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Comparative study of digestion methods EPA 3050B (HNO_3–H_2O_2–HCl) and ISO 11466.3 (aqua regia) for Cu, Ni and Pb contamination assessment in marine sediments

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ABSTRACT

Knowing the metal extraction capacity of a digestion method is crucial for a better environmental interpretation of metal concentrations determined in sediments. One of the main problems at the present is the lack of harmonization of information obtained by two of the most popular sediment partial digestion methods: ISO 11466.3 (aqua regia) and EPA 3050B (HNO_3–H_2O_2–HCl). In the present work, the amount of Cu, Ni and Pb leached by using both methods was compared with the total content of those elements in marine sediments collected, as an example, from the Cienfuegos Bay, Cuba. Similar amounts of Cu were extracted by both methods; while leaching of Ni and Pb were different. Generally, the EPA method extracted more Ni than the ISO method. In contrast, Pb was extracted in a larger amount by the ISO method. Some explanations are given for the observed results. X-ray Diffraction, X-ray Fluorescence, Particle Induced X-ray Emission Spectrometry and Energy Dispersive X-ray coupled to Scanning Electron Microscopy were employed for this purpose. On the other hand, none of the methods studied extracted simultaneously the fraction of all the metals, probably provided by human activity (Theoretical Anthropogenic Fraction) in both sediments studied. The use of ISO 11466.3 or EPA 3050B is recommended since the analytical performance parameters of both, in combination with Flame Atomic Absorption Spectrometry, are adequate. For a better environmental interpretation of the analytical results, information on the extraction efficiency of the selected method for specific elements and sediments under study should also be provided, together with the determined concentrations.

1. Introduction

Sediments have been frequently used for the evaluation of metal contamination in sea ecosystems (Arain et al., 2008). Among metals, Cu, Pb, and Ni are ubiquitous indicators of human pollution, both urban and industrial (Forstner and Wittmann, 1979; Salomons and Forstner, 1984). These are some of the most measured elements in sediments of Cuban bays (Gonzalez, 1989, 1991). In particular, a high concentration of Ni was found along the whole Cienfuegos Bay and considerably high peaks in front of the power plant; while concentration peaks of Cu and Pb were located in sampling points near Cienfuegos city (Pérez Santana et al., 2007).

Nowadays, most analytical techniques used to quantify metal contents in sediments demand the transformation of the sample to aqueous solution (Sneddon et al., 2006). However, there is no international consensus about a unique digestion method that allows the homogeneous interpretation of results obtained in different parts of the world. Single-step (Agemian and Chau, 1976; DIN 38414, 1983; ISO 11466.3, 1995; UNEP/IOC/IAEA, 1995; USEPA 3050B, 1996; Ščančar et al., 2000; Sutherland, 2002; Sahuquillo et al., 2003; Snape et al., 2004; Larner et al., 2008; Townsend et al., 2009) and sequential extractions (Quevauviller et al., 1997; Craba et al., 2004; Pérez Santana et al., 2007) methods have been employed for this purpose.

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Single-step methods are very convenient for routine service analysis of many samples due to their quickness and simplicity. In this group, different solvents or mixtures of them have been used. For example, dilute HCl (Agemian and Chau, 1976; Sutherland, 2002; Snape et al., 2004; Larner et al., 2008; Townsend et al., 2009) and HOAc (UNEP/IOC/IAEA, 1995; Ščančar et al., 2000; Sahuquillo et al., 2003) are two of the most employed. Several mixtures as for example, HNO_3–H_2O_2–HCl (USEPA 3050B, 1996) and HCl–HNO_3 (ISO 11466.3, 1995) have also been used. In this context, we selected EPA 3050B (USEPA 3050B, 1996) and ISO 11466.3 (ISO 11466.3, 1995), which are probably among the most internationally recognized.

Both methods are based on strong acid digestions. In the case of EPA 3050B, the sediment is digested with repeated additions of nitric acid and hydrogen peroxide, followed by additions of hydrochloric acid to the initial digestate. In the second method, the sample is pre-digested overnight in a HCl + HNO_3 mixture (aqua regia) and then heated, filtered and diluted with HNO_3. These procedures are not total digestion techniques for most samples. For example, elements linked to the structures of silicates are not normally dissolved.

The close dependence of the amount of metal extracted by the EPA 3050B and ISO 11466.3 methods with the chemical and physical characteristics of sediments is not always known and then, apparently contradictory results are obtained in environmental studies (Ščančar et al., 2000; Sastre et al., 2002; Pavlíčková et al., 2003; Jakubowska et al., 2006). In some of the revised reports (Ščančar et al., 2000; Sastre et al., 2002), the extracted amount of metals has been related to the compositional/structural characteristics of the sample, while in other works (El Samrani et al., 2005; Bettiol et al., 2008) both the extraction capability of these methods, and the dependence regarding the type of sample and determined element, have been experimentally justified. In addition, ISO 11466.3 (Sahuquillo et al., 2003; Diz, 2005) and EPA 3050B (Diz, 2005; Burton et al., 2005) have been considered as total extraction methods, without an explanation or experimental verification, despite the fact that they do not totally transform some components of many types of sediments to aqueous solution.

The objective of this work was to compare the leaching percentage of Cu, Ni and Pb, extracted by ISO 11466.3 and EPA 3050B methods from six marine sediments selected as representative samples of the Cienfuegos Bay, Cuba. X-ray Diffraction (XRD), Energy Dispersive X-ray Fluorescence (ED-XRF), Particle Induced X-ray Emission Spectrometry (PIXE) and Energy Dispersive X-ray Scanning Electron Microscopy (EDX-SEM) characterization techniques were used to try to explain the behavior of the "metal-sediment-digestion method" system studied.

On the other hand, it was also important to know how the amount of metal extracted by the selected partial digestion methods ISO 11466.3 and EPA 3050B compared to the Theoretical Anthropogenic Fraction (TAF) of metal. TAF is defined in this work as the amount of metal present in the sediment that could be provided by human activity. TAF was determined as the difference between the total metal content in each analyzed sediment and the total metal content in sediment collected in a non-polluted zone. In previous studies (Pérez Santana et al., 2007), the sum of the metal content extracted by the first three steps of the Community Bureau of Reference of the Commission of the European Communities (BCR) sequential extraction method was close to the TAF of the metal. The BCR method is relatively complex and laborious. Thus, it makes sense to continue the search for a single-step method, which allows only the extraction of the TAF. In addition to both strong acid digestion methods EPA and ISO, the Acetic acid (HOAc) extraction method (UNEP/IOC/IAEA, 1995) was also studied for comparison. The Acetic acid method is one of the weakest treatments that is used to effectively remove the weak links of the metals in the sediment and is considered by some authors (UNEP/IOC/IAEA, 1995; Sahuquillo et al., 2003; Ščančar et al., 2000) as a good estimate of anthropogenic pollution. The efficiency in the estimation of TAF by the selected digestion methods was evaluated in two representative samples.

2. Materials and methods

Six sediment samples (S2, S5, S6, S9, S10 and S17), representative of the metal pollution level of the Cienfuegos Bay, Cuba were selected for this study. An increase in pollution level was previously reported (Pérez Santana et al. 2007) from sample S2 to S10 located in the northern zone. Sample S10 was the most contaminated in the whole bay, while sample S17 was the most contaminated in the southern zone (Fig. 1).

Surface sediments (first 10 cm layer) were taken from sampling points with a Petersen dredge. Samples were carefully screened for determining the presence of animals and detritus and then put in polyethylene containers and stored at 4 °C. Before the analysis, samples were sieved to 2 mm and homogenized, each replicate subsample was then hand ground to a powder and left at 40 °C for 48 h.

Four standardized sample treatment methods were employed: ISO 14869-1:2001 total digestion method (ISO 14869-1, 2001), and three different partial digestion methods: EPA 3050B (USEPA 3050B, 1996), ISO 11466.3 (ISO 11466.3, 1995) and the Acetic acid method (UNEP/IOC/IAEA, 1995).

USEPA 3050B method: Around 0.25 g of sample were accurately weighed in a 250 ml Pyrex Erlenmeyer flask and 10 ml of 8.5 M HNO_3 were added. The solution was heated on a hot plate to ~95 °C without boiling and this temperature was maintained for 15 min. After cooling to less than 70 °C, 5 ml of 17 M HNO_3 were added and the sample was refluxed at ~95 °C without boiling until the volume was 5 ml. Addition of 5 ml of 17 M HNO_3 was repeated a second time. Thereafter, the sample was evaporated to ~5 ml without boiling. After cooling to less than 70 °C, 2 ml of water were added followed by the slow addition of 3 ml of 8.8 M H_2O_2. The solution was then heated until effervescence subsided. Later, 4 ml

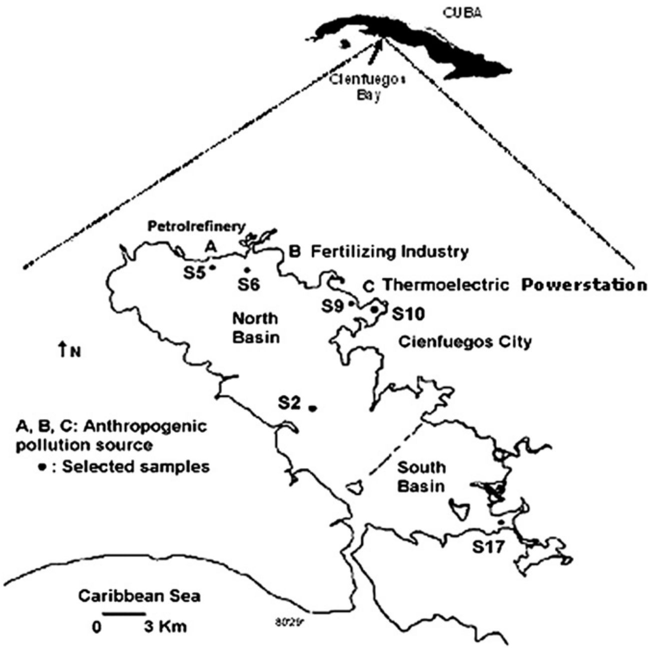

Fig. 1. Map of sampling points in the Cienfuegos Bay.

Table 1
Accuracy ± relative standard deviation of FAAS analysis as recovery percentage for three digestion triplicates.

Analyte	EPA 3050B			ISO 11466.3		
	Concentration added/mg/L					
	100	200	300	100	200	300
Cu	104 ± 4.6	106 ± 3.5	97 ± 5.5	110 ± 2.4	93 ± 2.9	91 ± 2.1
Ni[a]	89 ± 2.0	95 ± 3.3	90 ± 1.7	105 ± 3.6	92 ± 3.1	111 ± 1.9
Pb	90 ± 1.8	109 ± 3.0	102 ± 2.1	114 ± 1.1	94 ± 4.0	89 ± 1.5

[a] For Ni, added concentrations were 150, 200 and 350 mg/L, respectively.

more of 8.8 M H_2O_2 in 1-mL aliquots were added and solution refluxed. After cooling to less than 70 °C, 10 ml of 12 M HCl were added and the sample was refluxed for 15 min. without boiling. After cooling to room temperature, the sample was filtered and diluted to 25 ml with double distilled water.

ISO 11466.3 method: around 0.250 g of sediment were accurately weighed and placed in a 250 ml Pyrex Erlenmeyer flask. First, the pre-digestion step was done at room temperature for 24 h with 10 ml of a (3:1) mixture of 12 M HCl and 17 M HNO_3. Then, the suspension was digested on hot plate at 130 °C for 15 min. The obtained suspension was cooled until room temperature, filtered through an ashless Whatman 41 filter and, finally, diluted to 25 ml with 0.17 M HNO_3.

Acetic acid method: Around 2 g of sample were accurately weighed in a 50 ml polyethylene flask with cap and 25 ml of 50% acetic acid (v/v) were added. Then, the flask was shaken mechanically during 24 hours. The suspension was filtered and diluted to 50 ml with double distilled water.

ISO 14869-1:2001 method: around 0.250 g sample were accurately weighed in a 250 ml polytetrafluoroethylene Erlenmeyer flask and 5 ml of 8.5 M HNO_3 were added. The solution was heated on a hot plate without boiling until the volume was reduced to 1 ml and then the sample was cooled. This step was repeated two more times until a total amount of 15 ml of 8.5 M HNO_3 was added. After cooling, 5 ml of HF and 1.5 ml of $HClO_4$ were added and the sample was refluxed. A second portion of 5 ml of HF and 1.5 ml of $HClO_4$ were added one more time and the sample was kept overnight at room temperature, after which, the volume was reduced again up to 1 ml by refluxing. After cooling, 5 ml of double distilled water plus 2 ml HCl conc. were added with light heating to dissolve the residue. After cooling to room temperature, the sample was filtered and diluted to 50 ml with double distilled water. The total content of elements was used to calculate the TAF values and extraction efficiency of the three partial digestion methods studied for the elements determined.

Extraction efficiency (EE), expressed in percentage, is defined in this work as the ratio of the amount of element extracted by a partial digestion method to the total amount of the same element determined in the sediment by a total digestion method.

Sample blanks were carried out through the entire analytical procedure (sample preparation plus quantification technique) for all digestion methods employed.

Cu, Ni and Pb were quantified by Flame Atomic Absorption Spectrometry (FAAS) in all the sediment solutions. A Pye Unicam 9100 (Philips, Holland) Atomic Absorption Spectrometer with an acetylene/air ($1.5\ \mathrm{L\,min^{-1}}/5.0\ \mathrm{L\,min^{-1}}$) flame and a bandpass of 0.2 nm was used. The absorption lines were: Cu 324.8 nm, Ni 232.0 nm and Pb 217.0 nm. For all the analytes, the compromise observation height was set at 10 mm. The intensity of the current was 4, 12 and 12 mA for the Cu, Ni and Pb lamps, respectively.

Mean concentrations of three digestion triplicates of sediment and the confidence interval for $\alpha = 0.05$ and $n - 1 = 2$ degrees of freedom were calculated for statistical comparison.

The evaluation of the analytical performance of the total digestion method ISO 14869-1:2001 with FAAS quantification was made by determining Ni and Pb in the IAEA-405 Certified Reference Material (CRM) and by a spike recovery study for Cu (since its concentration is not reported in the CRM IAEA-405). The recovery was 106%, 91% and 90% for Cu, Ni and Pb, respectively. The precision, evaluated directly on Cienfuegos sediments as the Relative Standard Deviation (RSD), was better than 6% for all determined metals. On the other hand, the performance of the EPA 3050B and ISO 11466.3 methods with FAAS quantification was evaluated by a spike recovery study. The metal concentrations added were 100, 200 and 300 $\mathrm{mg\,kg^{-1}}$, with the exception of spikes of 150 and 350 $\mathrm{mg\,kg^{-1}}$ for Ni in the sample treated by the aqua regia method. The mean percentage recovery was 102%, 91% and 100% for Cu, Ni and Pb, respectively, for the EPA 3050B method, and it was 98%, 103% and 99% for the ISO method. In summary, all the methods studied showed good analytical performance (Table 1). The detection limit (Table 2) was calculated as the concentration of the analyte that gives a net signal equal to three times the standard deviation of the background level. Background level was estimated as the average of ten replicates of digestion sample blank. In general, small variations of detection limits were observed among digestion methods studied. Nevertheless, the detection limit of Pb slightly increased, when the acetic acid method was used.

The mineralogical composition of the sediments, before and after the treatment by both partial digestion methods ISO and EPA, was obtained by X-ray Diffraction method (XRD), using CuKα radiation ($\lambda = 1.54183$ Å) in a HZG4 goniometer (Carl Zeiss, Jena) equipped with Enraf-Nonious Diffractis 581 generator and a graphite monochromator. The XRD pattern was recorded from 6° to 60° (2θ) in 0.05° steps and 3 s counting time. Elemental qualitative analysis of sediment residues was made with an Energy Dispersive X-ray Fluorescence Spectrometer (ED-XRF), model ED 2000, (Oxford Instruments, England) with silver tube and vacuum pump. The Particle Induced X-ray Emission Spectrometry (PIXE) qualitative analysis used to corroborate the presence of sulphur in the sediments was carried out by the 3 MV Tandetron accelerator using a 2 MeV proton beam. The acquisition time of the spectra was ~300 s. The beam spot at the target position was ~1 $\mathrm{mm^2}$. The characteristic X-rays were detected by a lithium-doped silicon detector, with an energy resolution of about 155 eV at 5.9 keV. The electronics consisted of a Telennec245 amplifier associated with a PCA3 multi-channel analyzer (Oxford Instruments, England).

Table 2
Detection limits[a] of methods for ten replicates of digestion sample blank.

Digestion methods	Cu	Ni	Pb
ISO 14869-1:2001	1.3	1.0	4.9
EPA 3050B	1.0	1.0	2.3
ISO/DIS 11466.3	1.1	2.6	6.4
Acetic acid	4.4	4.2	9.5

[a] Expressed in $\mathrm{mg.kg^{-1}}$.

Semiquantitative determination of Mg, Al, Si, K, Ca, Fe, and Mn, macro components in the sediment residues, was performed by using the Vega TESCAN 5130 SB Energy Dispersive X-ray Scanning Electron Microscope with Inca 350 micro analyzer, liquid nitrogen cooled, and with a resolution of 128 eV.

3. Results and discussions

3.1. *Leaching of metals by EPA 3050B and ISO 11466.3 partial digestion methods*

Extraction efficiency of EPA 3050B and ISO 11466.3 was calculated from the concentrations shown in Fig. 2. Thus, EE of Cu varied for the different samples. EE was ~100% for S9 and it was between 70% and 85% for the remaining samples. Significant differences were not found in the amounts of Cu (Fig. 2a) extracted by both partial methods in each studied sample. These results contradict those reported by Pavlíčková et al. (2003), where the ISO 11466.3 method extracted 12% more Cu than the HNO_3–H_2O_2 method. This last method is similar to the EPA 3050B used in the present work. This disagreement between EE of Cu by the EPA 3050B and ISO 11466.3 reported by Pavlíčková and the EE obtained in the present work could be explained by possible differences in composition of the samples studied in both cases. Unfortunately, the composition of the samples studied by Pavlíčková was not provided and thus, it was impossible to confirm this hypothesis.

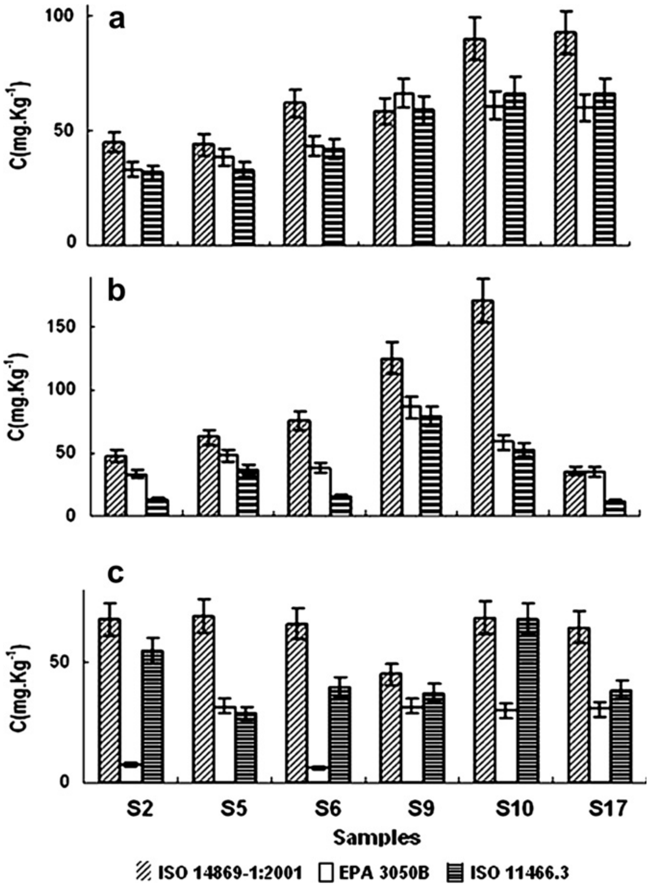

Fig. 2. Comparison of the concentrations of Cu (a), Ni (b) and Pb (c) extracted from sediments by the total digestion method (ISO 14869-1:2001 – ▨) and the partial digestion methods (EPA 3050B – ☐ and ISO 11466.3 – ▤). The confidence interval calculated for $\alpha = 0.05$ and $n = 3$ are included for each measured concentration.

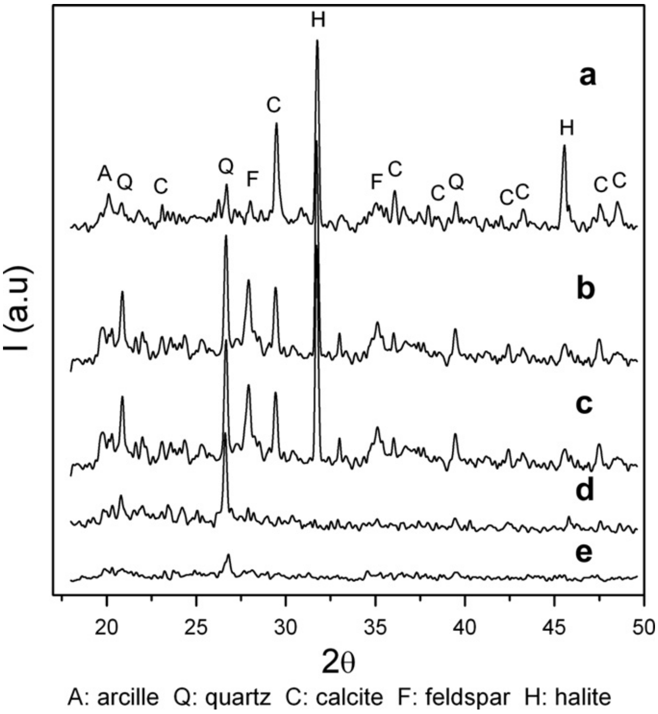

Fig. 3. X-ray diffraction patterns of sediments S5 (3a), S9 (3b) and S10 (3c) before digestion and of the residues of sediment S9 after digestion by EPA 3050B (3d) and ISO 11466.3 (3e) methods. Diffraction patterns of each sample are in different scales.

The non-extracted fraction of Cu could be associated with the silicate phase (quartz), whose presence was verified by X-ray diffraction analysis in all the sediments studied and their residues (see some examples shown in Fig. 3a–c for sediments and Fig. 3d, e for residues, respectively). Therefore, the fraction of Cu associated to this phase was not extracted. The presence of Si, as major element in the residue, was also confirmed by EDX-SEM (Table 3).

On the other hand, the differences in the EE of Cu in the samples can be explained by the different amounts of Cu associated with the silicate phase in each one. For example, the silicate phase (quartz) content of samples S9 and S10 are very comparable as can be confirmed by their similar X-ray diffraction patterns obtained before digestion (Fig. 3b and c). However, the EE of Cu in sample S10 by both partial methods is smaller (~70%) than the corresponding value in sample S9 (~100%). Thus, the amount of Cu linked to the silicate phase is probably larger in sample S10 than in sample S9.

Table 3
Major element concentrations of sediment residues determined by EDX-SEM technique.

Residue of samples	Digestion methods	Metal concentration (%)						
		Mg	Al	Si	K	Ca	Fe	Mn
S2	EPA 3050B	0.1	2.2	33.3	0.6	0.2	0.5	▲
	ISO 11466.3	0.9	7.8	26.4	1.0	0.3	3.4	▲
S5	EPA 3050B	▲	3.2	40.6	0.7	0.4	0.5	▲
	ISO 11466.3	1.0	9.5	30.2	1.2	0.5	4.7	0.5
S6	EPA 3050B	0.2	2.2	31.7	0.5	▲	0.5	▲
	ISO 11466.3	0.9	7.7	22.1	0.5	▲	2.9	▲
S9	EPA 3050B	0.2	3.2	31.4	0.2	0.6	▲	▲
	ISO 11466.3	0.6	9.2	27.3	1.0	0.2	4.0	▲
S10	EPA 3050B	0.4	3.8	33.6	0.2	0.7	▲	▲
	ISO 11466.3	0.9	8.3	26	1.1	0.4	3.2	▲
S17	EPA 3050B	▲	2.5	33.5	0.6	0.2	0.5	▲
	ISO 11466.3	0.5	8.2	30.1	1.3	0.9	0.8	3.3

▲, Concentration is below the instrumental detection limit (0.1%) of the EDX-SEM technique used.

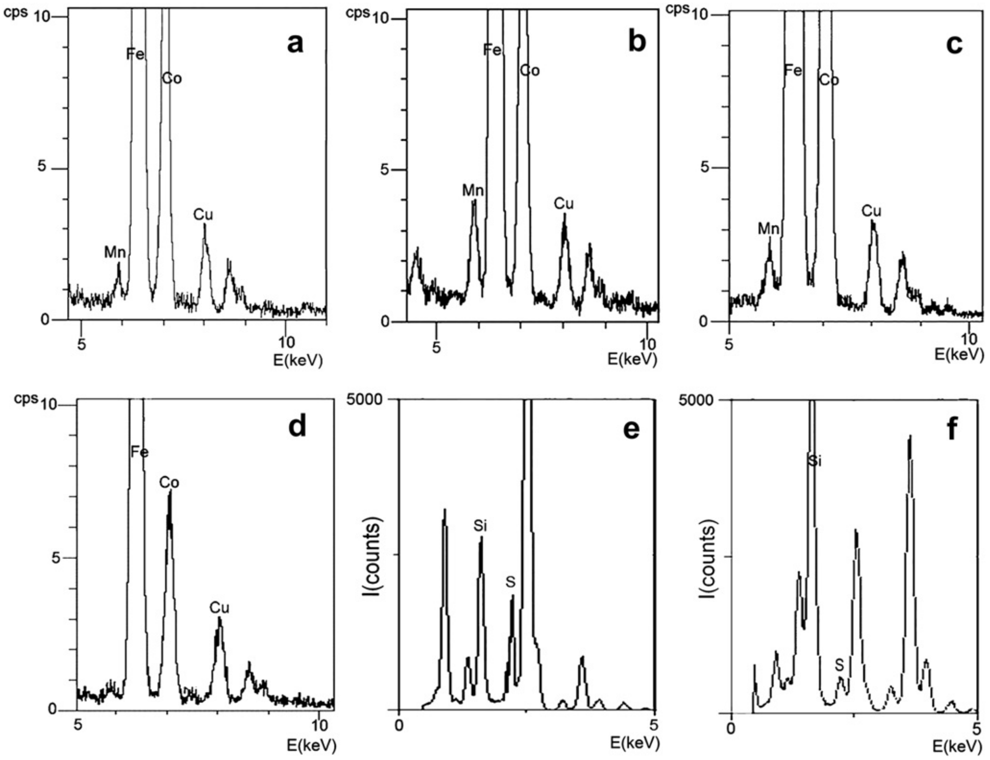

Fig. 4. X-ray fluorescence spectrum of residues obtained from: sediment S9 treated by EPA 3050B (4a); sediment S9 treated by ISO 11466.3 (4b); sediment S10 treated by EPA 3050B (4c) and sediment S10 treated by ISO 11466.3 (4d). The PIXE spectra of sediments S2 (4e) and S17 (4f) were also included.

The presence of Cu in the residues was corroborated by ED-XRF qualitative analysis (Fig. 4).

The EE of Ni by using both partial methods was less than 100% for all samples, except for S17 treated with EPA 3050B. In particular, the EE of Ni by both methods was similar in samples S9 and S10 (Fig. 2b). This behavior is like that observed for the extraction of Cu from all samples and therefore, the explanation in this case would be the same as that given above for Cu. Besides that, the ISO 11466.3 extraction efficiency was 41.2%, 17.7%, 28.9% and 63.9% smaller than that obtained by EPA 3050B for samples S2, S5, S6 and S17, respectively.

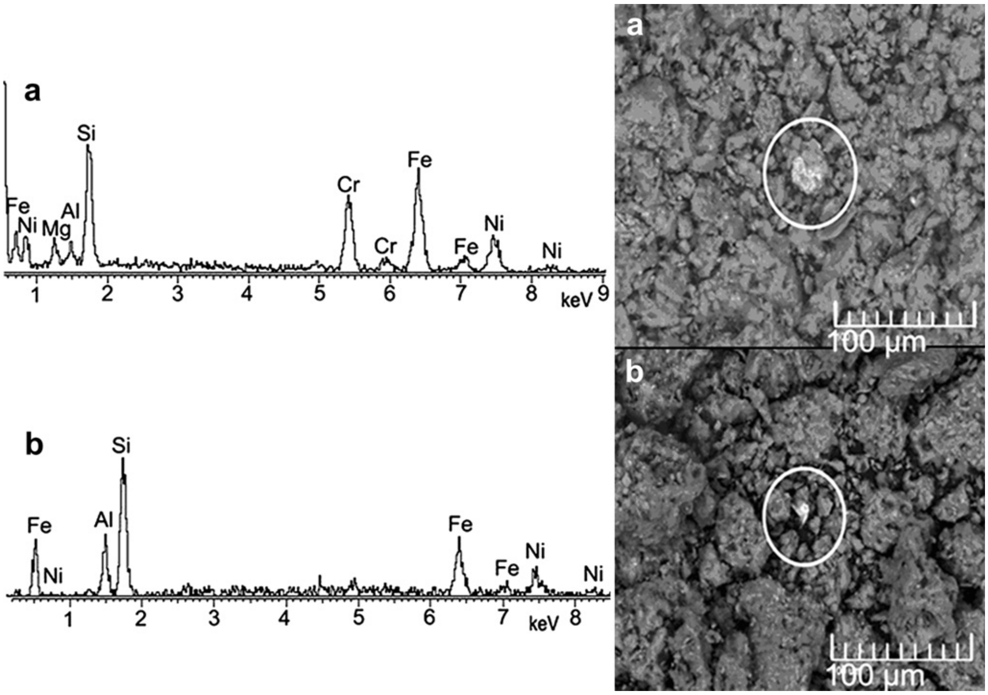

Fig. 5. Shiny particles (inside circles in right side) observed in the mixed residues of sample S9 after treatment by the EPA 3050B (5a) and ISO 11466.3 (5b) methods, and the EDX-SEM spectra of the particles (left side).

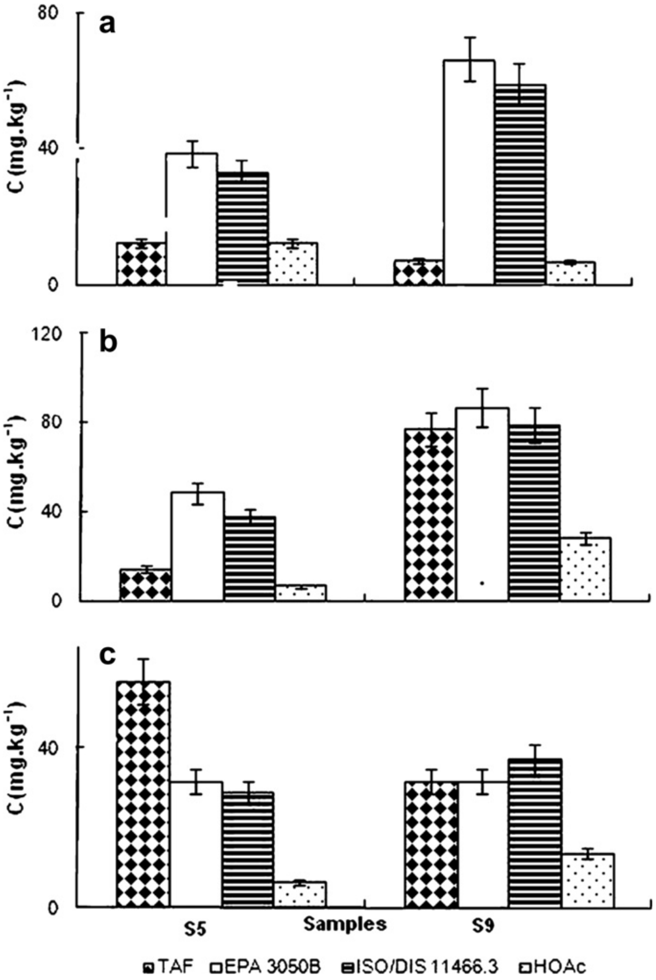

Fig. 6. Comparison of the Theoretical Anthropogenic Fraction (◆◆) estimated for each metal with the concentrations of Cu (6a), Ni (6b) and Pb (6c), extracted by the EPA 3050B (☐), ISO 11466.3 (▤) and HOAc (▨) methods. Mean concentrations of three digestion triplicates and the confidence intervals ($\alpha = 0.05$, $n - 1 = 2$) are shown.

The more complex behavior of Ni respect to Cu could be explained assuming that the non-extracted fraction of Ni is not only associated to the silicate phase. In this context, it should be noted that the concentration of Fe is higher in residues obtained by ISO 11466.3 for S2, S5 and S6 (Table 3). Consequently, it is possible to assume that some part of the Ni is associated to iron oxides and hydroxides, which are not completely dissolved by the aqua regia (Ščančar et al., 2000) used in the ISO 11466.3 method. Contrastingly, iron oxides and hydroxides are dissolved in hot diluted HNO_3 (Krasnodębska-Ostręga et al., 2001), as that used in EPA 3050B. In the case of sample S17, the association of Ni to the manganese oxides and hydroxides can explain the poor extraction of Ni by method ISO 11466.3, as is corroborated by the high content of Mn observed in the corresponding residue (Table 3).

The possible link between Ni and Fe is corroborated by the simultaneous presence of both elements in the residues of the sediments obtained after the application of both partial digestion methods. See, for example, the XRF spectrum measured by EDX-SEM on shiny particles found in the residues of sample S9 (Fig. 5).

The EE of Pb by using both partial methods was less than 100% for all samples, except for S10 treated with the ISO 11466.3 method. This result indicated that some fraction of Pb could be associated to a silicate phase for all the studied samples, except for sample S10. Specifically, the EE of Pb by both methods was similar in samples S5 and S9 (Fig. 2c). As in the case of Cu, this behavior can be explained by the possible association of the non-extracted fraction of Pb to the silicate phase (quartz). For the rest of the samples, the ISO 11466.3 method leached a higher amount of Pb than the EPA 3050B method (Fig. 2c). This fact was particularly significant in samples S2, S6 and S10. The poor extractability of Pb by the EPA 3050B method is explained if we assume that a part of this metal is present in those samples as lead sulphide (PbS). The nitric acid oxidizes the PbS to a slightly soluble salt ($PbSO_4$), which remains in the residues. In contrast, the presence of HCl (aq), as part of the aqua regia used in the ISO 11466.3 method, favours the formation of a lead chloride complex, which is highly soluble and is then easily extracted. The presence of sulphur (S) in the studied sediments was confirmed by PIXE. See, for example, the PIXE spectra of sediments S2 and S17 in Fig. 4e and f, respectively.

3.2. Efficiency of the digestion method in the estimation of the theoretical anthropogenic fraction of metal

The efficiency of the 3050B, ISO 11466.3 and acetic acid methods in the estimation of the TAF of Cu, Ni and Pb was evaluated on samples S5 and S9, which are representative (Alonso-Hernández et al., 2006) of medium and high pollution level of metals in the northern basin of the bay, respectively. S8 was selected as the non-polluted sample because it shows the lowest concentration of Cu, Ni and Pb with 31.7 mg kg^{-1}, 34.8 mg kg^{-1} and 12.2 mg kg^{-1} (Alonso-Hernández et al., 2006; Pérez Santana et al., 2007), respectively in the zone.

The metal concentrations extracted by each method and the TAF of metals are shown in Fig. 6. For sample S5, the amount of Cu and Ni extracted by the acetic acid method is closer to the TAF than that extracted by the other two methods, which overestimated the TAF of both metals. Contrastingly, in the same sample S5, the three partial leaching methods underestimated the TAF for Pb. For sample S9, the Acetic acid method only estimated well the TAF of Cu, while the TAF of Ni and Pb are better estimated by either of the other two methods. Therefore, it is possible to suppose that the TAF of Cu in both samples, and the TAF of Ni in S5, are mostly associated to the fraction of sediment easily interchangeable and weakly absorbed ($CaCO_3$). Besides that, the TAF of Ni and Pb in S9 should be associated to some less mobile fraction of sediment, for example, the organic matter provided by the thermoelectric powerstation near the sampling site.

4. Conclusions

The EPA 3050B and ISO 11466.3 methods extract the total metal content or a fraction (10–100%) of it, depending on the sample and the element. The non-extracted fraction of Cu by both methods seems to be associated to the silicate phase of the sediment, which is very poorly attacked by the solvents used. The more complex behaviors observed for Ni and Pb need additional explanations. Thus, the probable association of Ni to Fe/Mn oxides and hydroxides, which are not completely dissolved by the aqua regia, could explain the smaller amount of Ni extracted by the ISO method. On the other hand, the possible presence of Pb as PbS could explain the higher EE of this metal by the ISO method due to the action of HCl (aq), which favours the easy soluble lead chloride complex formation.

None of the evaluated digestion methods extracted the Theoretical Anthropogenic Fraction of all the metals in both sediments studied. Nevertheless, the estimation of TAF should be extended to others samples of the ecosystem under study in order to reach definitive conclusions.

The results obtained in this work indicate the need of a detailed study of metal extraction capability of the EPA 3050B and ISO 11466.3

partial digestion methods in specific sediments of the ecosystem under study. Thus, the behaviors observed in the present work and the possible explanations given might contribute to the best assessment of metal contamination in other marine ecosystems.

From the practical point of view, our recommendation is to use either of the two partial methods, EPA 3050B or ISO 11466.3, but after knowing the extraction efficiency of the method selected for the specific element of interest in the sediment under study. The extraction efficiency, as well as the determined metal concentrations should be taken into account for a better environmental interpretation of the analytical results.

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