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# Matrix solid phase dispersion-assisted BCR sequential extraction method for metal partitioning in surface estuarine sediments

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ABSTRACT

The BCR (the Community Bureau of Reference) of the European Union sequential extraction scheme for metal partitioning in estuarine sediments has been accelerated by using a matrix solid phase dispersion (MSPD) approach. The MSPD assisted BCR procedure consists of passing the extractants proposed by conventional BCR protocol (0.11 M acetic acid, 0.1 M hydroxylammonium chloride and 8.8 M hydrogen peroxide plus 1 M ammonium acetate) through the dispersed sample packaged inside a disposable syringe. Different silica-, magnesium- and aluminium-based materials were tested as dispersing agents and sea sand was found to offer the best performances. Variables for assisting the three stages of the BCR protocol were optimized, and accurate results were obtained when assisting the first and the third stages (exchangeable and oxidizable fractions, respectively). However, lack of accuracy was observed when assisting the second step (reducible fraction) and this result agrees with most of the assisted BCR procedures for which extracting the reducible fraction is the most troublesome stage. The organic matter oxidation (third stage) was successfully assisted by passing hydrogen peroxide at 50 °C through the dispersed sample inside de syringe just before passing ammonium acetate. Therefore, the time-consuming and unsafe conventional organic matter oxidation processes, commonly performed even for microwave/ultrasounds assisted BCR procedures, are totally avoided. Inductively coupled plasma-mass spectrometry (ICP-MS) was used as a selective detector. The target elements were Cd, Co, Cr, Mn, Ni, Sr and Zn (first stage), Cd, Co and Ni (second stage), and Co, Cr, Mn, Ni, Sr and Zn (third stage). Repeatability of the method ($n=7$) was good, and RSDs values of 9, 10, 10, 8, 8, 3 and 8% was obtained for Cd, Co, Cr, Mn, Ni, Sr and Zn, respectively (first stage); 10, 9 and 9% for Cd, Co and Ni, respectively (second stage); and 6, 2, 3, 4, 7 and 9% Co, Cr, Mn, Ni, Sr and Zn, respectively (third stage). The procedure was also validated by analysing two certified reference materials (CRM 601 and CRM 701). Good accuracy was obtained for the target elements extracted at the first stage: Cd ($4.0\pm0.1$ and $7.3\pm0.09$ $\mu g\,g^{-1}$ in CRM 601 and CRM 701, respectively), Cr ($0.36\pm0.008$ and $2.21\pm0.08$ $\mu g\,g^{-1}$ in CRM 601 and CRM 701, respectively), Ni ($8.0\pm0.3$ and $15.4\pm0.3$ $\mu g\,g^{-1}$ in CRM 601 and CRM 701, respectively) and Zn ($262\pm3$ and $203\pm3$ $\mu g\,g^{-1}$ in CRM 601 and CRM 701, respectively). Also, good accuracy was observed for elements extracted at the third step: Cd ($1.8\pm0.09$ and $0.29\pm0.03$ $\mu g\,g^{-1}$ in CRM 601 and CRM 701, respectively), Cr ($145\pm4$ $\mu g\,g^{-1}$ in CRM 701), Ni ($8.2\pm0.7$ and $15.1\pm0.5$ $\mu g\,g^{-1}$ in CRM 601 and CRM 701, respectively) and Zn ($45\pm0.7$ $\mu g\,g^{-1}$ in CRM 701).



## 1. Introduction

The toxicity of metals in the aquatic environment depends on the physicochemical form of the metals (simple or complex ions, oxides or hydroxides, or hydro-soluble organometallic complexes, etc.); and also, on the type of particle to which the metal is adsorbed or retained, and on the nature and strength of the adsorption/retention mechanisms [1]. Thus, factors that control the concentration of trace metals in sediments can be split into physical and chemical factors, all of them closely interrelated. Physical factors refer to physical properties of the sediment particles, and they play a key role in the concentration of trace metals onto or within the different constituents. These properties are: particle size (which is one of the most significant factors controlling the retention capacity and concentration of metals in sediments) [2], spherical surface, specific weight, surface charge, porosity, and permeability, among others [2,3]. Chemical factors are those related to physical–chemical mechanisms by which trace metals are retained

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in the sediment particles. The most important are adsorption, precipitation/coprecipitation, formation of organometallic bonds, incorporation into crystalline structures by substitution, and cation exchange. The formation of organometallic bonds can attach metals to organic matter by *in situ* or biological processes, while the incorporation into crystals occurs by metal replacements in the solid, mainly due to solid–liquid interactions in which the ionic radio and charges of the replaced and the target metals play an important role [2,4]. Cation exchange refers to a material's capacity for capturing dissolved cations and gives off an equivalent amount of another cation to the solution. The mechanisms of this process are not entirely clear, but it is related to the negative charges in the structure of clays (Si–OH, HO–Al–OH and Al–OH interactions) and iron (Fe–OH), and to organic matter through –COOH and –OH functional groups [5].

Taking to account the different physical and chemical factors described above, it is evident that metals can be associated to different components of sediments or soils, and the strength of this association will be different. As a function of the strength of this association, there are fractions of metals that could easily interact (metals less adsorbed or retained onto/within sample constituents) or not with other environmental compartments (water or biota). This information is useful for characterizing environmental samples based on metal mobility or availability. These metal fractionation studies can be performed by using single or sequential extractions (different extractants with increasing extraction capacity) [6], and both often address operationally defined fractions which identify certain groups of elements without clear identification [7,8]. Although in some cases a particular single extraction can be related to a certain chemical form of a metal, such extraction procedures are mainly developed for isolating a particular metal-containing matrix phase; e.g., a water soluble fraction or an exchangeable fraction, which will be more available; a carbonate bound fraction, which could be available under acid conditions; manganese and iron oxides and moderately reducible oxides, which contain metals that are easily or moderately reducible; and an organically bound fraction, which contains metals bound to easily extractable organic matter.

Different sequential extraction procedures to assess the fraction of metals belonging to some of the defined phases listed above have been developed in recent years. Most of these methodologies are based on the use of specific reagents to release certain fractions [6,9,10], but also methods using non-specific reagents, such as nitric acid, have been proposed [11,12]. However, comparisons among different laboratories in assessing metal mobility or availability can be difficult because the significance of the analytical results is dependent on the extraction procedure used. This fact has led to the development and establishment of standardized single and sequential extraction protocols by the Standard, Measurements and Testing Programme (SM&T, formerly BCR – The Community Bureau of Reference) of the European Union [13]. The BCR protocol proposes a standardized three-stage extraction procedure (BCR EUR 14763 EN) [14], which consists of a first stage (extraction with 0.11 M acetic acid) to release elements weakly absorbed on the sample surface, elements involved in ion-exchange processes and elements co-precipitated with carbonates (water-soluble, exchangeable, and carbonate bound phases); a second stage (extraction with 0.11 M hydroxylammonium chloride) to mobilize easily or moderately reducible phases (iron/manganese oxides); and a third stage (digestion with 8.8 M hydrogen peroxide and extraction with 1.0 M ammonium acetate) to extract elements related to organically bound and sulfide fraction phases. This BCR protocol has been applied for metal partitioning studies in several environmental samples such as marine and river sediments, soils, sludge and atmospheric particulate matter [15–17].

However, a practical drawback of BCR procedures is the long time required to complete the whole procedure. This is mainly due to the long extraction time for each stage (16 h), additional centrifugation and rinsing steps, and an organic matrix digestion process during the third stage. Therefore, several attempts have been developed to speed up the BCR protocol. Ultrasound energy (ultrasonic water baths and ultrasonic probes) [5,18–22] and microwave energy [23–25] have been commonly used for assisting the three stages of the BCR scheme. Good agreement between extractability of certain fractions (mainly exchangeable and carbonate-bound) after ultrasound assistance and conventional BCR procedures has been obtained [15]; however, significant changes on the amount of metal extracted have been reported when using microwave heating [15,18].

The objective of the current work has been the novel application of matrix solid phase dispersion (MSPD) to speed up the BCR protocol. MSPD, firstly introduced by Barker et al. [26], is an appealing sample pre-treatment used mainly for isolating organic compounds from solid and semi-solid samples [27,28], and recent applications have focused on the extraction of organometallic compounds [29,30]. MSPD consists of solid sample architecture disruption by mechanical blending with a solid support bonded-phase [27,28,31]. After blending, a new sample matrix-solid support phase is formed and analytes tend to be more weakly bonded to it. This new sample-support mixture is then transferred to a cartridge or syringe, the extractants are directly added (extraction can be performed by gravity or vacuum-assisted), and the extracts are finally obtained without need of a centrifugation step. Because analytes are more weakly associated to the new sample-support phase, extraction can occur using less-toxic reagents/solvents and mild operating conditions. In addition, the possibility of performing a clean-up step simultaneously or just before extraction is another practical advantage derived from MSPD procedures. In this work, the possibilities of MSPD for assisting the BCR protocol for metal partitioning studies in marine sediment samples have been investigated for each stage of the BCR protocol. Special attention has been devoted for avoiding the long organic matrix oxidation process during the third stage of the BCR protocol when releasing the metal fraction associated with the organically bound phase.

## 2. Materials and methods

### 2.1. Instrumentation

Metals were determined by using an 820-MS inductively coupled plasma mass spectrometer (Varian, Mulgrave, Australia), equipped with a SPS3 autosampler (Varian) and a MicroMist nebulizer (Varian). A vacuum manifold station (Waters, Milford, MA, USA) connected to a vacuum pump (Millipore Co., Bedford, MA, USA) was used for MSPD. A Nahita glass mortar (50 mL capacity) with a glass pestle (Auxilab S.L., Beriáin, Navarra, Spain) was used to achieve sample dispersion. Dispersed samples were packaged in 10 mL Injekt plastic syringes (Braun, Melsungen, Germany), between 10 mL replacement 20 μm polyethylene frits (Supelco, Bellefonte, PA, USA). A Vibromatic-384 mechanical shaker from Selecta (Barcelona, Spain) was used for performing conventional BCR. Extracts were separated by using a Centromix model 540 centrifuge (Selecta), while organic matter was digested by using a PL 3920 hotplate from Raypa (Barcelona, Spain). An ORION 720A plus pH-meter with a glass–calomel electrode (ORION, Cambridge, UK) was used for pH measurements. Albet cellulose acetate syringe filters (0.45 μm) were from Albet-Hahnemuehle (Dassel, Germany). Surface estuarine sediments were freeze-dried by using a LYPH–LOCK 6 L freeze dry system, model 77530 from Labconco Corporation (Kansas City, MO, USA), and the dried <63 μm fraction

was obtained by sieving with nylon mesh sieves (CISA, Barcelona, Spain). Scanning electron microscope JEOL 6360-LV (Tokyo, Japan) was used for SEM pictures.

### 2.2. Reagents

Ultrapure water, resistivity 18 MΩ cm, obtained from a Milli-Q water-purification system (Millipore, Bedford, MA, USA) was used throughout this work. Single standard solutions (1000 mg $L^{-1}$) of Cd, Co, Cr, Cu, Mn, Mo, Ni, Sr, V and Zn were from Merck (Darmstadt, Germany). Diatomaceous earth, 95% $SiO_2$; C18 octadecyl-functionalized silica gel; and active magnesium silicate (Florisil), 60–100 mesh, used as dispersing agents, were from Aldrich Chemical Co. (Milwaukee, WI, USA). Alumina, aluminium oxide 90 active neutral, 70–230 mesh (also used as a dispersing agent) was from Merck, while sea sand (washed) QP, $SiO_2$ was from Panreac (Barcelona, Spain). Acetic acid (0.11 M) was prepared from 99.8% acetic acid (Panreac). Hydroxylamine hydrochloride (0.1 M) was from analytical reagent grade hydroxylamine hydrochloride (Merck). Ammonium acetate (1 M) was prepared from analytical reagent grade ammonium acetate (Aldrich). Nitric acid, 65%, and hydrogen peroxide, 33% (m/v), were obtained from Panreac. Tellurium chloride, scandium (in nitric acid), and germanium (in water) standard solutions, 10,000 mg $L^{-1}$, used as internal standards, were from SCP Science (Montreal, Canada). Lake sediment (CRM 601) and lake sediment (CRM 701) certified reference materials were obtained from the Community Bureau of Reference of the European Union (Brussels, Belgium).

To avoid metal contamination, all glassware and plastic ware was washed and kept for 48 h in 10% (v/v) nitric acid, then rinsed several times with Milli-Q water before use.

### 2.3. Estuarine sediment samples

Surface estuarine sediment samples were collected in triplicate from different sampling points along the Ría de Arousa estuary (northwestern Spain) by a van Veen grab. Samples were subjected to a freeze dry procedure at −40 °C. After sieving, the <65 μm grain size was isolated, and this fraction was stored in polyethylene bottles with hermetic seals [25].

### 2.4. Conventional three-stage BCR procedure

The extractants used (0.11 M acetic acid for the first step; 0.1 M hydroxylammonium chloride at pH 2 for the second step; and hydrogen peroxide digestion and 1 M ammonium acetate at pH 2 for the third stage), as well as the extraction conditions for conventional three-stage BCR procedure were performed as described elsewhere [14]. The procedure implies different extraction steps of 16 h and an organic matrix oxidation for 2 h.

### 2.5. Matrix solid phase dispersion assisted-BCR procedure

Approximately 0.5 g of dried sediment was weighted and then blended thoroughly with 1.0 g of sea sand (dispersing agent mass to sample mass ratio of 2) in a glass mortar (50 mL capacity) for 5 min using a glass pestle. This mixture was quantitatively transferred by using a powder funnel to a 10 mL syringe containing a 20 μm polyethylene frit. Then, a second polyethylene frit was placed at the top of the syringe and was slightly compressed with a syringe plunger to remove air and avoid preferential channels. The syringe was placed in a vacuum manifold station and 10 mL of 0.11 M acetic acid was added. A vacuum pump was then connected to obtain a drop by drop elution, and further volumes of extractant (0.11 M acetic acid) were added to complete 25 mL (this operation took approximately 30 min). The eluated extract (first fraction) was finally made up to 25 mL with Milli-Q water and stored in PTFE bottles (4 °C) before analysis. After 0.11 M acetic acid elution, the sediment-dispersing agent mixture was washed by passing 10 mL of Milli-Q water (drop by drop elution). This washing step took 20 min. Then, 25 mL (10 + 10 + 5 mL) of 0.1 M hydroxylammonium chloride (pH adjusted to 2 with nitric acid) was added, and the second fraction was obtained in 40 min (drop by drop elution). This second extract was made up to 25 mL with Milli-Q water and was kept at 4 °C before analysis. A second rinsing step was then performed by using 10 mL of Milli-Q water (drop by drop elution for 20 min). Then, 10 mL of 8.8 M hydrogen peroxide, heated to 50 °C, was added, and a complete drop by drop elution was achieved in 60 min. Then, 25 mL (10 + 10 + 5) of 1.0 M ammonium acetate (adjusted at pH 2 with nitric acid) was passed through the syringe under pressure (drop by drop) for about 30 min. Both hydrogen peroxide and ammonium acetate extracts were combined and made up to 50 mL with Milli-Q water. This third extract was also stored in a PTFE bottle and was kept at 4 °C before analysis. In some of the samples, dispersing agent particles or sample particles reached the solution as a consequence of the reaction between organic matter and hydrogen peroxide inside the syringe. In these cases, the hydrogen peroxide–ammonium acetate solution was filtered through a 0.45 μm cellulose acetate syringe filter. Matrix blanks were prepared by packing 1 g of sea sand (dispersing agent) and performing all steps described above. Low or negligible values for blanks were obtained for all target elements and BCR stages.

**Table 1**
Operating ICP-MS conditions.

| | | |
|---|---|---|
| General | Radiofrequency power/W | 1400 |
| | Peristaltic pump speed/mL $min^{-1}$ | 0.45 |
| | Stabilization delay/s | 35 |
| | Number of replicates | 3 |
| | Nebulizer type | MicroMist |
| Gas flows/L $min^{-1}$ | Nebulizer | 0.99 |
| | Plasma | 17.0 |
| | Auxiliary | 1.65 |
| | Sheath | 0.24 |
| | Skimmer cone | Nickel |
| | Sampler cone | Nickel |
| Torch alignment/mm | Sampling death | 8.0 |
| Ion optics/V | First extraction lens | −12 |
| | Second extraction lens | −180 |
| | Third extraction lens | −220 |
| | Corner lens | −202 |
| | Mirror lens right | 30 |
| | Mirror lens left | 25 |
| | Mirror lens bottom | 34 |
| | Entrance lens | 3 |
| | Fringe bias | −2.2 |
| | Entrance plate | −39 |
| | Pole bias | 0 |
| CRI/mL $min^{-1}$ | Skimmer gas source | $H_2$ |
| | Sampler gas source | OFF |
| | Skimmer flow | 40 |
| | Sampler flow | 0 |
| Mass-to-charge-ratio | Cd | 114 |
| | Co | 95 |
| | Cr | 52 |
| | Mn | 55 |
| | Ni | 60 |
| | Sr | 88 |
| | Zn | 66 |

### 2.6. ICP-MS measurements

Multi-element determinations were performed by ICP-MS using the operating conditions given in Table 1. The use of $H_2$ in the collision cell at a flow rate of 40 mL $min^{-1}$ gave the best sensitivity as

well as minimizing possible polyatomic interferences for the target elements [32]. Determinations were performed by using aqueous standards in 0.11 M acetic acid (first stage), in 0.1 M hydroxylammonium chloride (second stage), and 8.8 M hydrogen peroxide 1 M ammonium acetate (third stage), covering metal concentrations within the 0–1000 $\mu g\,L^{-1}$ range. Scandium (Sc45) was used as an internal standard for Cr and Mn; germanium (Ge72) for Co, Ni, Sr and Zn; and tellurium (Te125) for Cd. All internal standards were used at a concentration of 500 $\mu g\,L^{-1}$.

## 3. Results and discussion

### 3.1. Preliminary studies: selection of the solid support at the first stage of MSPD-assisted BCR

Different surface estuarine sediment samples were pooled (approximately 100 g) and the pool was analyzed for conventional BCR procedure in triplicate. Results showed metal concentrations higher than the limit of detection for Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Se, Sn, Sr, V and Zn after the first extraction step (exchangeable and acid soluble fraction). Then, a screening experiment was performed to select the proper solid support (dispersing agent) for MSPD. Different silica-based materials, such as octyl-bonded silica (C18); diatomaceous earth (DE); and sea sand, were tested. These agents are believed to facilitate disruption and dispersion, especially for biological materials [26], as well as for on column clean up procedures [26,27]. In addition to silica-based supports, magnesium- and aluminium-based materials, such as Florisil (synthetic magnesia–silica gel) and alumina (aluminium oxide) were also tested as dispersing agents for estuarine sediments. For each dispersing agent, three MSPD assisted BCR replicates were performed with 0.5 g of a dry pooled estuarine sediment and 2.0 g of supporting agent (dispersing mass to sample mass ratio of 4), and eluting with 20 mL of 0.11 M acetic acid. Matrix blanks (2.0 g of each supporting agent) was also prepared. Negligible concentrations were obtained for some elements such as Cu, Mo, Pb and Se when performing MSPD assisted BCR. However, high concentrations were measured for the remaining metals mainly when using DE, alumina and sea sand as solid supports (Fig. 1). Results from Fig. 1 show that Cd and Co concentrations in acetic acid extracts after MSPD with alumina, DE and sea sand as dispersing agents are comparable with those obtained after the conventional BCR method. However, higher Cr, Mn, Ni, Sr and Zn concentrations have been found in the extracts for the use of DE, while alumina offered higher Cr and Ni concentrations and lower Mn, Sr and Zn levels when comparing to conventional BCR. These high concentrations can be explained by assuming that dispersion with these supporting agents allows the extraction of the target elements bound to other phases different to the exchangeable bound phase. In addition, higher metal concentrations than those obtained after conventional BCR could also be attributed to a certain contamination from the slid support material. In fact, high matrix blanks for these elements were obtained when using alumina and DE as dispersing agents. Finally, Florisil and C18 gave low or negligible values for all selected elements.

Although silica-based materials (DE) have been found as adequate when extracting organometallic compounds (arsenicals) from biological materials [29], there is no data on the application of these materials for extracting inorganic compounds (metals). The evaluation of data from Fig. 1, led us to choice sea sand as a compromise solid support for MSPD assisted BCR. Scanning electron microscopy (SEM) pictures taken from estuarine sediment and dispersed estuarine sediment with sea sand as a solid support (Fig. 2A and B) were obtained, and dispersed sample (B) offers large agglomerates, which can indicate certain association between the sample constituents and the dispersing agent after blending. According to MSPD theory a new sample-dispersing agent mixture is formed, and weaker interactions between analytes and the dispersed matrix than those in the original sample could be obtained [26,33].

### 3.2. Effect of the sea sand mass to sample mass ratio and the volume of 0.11 M acetic acid for the first stage of MSPD-assisted BCR

Different dispersing agent mass to sample mass ratios, ranging from 2 to 8, were investigated. A sample mass of 0.5 g was used for all experiments, and sea sand masses from 1 g (sea sand mass to sample mass ratio of 2) to 4 g (sea sand mass to sample mass ratio of 8) were tested in triplicate. Elution was performed by using 20 mL of 0.11 M acetic acid. Table 2 gives results for selected elements after blank substration. It can be seen that this variable is not important when extracting Cd, Co, Ni and Sr (similar concentrations after MSPD assisted BCR with all sea sand mass to sample mass ratios and after conventional BCR). However, efficiencies for Cr, Mn and Zn extraction are low when dispersing with higher sea sand masses. Therefore, a sea sand mass to sample mass ratio of 2 (0.5 g of sample and 1 g of sea sand) could be chosen as a compromise condition. Typical value for this parameter is within 2 and 4 in most of MSPD applications [27].

The volume of extractant (0.11 M acetic acid) was studied within the 10–25 mL range. Fig. 3 shows that the extraction capacities increase when the extractant volume is higher. Therefore, concentrations of the selected elements in the acetic acid extracts after eluting with 20 and 25 mL are similar to those obtained after conventional BCR; and 25 mL of 0.11 M acetic acid was finally chosen. This extractant volume is quite near the recommended acetic acid volume for conventional BCR [14].

### 3.3. Effect of the volume of 0.1 M hydroxylammonium chloride for the second stage of MSPD-assisted BCR

After fixing conditions for extracting the target elements in the first step (exchangeable fraction), a number of experiments were performed to release the reducible fraction. Several volumes (within the 10–25 mL range) of 0.1 M hydroxylammonium chloride at pH 2.0 were tested in triplicate. A blank was also obtained for each volume of extractant. For all cases, extracts were diluted to 25 mL with ultrapure water. After measurements, metal concentrations were compared with those metal concentrations obtained after conventional BCR (Fig. 4). It can be seen that the extraction efficiency is higher when the extractant volume increases, and Cd, Co and Ni concentrations are similar to those found after conventional BCR when using a volume of 25 mL. However, extractive efficiencies for other target elements were low, even when using high extractant volumes. Higher concentrations of hydroxylammonium chloride, up to 0.5 M as originally proposed by Rauret et al. [34] and later by other authors [35], were tested; but lack of accuracy for Cr, Sr and Zn was still observed. This result agrees with those obtained by several authors when assisting BCR, which have shown inaccurate values for the reducible fraction [15,17]. In addition, as pointed out by Davidson et al. [35], unacceptable variability in results obtained for the reducible fraction during certification of the certified reference material CRM 601, led to a re-evaluation of the extraction protocol by increasing hydroxylammonium chloride concentration and by decreasing the pH of the extractant to 1.5 [36], or even to the replacement of hydroxylammonium chloride by ammonium oxalate [35], a reagent commonly used by soil scientists to estimate amorphous iron oxides in soil. The low extractive efficiencies for the reducible fraction by the proposed MSPD-assisted BCR will be more noticeable when discussing the analysis of CRMs. Lack of accuracy will be observed for both CRM 601 and CRM 701,

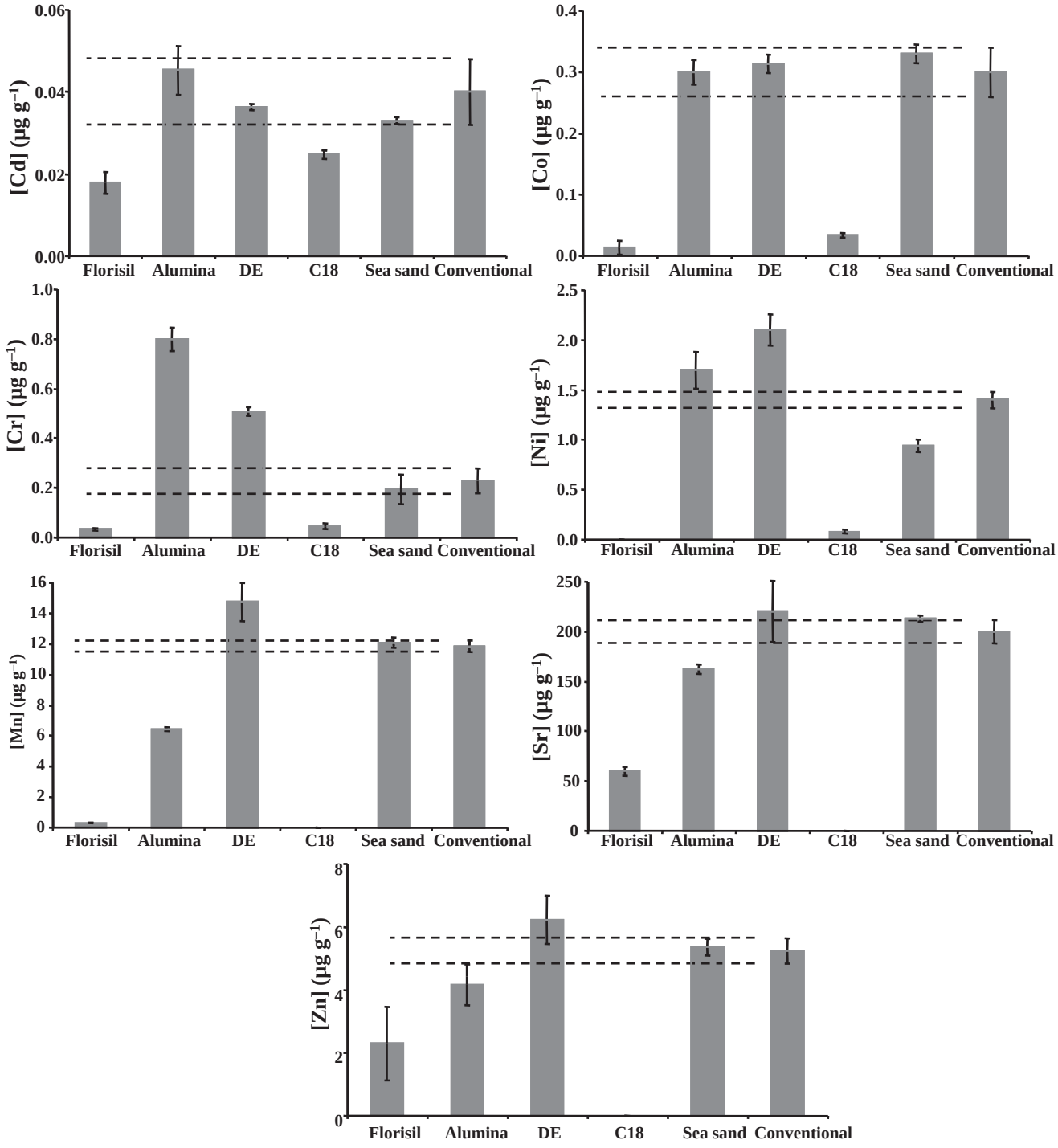


**Fig. 1.** Effect of the nature of the solid support for the first stage of the MSPD assisted BCR procedure when extracting Cd, Co, Cr, Mn, Ni, Sr and Zn from surface estuarine sediments ($N = 3$).

**Table 2**
Effect of the dispersing agent (sea sand) mass to sample mass ratio (DA/S ratio) for assisting first BCR stage.

| DA/S ratio | Concentrations expressed as µg g$^{-1}$ [a] | | | | | | |
|---|---|---|---|---|---|---|---|
| | Cd | Co | Cr | Mn | Ni | Sr | Zn |
| 2 | 0.040 ± 0.00099 | 0.29 ± 0.020 | 0.24 ± 0.025 | 11.8 ± 0.317 | 1.5 ± 0.033 | 200 ± 1.66 | 4.7 ± 0.27 |
| 3 | 0.044 ± 0.0013 | 0.30 ± 0.025 | 0.22 ± 0.033 | 12.1 ± 0.374 | 1.5 ± 0.215 | 211 ± 3.88 | 4.5 ± 0.49 |
| 4 | 0.046 ± 0.0027 | 0.28 ± 0.0014 | 0.21 ± 0.034 | 12.1 ± 0.446 | 1.6 ± 0.139 | 228 ± 5.06 | 4.4 ± 0.12 |
| 5 | 0.042 ± 0.0040 | 0.29 ± 0.010 | 0.19 ± 0.016 | 10.1 ± 0.547 | 1.6 ± 0.018 | 221 ± 6.86 | 3.9 ± 0.36 |
| 6 | 0.044 ± 0.0012 | 0.27 ± 0.019 | 0.18 ± 0.026 | 8.64 ± 0.864 | 1.7 ± 0.148 | 230 ± 1.09 | 3.9 ± 0.13 |
| 8 | 0.043 ± 0.0033 | 0.27 ± 0.032 | 0.19 ± 0.022 | 6.15 ± 0.771 | 1.6 ± 0.173 | 234 ± 1.06 | 3.6 ± 0.071 |
| Conventional BCR | 0.041 ± 0.0078 | 0.30 ± 0.040 | 0.23 ± 0.050 | 11.8 ± 0.373 | 1.4 ± 0.080 | 199 ± 11.5 | 5.2 ± 0.40 |

[a] $N = 3$.

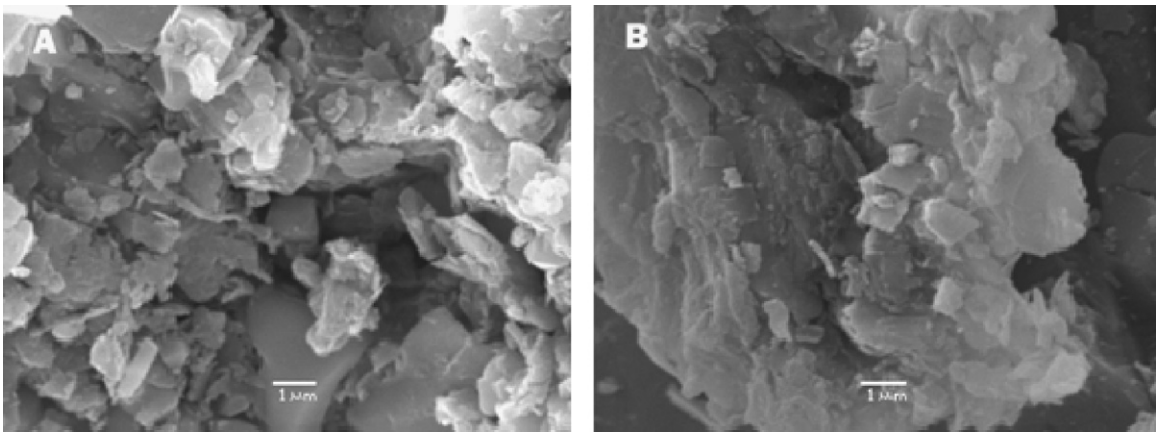


**Fig. 2.** Scanning electron microscopy pictures for estuarine sediment (A) and dispersed estuarine sediment (B).

even for elements such as Cd, Co and Ni, which offered similar concentrations in the hydroxylammonium chloride extracts after MSPD-assisted BCR and conventional BCR procedures when using the pooled sediment.

### 3.4. Evaluation of an integrated hydrogen peroxide digestion and ammonium acetate extraction for the third stage of MSPD-assisted BCR

One of the main drawbacks of the conventional BCR procedure is the long hydrogen peroxide digestion step (which has to be performed twice) as a previous step for extracting the oxidizable fraction (organic matter/sulfide bound). Several experiments were performed with the residue from step 2 inside the MSPD syringe to digest the organic matter before extraction. Preliminary experiments consisted of passing different volumes (5 and 10 mL) of 8.8 M hydrogen peroxide (at room temperature as well as heating to 50 °C) before eluting with 1.0 M ammonium acetate (at pH 2). Higher concentrations in the extracts before passing ammonium acetate were obtained when using 10 mL of 8.8 M hydrogen peroxide previously heated to 50 °C (Fig. 5). Fig. 5 also shows that concentrations of the target elements in the hydrogen peroxide solution (without ammonium acetate extraction) were lower than those obtained after performing conventional BCR. In addition, the omission of the hydrogen peroxide digestion stage was also considered, and several extractions with residues from step 2 were performed with 1.0 M ammonium acetate. Results plotted in Fig. 5 show that an oxidation step is necessary to obtain the complete release of the oxidizable fraction by ammonium acetate. Therefore, comparable concentrations of the target elements in hydrogen peroxide–ammonium acetate extracts after MSPD assisted BCR and conventional BCR procedures were obtained when performing an on-column oxidation with 10 mL of 8.8 M hydrogen peroxide at 50 °C, followed by an extraction with 25 mL of 1.0 M ammonium acetate (Fig. 5). Although the omission of the oxidation stage was not possible, the developed on-column organic matter digestion is faster and safer than the digestion step proposed by conventional BCR and by most of the microwave/ultrasounds-assisted BCR procedures, which perform a conventional hydrogen peroxide oxidation step before assisting ammonium acetate extraction [25].

### 3.5. Analytical performances

The limit of detection (LOD) and the limit of quantification (LOQ), based on the $3\sigma/m$ and $10\sigma/m$ criterion ($\sigma$, the standard deviation of eleven measurements of a blank; and $m$, the slope matched calibrations) were calculated. Table 3 lists the LODs and LOQs, expressed as ng g$^{-1}$, for each stage of the assisted BCR procedure. It can be seen that these values are low enough to perform the fractionation of the selected elements in estuarine sediments.

The repeatability of the over-all procedure was also assessed by subjecting the pooled sediment sample seven times to the optimized MSPD assisted BCR procedure, and by determining the selected elements in all extracts (seven extracts) from each BCR stage. RSD values (also listed in Table 3) are lower than 10% for all cases.

Accuracy of the proposed assisted BCR method was assessed by analyzing the two certified CRM 601 and CRM 701 lake sediments, which offer certified concentrations for Cd, Cr, Ni and Zn in the first BCR step (CRM 601 and CRM 701); for Cr, Ni and Zn (CRM 601) and Cd, Cr, Ni and Zn (CRM 701) in the second BCR step; and for Cd and Ni (CRM 601) and Cd, Cr, Ni and Zn (CRM 701) in the third BCR stage. Each CRM was prepared in triplicate following the optimized MSPD assisted BCR procedure, and extracts from each BCR stage were analyzed in triplicate by ICP-MS. Table 4 shows concentrations found in each CRM after subtracting matrix blanks. Accurate results were obtained for Cd, Cr, Ni and Zn in both CRM 601 and CRM 701 after MSPD assisted first stage BCR (exchangeable fraction) and for Cd and Ni in CRM 601, and Cd, Cr, Ni and Zn in CRM 701 at the third stage of the assisted

**Table 3**
Limits of detection, limits of quantification and repeatability.

| | Repeatability, RSD[a]/% | LOD[b]/ng g$^{-1}$ | LOQ[b]/ng g$^{-1}$ |
|---|---|---|---|
| *First stage* | | | |
| Cd | 9 | 0.0557 | 0.186 |
| Co | 10 | 0.133 | 0.444 |
| Cr | 10 | 2.99 | 9.96 |
| Mn | 8 | 1.71 | 5.70 |
| Ni | 8 | 0.498 | 1.66 |
| Sr | 3 | 1.22 | 4.07 |
| Zn | 8 | 39.8 | 133 |
| *Second stage* | | | |
| Cd | 10 | 0.796 | 2.65 |
| Co | 9 | 0.250 | 0.833 |
| Ni | 9 | 2.97 | 9.90 |
| *Third stage* | | | |
| Co | 6 | 4.60 | 15.3 |
| Cr | 2 | 121 | 403 |
| Mn | 3 | 23.0 | 76.7 |
| Ni | 4 | 171 | 570 |
| Sr | 7 | 48 | 160 |
| Zn | 9 | 134 | 447 |

[a] $N = 7$.
[b] $N = 11$.

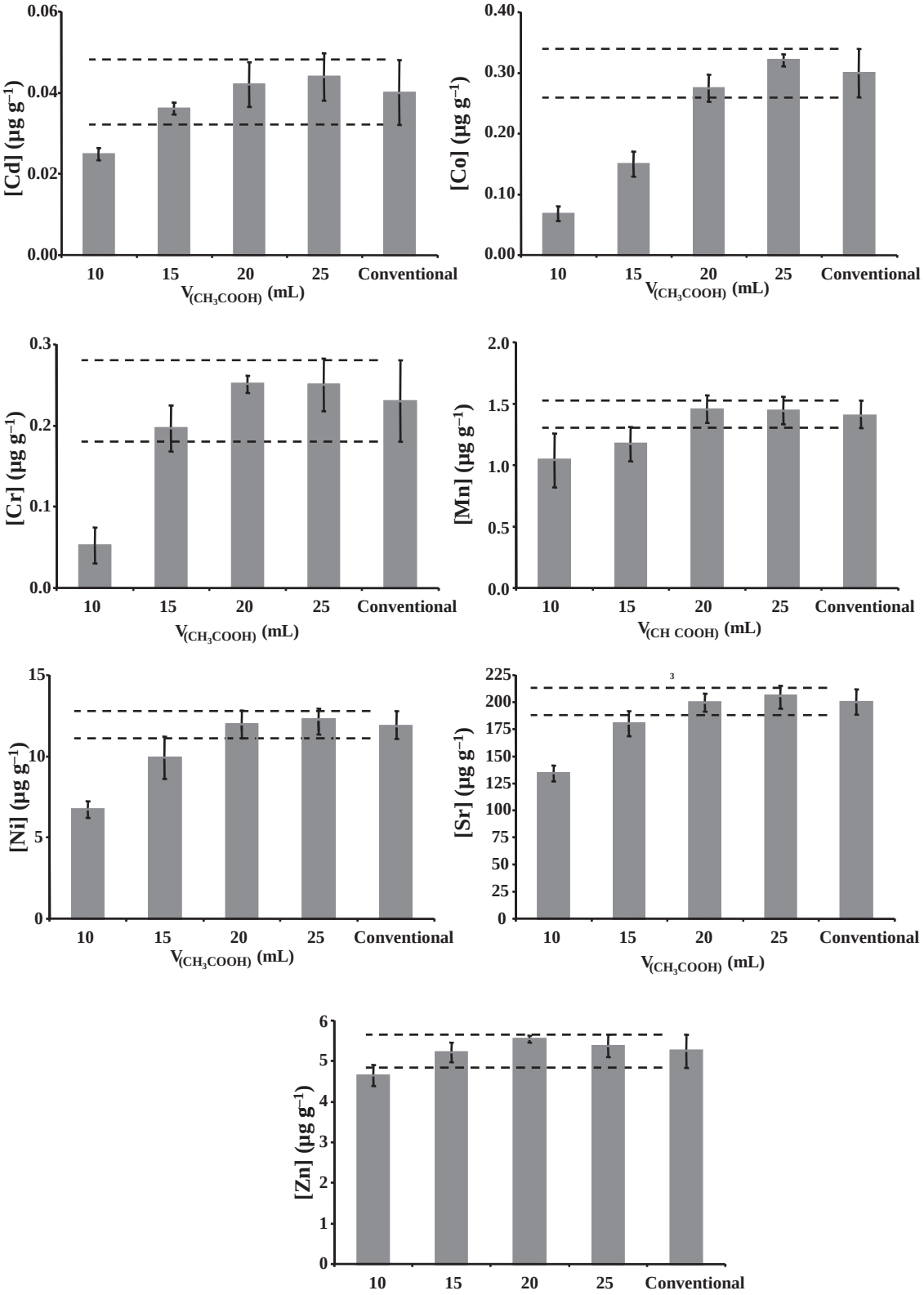


**Fig. 3.** Effect of the volume of 0.11 M acetic acid solution for the first stage of the MSPD assisted BCR procedure when extracting Cd, Co, Cr, Mn, Ni, Sr and Zn from surface estuarine sediments ($N = 3$).

BCR procedure (oxidizable fraction). This fact has been confirmed after applying a *t*-test (95% confidence interval eight degrees of freedom), showing $t_{cal}$ (Table 4) lower than $t_{tab} = 2.31$ for Cd, Cr, Ni and Zn in extracts from the first and third stages of the BCR protocol. Lack of accuracy was observed when analyzing both CRMs for Cd, Cr, Ni and Zn in extracts derived from the second BCR stage, even for elements such as Cd and Ni, whose extracted concentrations were similar to those from the second stage of conventional BCR (Fig. 4). Concentration found in both CRMs when analyzing extracts from the second stage were from 20 to 50 fold

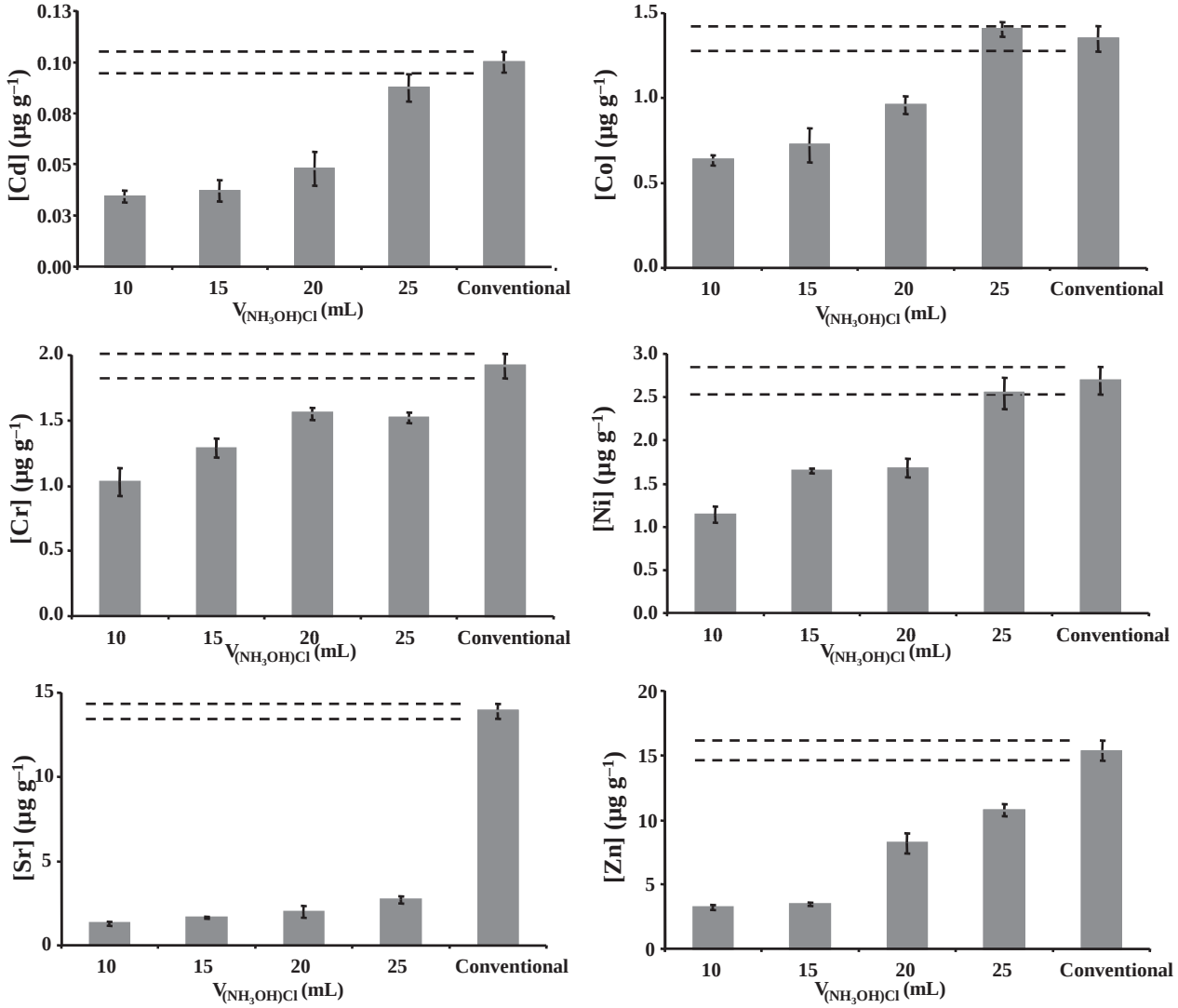


**Fig. 4.** Effect of the volume of 0.1 M hydroxylamine hydrochloride solution for the second stage of the MSPD assisted BCR procedure when extracting Cd, Co, Cr, Ni, Sr and Zn from surface estuarine sediments ($N = 3$).

lower than the certified concentrations. As previously noted, good agreement between extractability of exchangeable/carbonate-bound and oxidizable (organic matter-bound) fractions after ultrasound/microwave assistance and conventional BCR procedures has been reported [15]; but lack of accuracy is commonly found for the reducible fraction. The low metal recoveries of the reducible fraction could be explained by assuming the presence of certain organic compounds having characteristics of recalcitrant organic matter, which are able to bound the target metal and decease the extraction efficiency.

**Table 4**
Analysis of CRM 601 (lake sediment) and CRM 701 (lake sediment) by the proposed MSPD assisted BCR method.

| | First stage | | | Third stage | | |
|---|---|---|---|---|---|---|
| | Certified/(g g$^{-1}$ | Found/(g g$^{-1}$ [a] | $t_{cal}$ [b] | Certified/(g g$^{-1}$ | Found/(g g$^{-1}$ [a] | $t_{cal}$ [b] |
| *CRM 601* | | | | | | |
| Cd | 4.14 ± 0.230 | 4.04 ± 0.150 | 2.00 | 1.83 ± 0.200 | 1.76 ± 0.0898 | 2.30 |
| Cr | 0.36 ± 0.040 | 0.36 ± 0.0077 | 0.00 | –[c] | | – |
| Ni | 8.01 ± 0.730 | 7.98 ± 0.340 | 0.26 | 8.55 ± 1.04 | 8.23 ± 0.729 | 1.32 |
| Zn | 264 ± 5.00 | 262 ± 2.97 | 2.02 | –[c] | | – |
| *CRM 701* | | | | | | |
| Cd | 7.34 ± 0.350 | 7.28 ± 0.0849 | 2.12 | 0.270 ± 0.0600 | 0.293 ± 0.0310 | 2.22 |
| Cr | 2.26 ± 0.160 | 2.21 ± 0.0847 | 1.77 | 143 ± 7.00 | 145 ± 3.87 | 1.55 |
| Ni | 15.4 ± 0.900 | 15.4 ± 0.301 | 0.00 | 15.3 ± 0.900 | 15.1 ± 0.481 | 1.25 |
| Zn | 205 ± 6.00 | 203 ± 3.23 | 1.86 | 45.7 ± 4.00 | 45.4 ± 0.677 | 1.33 |

[a] $N = 9$.
[b] $t_{cal} = (\left| [\,]_{certtified} - [\,]_{found} \right| \sqrt{N})/SD_{found}$, $N = 9$; $t_{tab}$ (95%, 8) = 2.31.
[c] Not given.

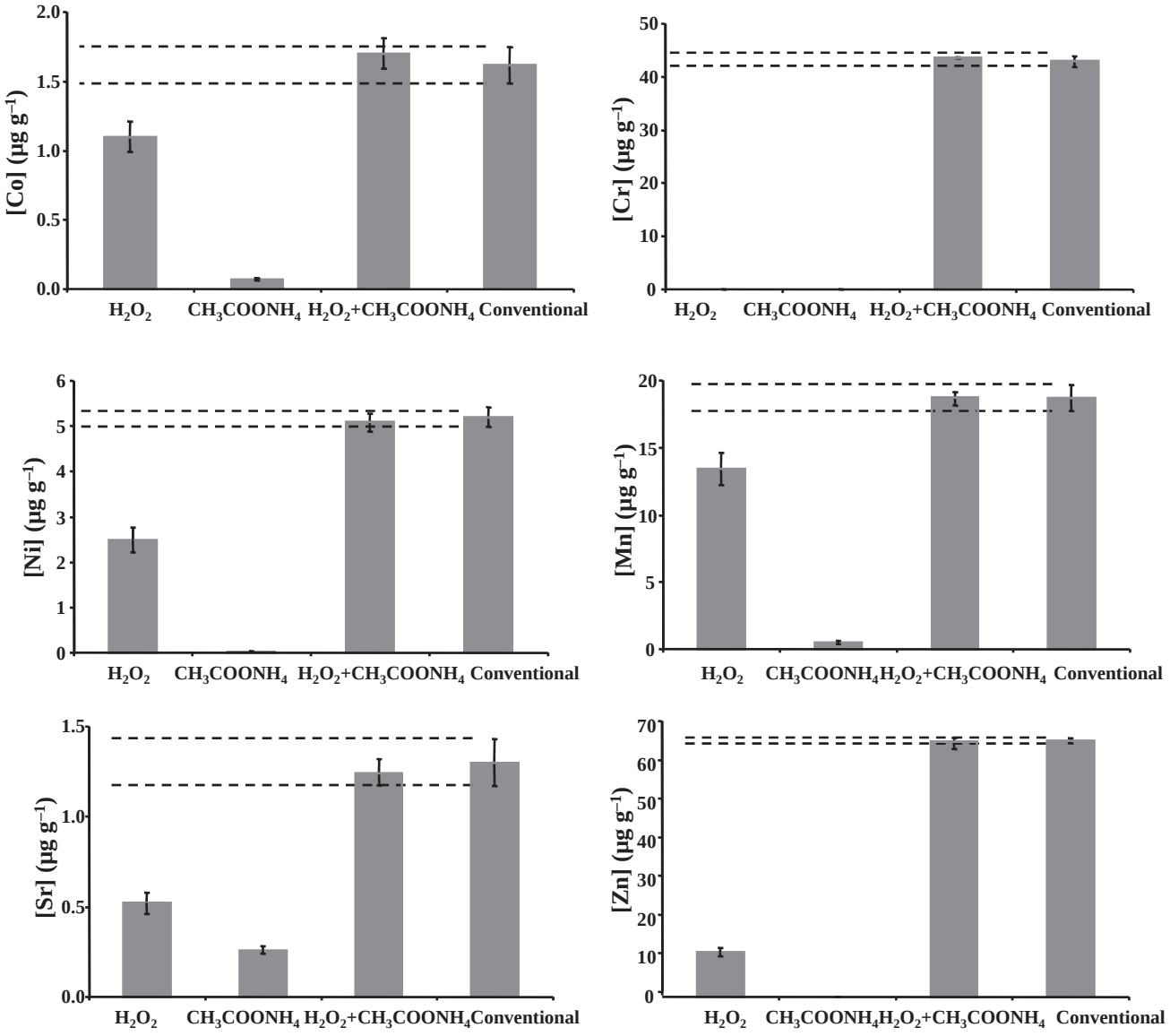


**Fig. 5.** Effect of the on-column organic matter decomposition – ammonium acetate extraction for the third stage of the MSPD assisted BCR procedure when extracting Co, Cr, Mn, Ni, Sr and Zn from surface estuarine sediments ($N = 3$).

## 4. Conclusions

The application of MSPD to assist the BCR sequential extraction procedure has offered accurate results for certain metals loosely bound or bound to carbonate (exchangeable fraction) and for those metals bound to organic matter (oxidizable fraction). However, metals bound to iron/manganese oxides (reducible fraction) were not accurately extracted by the proposed MSPD assisted BCR procedure. These results are quite similar to those obtained by other authors when assisting the second step of the BCR protocol, meaning that the extraction of the reducible fraction is the most troublesome stage. However, although lack of accuracy has been observed for the reducible fraction, the proposed MSPD assisted BCR offers several advantages over the conventional BCR protocol and other microwave/ultrasounds assisted BCR procedures. First, all sequential extractions are performed with the dispersed sample inside a syringe, thus repetitive centrifugation steps to isolate the extracts and the rinsing wastes are not necessary. Therefore, the proposed procedure is fast, while losses of the residue are avoided because transfers of sample-extract mixtures to centrifuge tubes are not needed. This is especially important when assisting with microwave energy, for which the extraction is performed in high pressure reactors. Second, the oxidation step is successfully assisted by passing heated hydrogen peroxide solution through the syringe containing the dispersed sample. In most of the microwave/ultrasounds assisted BCR methods, although the extraction with ammonium acetate could be assisted (speeded up), the hydrogen peroxide oxidation stage was conventionally performed. As noted above, this step is time-consuming and requires special safety operating conditions. These drawbacks are avoided when using MSPD for assisting the BCR protocol. Therefore, this application for assisting sequential extraction procedures can become a new trend of MSPD approaches; furthermore, the potential for assessing bioavailability, bioaccessibility and mobility of trace elements in other environmental samples such as soils or sludge should be explored.

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