,l^{-1}$) in stock solutions and extracts was achieved with a chromium concentration between 0 and 10 $\mu g\,l^{-1}$. However, the solutions injected in the graphite furnace have provided very sensitive signals with 7.5 $\mu g\,l^{-1}$. Accordingly, we selected 7.5 $\mu g\,l^{-1}$ as the maximum levels used in the calibration and standard addition graphs. Standard aqueous solutions containing chromium concentrations 0, 2.5, 5 and 7.5 $\mu g\,l^{-1}$ were used to obtain a calibration graph. The standard addition method was applied over the same range of concentrations using the three different extracts of the marine sediment sample obtained in the sequential extraction procedure developed previously. To compare slopes of the calibration and addition graphs for the three extracts, the *t*-test (95% significance level) [28] was applied and no differences were observed for extracts one and two in several samples but differences were observed for the extract three. Hence, aqueous calibration was used for extracts one and two and standard addition method was used for extract three in each sample.

3.1.4. Sensitivity

Sensitivity was studied by means of three parameters: limit of detection (LOD), limit of quantification (LOQ) and characteristic mass (m_o) [29].

The limits of detection and quantification were 0.7 and 2.5 $\mu g\,l^{-1}$, respectively, for extract one; 0.8 and 2.7 $\mu g\,l^{-1}$, respectively, for extract two; 0.3 and 0.9 $\mu g\,l^{-1}$, respectively, for extract three. Considering that we obtained 10 ml of acetic acid extract, 10 ml of hydroxylammonium hydrochloride extract and 12.5 ml of ammonium acetate extract from 0.25 g of sediment the limits of detection and quantification obtained are 0.028 and 0.10 $\mu g\,g^{-1}$, respectively, for extract one; 0.032 and 0.11 $\mu g\,g^{-1}$, respectively, for extract two; 0.015 and 0.045 $\mu g\,g^{-1}$, respectively, for extract three.

Characteristic mass, m_o, is defined as the mass of analyte in picograms required to give a signal of 0.0044 for the integrated absorbance, the results were 3.0 ± 0.5 pg, 2.8 ± 0.5 pg and 3.0 ± 0.8 pg for extracts one, two and three, respectively.

3.1.5. Precision and accuracy

The over-all procedure was applied to five marine sediment sub-samples obtaining a chromium concentration of $0.191 \pm 0.006\,\mu g\,g^{-1}$ for the first extract, $0.126 \pm 0.008\,\mu g\,g^{-1}$ for the second and $5.85 \pm 0.16\,\mu g\,g^{-1}$ for the third. The within-batch precision obtained were 3.3, 6.2 and 2.7% for extracts one, two and three, respectively, the best reproducibility was obtained for extracts one and three. To study the accuracy of the method, the Certified Reference Material CRM 601 and the Certified Reference Material CRM 701 were used (Table 3). The CRM 601 is a lake sediment that has only certified the chromium concentration for the first extraction; the certified value is $0.36 \pm 0.04\,\mu g\,g^{-1}$. This reference material was subjected to the conventional sequential

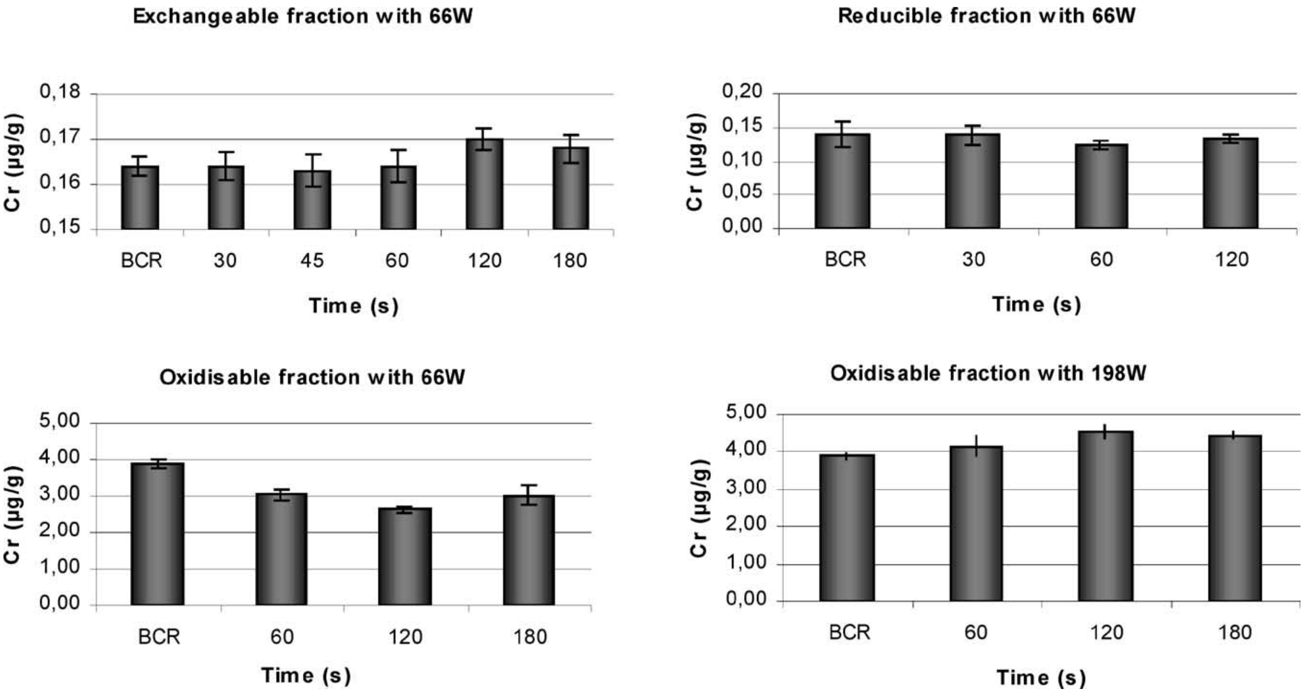

Fig. 2. Comparison of the chromium extracted with the conventional BCR protocol and the accelerated procedures, heating at different times and powers in the microwave oven.

extraction BCR procedure and the accelerated procedure developed in this work. Chromium concentrations found were $0.390 \pm 0.018\,\mu g\,g^{-1}$ and $0.390 \pm 0.015\,\mu g\,g^{-1}$, respectively.

We studied the extractable chromium content only in the first and the third step with our microwave extraction procedure, using the CRM 701, other certified lake sediment. This material follows a modified procedure in the reductive extraction with hydroxylammonium hydrochloride. Therefore, the second step is carried out following a new extraction protocol but the others follow the classic scheme. The authors of this new certified material used a concentration of $NH_2OH{\cdot}HCl$ (0.5 M) and an extractant at pH = 1.5, that was different from the conventional scheme proposed by the Bureau Community of Reference. For this reason, the first and the third steps were analysed with the accelerated optimised conditions, but not the second one. The conventional shake was followed to separate the reductive fraction. Results of our analysis are given in Table 3.

Table 3
Chromium concentration in certified material analysed with the optimised accelerated conditions using microwave energy

	Cr obtained ($\mu g\,g^{-1}$)	Certified and indicative values ($\mu g\,g^{-1}$)
CRM 601		
Fraction 1	0.39 ± 0.02	0.36 ± 0.04[c]
CRM 701		
Fraction 1	2.26 ± 0.04	2.26 ± 0.16
Fraction 2	43 ± 2[a]	46 ± 2
Fraction 3	138 ± 5	143 ± 7
Residual fraction	75 ± 3[b]	63 ± 7
Total extracted and residual	258 ± 10	254 ± 16
Total chromium content	282 ± 2[b]	272 ± 20

[a] Fraction two in the modified BCR protocol was applied by shaking.

[b] Total and residual fraction in CRM 701 were obtained analysing slurries by ETAAS [33]. The number of the analysis replicates in each sample was $n = 6$ and values of average and S.D. were consistent.

[c] Certified value ($\mu g\,g^{-1}$).

With these results, we can conclude that the method developed in this work is accurate for the first and third extractions. Results are comparable with the ones obtained with the conventional BCR procedure.

The recovery of the chromium added was studied for the three steps. Recoveries obtained with one of the marine sediment sample analysed were 97.4, 99.2 and 102.3% for extracts one, two and three, respectively. In all cases, we concluded that the recoveries obtained were suitable.

3.2. Applications

The developed method was applied to chromium fractionation in different marine sediment samples from Ria de Arousa (NW of Spain). The samples were collected by triplicate in the station points showed in Fig. 3a. Three subsamples from each sediment and two solutions were prepared for the accelerated sequential BCR extraction procedure, for chromium fractionation. The results were obtained (mean ± S.D.) by analysing six sub-samples in each sampling station of Arousa. The distribution along the estuary is shown in Fig. 3b–d. Exchangeable fraction (b) (first extract of the microwave accelerated BCR), reducible fraction (c) (second

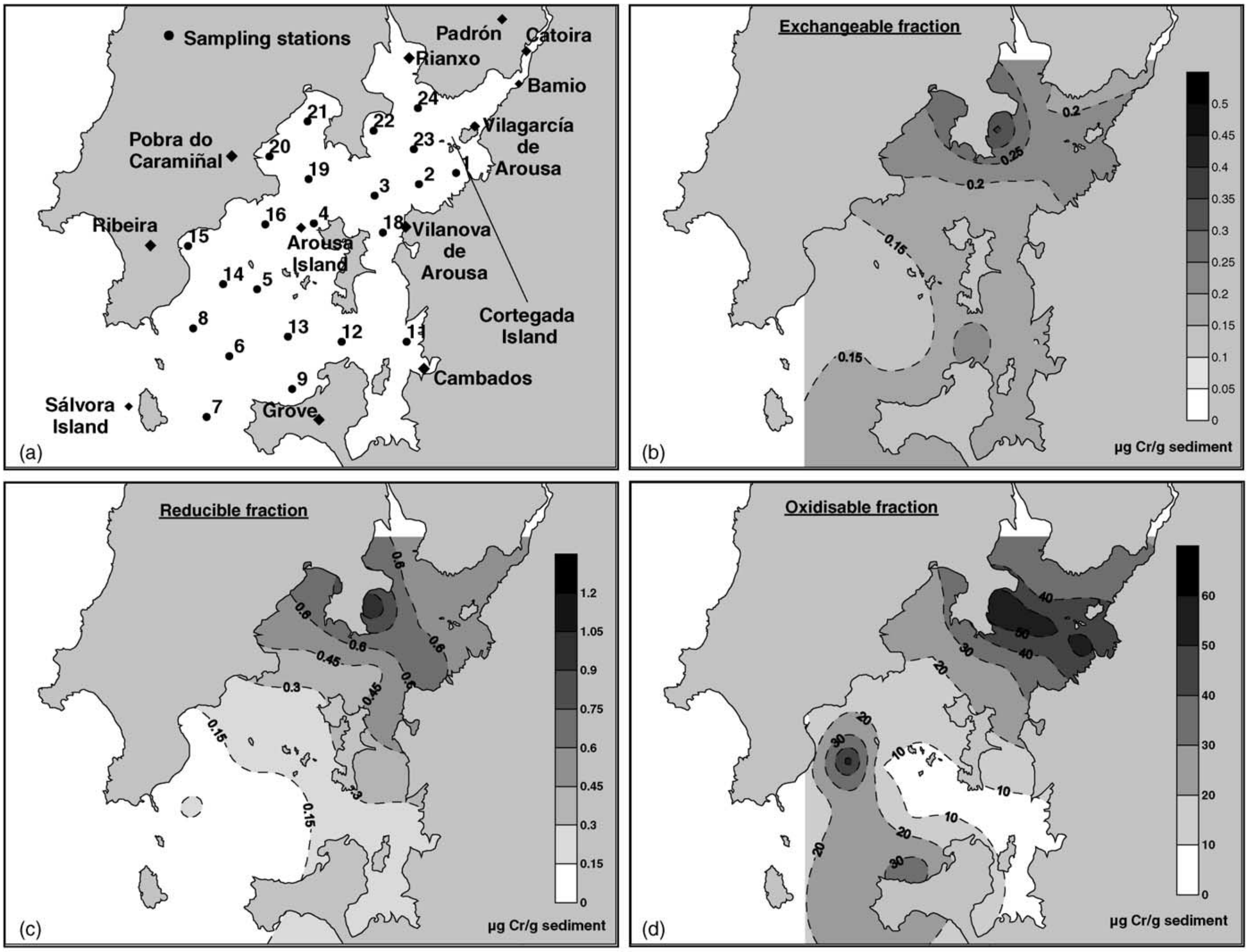

Fig. 3. (a) Sampling stations of marine sediment in Arousa estuary. (b) Chromium distribution along Arousa: exchangeable fraction. (c) Chromium distribution along Arousa: reducible fraction. (d) Chromium distribution along Arousa: oxidisable fraction.

extract) and oxidisable fraction (d) (third extract) are shown. Values obtained ranged from 0.10 to 0.36 $\mu g\,g^{-1}$ for the exchangeable fraction, 0.05 to 1.02 $\mu g\,g^{-1}$ for the reducible fraction and 5.5 to 60.0 $\mu g\,g^{-1}$ for the oxidisable fraction.

The most mobile fraction is the chromium water-soluble and chromium associated to soluble carbonates (first extract of the BCR procedure). Therefore, this fraction is the most easily exchangeable with the water column and the aquatic biota. The places that showed higher levels of exchangeable chromium were located in the northern margin of the estuary, around stations 21–24. Near to this area, anthropogenic activities are considerably higher than in the southern part of the estuary and the currents promote the accumulation of pollution close to the northern margin in the inner part of Arousa estuary. The lowest concentrations in this fraction were found near to the Atlantic Ocean end of the estuary. This suggests that the chromium concentration decreases gradually from the possible anthropogenic sources towards the ocean end.

The reducible fraction (second extract of the microwave accelerated BCR sequential extraction) is the chromium associated with iron or manganese oxides and hydroxides [13]. This sediment layer sometimes is very thin and the organic layer could be enough to obstruct the pollutant contact with the oxides. If chromium in the water column reaches the oxide layer, most of it will be trivalent chromium. The metal will combine with iron yielding a heterogeneous oxide, or it will suffer a redox reaction to be transformed into its trivalent state [30]. The behaviour of chromium distribution and the metal levels obtained in the reducible fraction agree with the results obtained in the first extract. Minimum chromium concentrations were close to the Atlantic Ocean end (between O Grove Peninsula, Sálvora Island and Ribeira). Higher chromium concentrations were found in the same area than the exchangeable fraction but also next to stations (one to two), in the southern margin of the inner estuary. Again, the lowest chromium concentrations were found close to the Atlantic Ocean end where chromium was slightly higher than the limit of detection (LOD = 0.032 $\mu g\,g^{-1}$ and minimum chromium detected was 0.050 $\mu g\,g^{-1}$).

The oxidisable fraction corresponded to the highest chromium levels obtained. This fraction is the chromium combined with the organic layer as well as the chromium associated with sulphur compounds. Although this fraction is not apparently available to the water column, this layer is the most important one in the sediments. Clams and other biota, filter and digest these organic compounds [31,32]. The inner part of the estuary is again the most contaminated. Chromium concentration range was between 40 and 60 $\mu g\,g^{-1}$ in this area. These results agree again with the higher chromium concentration in the reducible fraction. However, for the oxidisable fraction, reasonable high concentrations of chromium were also found close to the ocean end, next to stations eight and nine.

It is clear for the current research that chromium appears inside the estuary and is diluted towards the ocean. In our previous studies of Arousa about total chromium content in sediments, we found levels between 43 and 185 $\mu g\,g^{-1}$ with a similar distribution towards the ocean [33]. Carballeira and coworkers [34,35] suggested 30 $\mu g\,g^{-1}$ for chromium as the background value for this estuary (in the <63 μm fraction of the marine sediments). Therefore, according to our total metal content analysis in Arousa, the estuary could be described as a polluted area. However, results obtained with the fractionation protocol applied in the present study, show that the available fractions represent a low percentage of the total metal content.

4. Conclusions

Fast versions of the three-stage BCR sequential extraction have been developed. The use of microwave energy provides a large saving in extraction time relative to conventional shaking. The time is reduced from ca. 51 h, used in the conventional protocol, to approximately 3 h in the accelerated protocol. The results obtained with this method agree with those obtained by conventional procedure and therefore, the modified protocol is recommended. There are no changes in the sequential extraction composition of each extract, reflected clearly in results of the reference materials analysed. The developed method takes shorter than other methods proposed in the literature. For example, there are publications [36] where the researchers used an ultrasonic bath or a microwave oven for shortening the BCR protocol reducing the three steps to 100 s using microwave oven, working at 300 W, and 20 min for the first and second extraction using an ultrasonic bath. Therefore, the chromium concentration in the first fraction, obtained for these authors for the certified sediment sample BCR 601, using a microwave oven, did not agree with the certified value. There was also a time reduction [18] to 1 min for the first and second extraction and at 30 s for the third extraction for Cu, Cr, Cd, Pb, Ni determination in river sediments using an ultrasonic processor.

Chromium content in marine sediments shows that the metal appears in the river end, near to the anthropogenic activities, and it is transported along the estuary towards the open ocean. Maximum levels were obtained around the same place in the inner part of the estuary and close to the ocean end. The higher levels were analysed in the third extract of the BCR. They corresponded with the chromium associated to the organic layer. Although the exchangeable chromium is quite low, as well as the metal associated with the oxides layer, the metal combined to the organic materia was the highest percentage of the total metal extracted in Arousa sediments, and could be an uptake source to biota such as bivalves and organisms that live inside the Arousa sediments or filtrate the Arousa seawater.

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