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Comparative Study of Digestion Methods EPA 3050B (HNO-HO-HCl) and ISO 11466.3 (aqua regia) for Cu, Ni and Pb Contamination Assessment in Marine Sediments

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2 **ISO 11466.3 (aqua regia) for Cu, Ni and Pb Contamination Assessment in**
3 **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 (HNO₃-H₂O₂-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.

Keywords: digestion methods; sediments; percentage of leaching; anthropogenic fraction.

48 1. Introduction

49 Sediments have been frequently used for the evaluation of metal contamination in sea
50 ecosystems (Arain et al. 2008). Among metals, Cu, Pb, and Ni are ubiquitous indicators of
51 human pollution, both urban and industrial (Forstner & Wittmann 1979; Salomons & Forstner
52 1984). These are some of the most measured elements in sediments of Cuban bays
53 (Gonzalez H., 1989; Gonzalez H., 1991). In particular, a high concentration of Ni was found
54 along the whole Cienfuegos Bay and considerably high peaks in front of the power plant;
55 while concentration peaks of Cu and Pb were located in sampling points near Cienfuegos city
56 (Perez Santana et al., 2007).

57 Nowadays, most analytical techniques used to quantify metal contents in sediments demand
58 the transformation of the sample to aqueous solution (Sneddon et al. 2006). However, there is
59 no international consensus about a unique digestion method that allows the homogeneous
60 interpretation of results obtained in different parts of the world. Single-step (Agemian and
61 Chau, 1976; DIN 38414, 1983; ISO 11466.3, 1995; UNEP/IOC/IAEA, 1995; USEPA 3050B,
62 1996; Ščančar et al. 2000; Sutherland, 2002; Sahuquillo, 2003; Snape, 2004; Larner, 2008;
63 Townsend, 2009) and sequential extractions (Quevauviller et al., 1997; Craba et al. 2004;
64 Perez Santana et al., 2007) methods have been employed for this purpose.

65 Single-step methods are very convenient for routine service analysis of many samples due to
66 their quickness and simplicity. In this group, different solvents or mixtures of them have been
67 used. For example, dilute HCl (Agemian and Chau, 1976; Sutherland, 2002; Snape, 2004;
68 Larner, 2008, Townsend, 2009) and HOAc (UNEP/IOC/IAEA, 1995; Ščančar et al. 2000;
69 Sahuquillo, 2003,) are two of the most employed. Several mixtures as for example, HNO₃-
70 H₂O₂-HCl (USEPA 3050B, 1996) and HCl-HNO₃ (ISO 11466.3, 1995) have also been used. In
71 this context, we selected EPA 3050B (USEPA 3050B, 1996) and ISO 11466.3 (ISO 11466.3,
72 1995), which are probably among the most internationally recognized.

73 Both methods are based on strong acid digestions. In the case of EPA 3050B, the sediment
74 is digested with repeated additions of nitric acid and hydrogen peroxide, followed by additions
75 of hydrochloric acid to the initial digestate. In the second method, the sample is pre-digested
76 overnight in a HCl + HNO₃ mixture (aqua regia) and then heated, filtered and diluted with
77 HNO₃. These procedures are not total digestion techniques for most samples. For example,
78 elements linked to the structures of silicates are not normally dissolved

79 The close dependence of the amount of metal extracted by the EPA 3050B and ISO 11466.3
80 methods with the chemical and physical characteristics of sediments is not always known and
81 then, apparently contradictory results are obtained in environmental studies (Ščančar et al.
82 2000; Sastre et al. 2002; Pavlíčková et al. 2003; Jakubowska et al. 2006). In some of the
83 revised reports (Ščančar et al. 2000; Sastre et al. 2002), the extracted amount of metals has
84 been related to the compositional/structural characteristics of the sample, while in other works
85 (El Samrani, 2005; Bettioli et al. 2008) both the extraction capability of these methods, and the
86 dependence regarding the type of sample and determined element, have been experimentally
87 justified. In addition, ISO 11466.3 (Sahuquillo et al. 2003; Diz, 2005) and EPA 3050B (Diz,
88 2005; Burton et al. 2005) have been considered as total extraction methods, without an
89 explanation or experimental verification, despite the fact that they do not totally transform
90 some components of many types of sediments to aqueous solution.

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

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

115

116 **2. Materials and methods**

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

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

127 Four standardized sample treatment methods were employed: ISO 14869-1:2001 total
128 digestion method (ISO 14869-1, 2001), and three different partial digestion methods: EPA
129 3050B (USEPA 3050B, 1996), ISO 11466.3 (ISO 11466.3, 1995) and the Acetic acid method
130 (UNEP/IOC/IAEA, 1995).

131 **USEPA 3050B method:** Around 0.25 g of sample were accurately weighed in a 250 ml Pyrex
132 Erlenmeyer flask and 10 ml of 8.5 M HNO₃ were added. The solution was heated on a hot
133 plate to ~95 °C without boiling and this temperature was maintained for 15 min. After cooling
134 to less than 70 °C, 5 ml of 17 M HNO₃ were added and the sample was refluxed at ~95 °C
135 without boiling until the volume was 5 ml. Addition of 5 ml of 17 M HNO₃ was repeated a
136 second time. Thereafter, the sample was evaporated to ~5 ml without boiling. After cooling to
137 less than 70 °C, 2 ml of water were added followed by the slow addition of 3 ml of 8.8 M H₂O₂.
138 The solution was then heated until effervescence subsided. Later, 4 ml more of 8.8 M H₂O₂ in
139 1-mL aliquots were added and solution refluxed. After cooling to less than 70 °C, 10 ml of 12
140 M HCl were added and the sample was refluxed for 15 min. without boiling. After cooling to
141 room temperature, the sample was filtered and diluted to 25 ml with double distilled water.

142 **ISO 11466.3 method:** Around 0.250 g of sediment were accurately weighed and placed in a
143 250 ml Pyrex Erlenmeyer flask. First, the pre-digestion step was done at room temperature
144 for 24 h with 10 ml of a (3:1) mixture of 12 M HCl and 17 M HNO₃. Then, the suspension was
145 digested on hotplate at 130 °C for 15 min. The obtained suspension was cooled until room

146 temperature, filtered through an ashless Whatman 41 filter and, finally, diluted to 25 ml with
147 0.17 M HNO₃.

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

152 **ISO 14869-1:2001 method:** Around 0.250 g sample were accurately weighed in a 250 ml
153 polytetrafluoroethylene Erlenmeyer flask and 5 ml of 8.5 M HNO₃ were added. The solution
154 was heated on a hot plate without boiling until the volume was reduced to 1 ml and then the
155 sample was cooled. This step was repeated two more times until a total amount of 15 ml of
156 8.5 M HNO₃ was added. After cooling, 5 ml of HF and 1.5 ml of HClO₄ were added and the
157 sample was refluxed. A second portion of 5 ml of HF and 1.5 ml of HClO₄ were added one
158 more time and the sample was kept overnight at room temperature, after which, the volume
159 was reduced again up to 1ml by refluxing. After cooling, 5 ml of double distilled water plus 2
160 ml HCl conc. were added with light heating to dissolve the residue. After cooling to room
161 temperature, the sample was filtered and diluted to 50 ml with double distilled water. The total
162 content of elements was used to calculate the TAF values and extraction efficiency of the
163 three partial digestion methods studied for the elements determined.

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

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

169 Cu, Ni and Pb were quantified by Flame Atomic Absorption Spectrometry (FAAS) in all the
170 sediment solutions. A Pye Unicam 9100 (Philips, Holland) Atomic Absorption Spectrometer

171 with an acetylene / air ($1.5 \text{ L min}^{-1} / 5.0 \text{ L min}^{-1}$) flame and a bandpass of 0.2 nm was used.
172 The absorption lines were: Cu 324.8 nm, Ni 232.0 nm and Pb 217.0 nm. For all the analytes,
173 the compromise observation height was set at 10 mm. The intensity of the current was 4, 12
174 and 12 mA for the Cu, Ni and Pb lamps, respectively.

175 Mean concentrations of three digestion triplicates of sediment and the confidence interval for
176 $\alpha=0.05$ and $n-1=2$ degrees of freedom were calculated for statistical comparison.

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

195 The mineralogical composition of the sediments, before and after the treatment by both partial
196 digestion methods ISO and EPA, was obtained by X-ray Diffraction method (XRD), using
197 $\text{CuK}\alpha$ radiation ($\lambda = 1.54183 \text{ \AA}$) in a HZG4 goniometer (Carl Zeiss, Jena) equipped with Enraf-
198 Nonious Diffractis 581 generator and a graphite monochromator. The XRD pattern was
199 recorded from 6 to $60^\circ (2\theta)$ in 0.05° steps and 3 s counting time. Elemental qualitative analysis
200 of sediment residues was made with an Energy Dispersive X-ray Fluorescence Spectrometer
201 (ED-XRF), model ED 2000, (Oxford Instruments, England) with silver tube and vacuum pump.
202 The Particle Induced X-ray Emission Spectrometry (PIXE) qualitative analysis used to
203 corroborate the presence of sulphur in the sediments was carried out by the 3 MV Tandetron
204 accelerator using a 2 MeV proton beam. The acquisition time of the spectra was $\sim 300 \text{ s}$. The
205 beam spot at the target position was $\sim 1 \text{ mm}^2$. The characteristic X-rays were detected by a
206 lithium-doped silicon detector, with an energy resolution of about 155 eV at 5.9 keV . The
207 electronics consisted of a Telenec245 amplifier associated with a PCA3 multi-channel
208 analyzer (Oxford Instruments, England). Semiquantitative determination of Mg, Al, Si, K, Ca,
209 Fe, and Mn, macro components in the sediment residues, was performed by using the Vega
210 TESCAN 5130 SB Energy Dispersive X-ray Scanning Electron Microscope with Inca 350
211 micro analyzer, liquid nitrogen cooled, and with a resolution of 128 eV .

212

213 **3. Results and Discussions**

214 **3.1 Leaching of metals by EPA 3050B and ISO 11466.3 partial digestion methods**

215 Extraction efficiency of EPA 3050B and ISO 11466.3 was calculated from the concentrations
216 shown in Figure 2. Thus, EE of Cu varied for the different samples. EE was $\sim 100\%$ for S9
217 and it was between 70% and 85% for the remaining samples. Significant differences were not
218 found in the amounts of Cu (Fig.2a) extracted by both partial methods in each studied
219 sample. These results contradict those reported by Pavlíčková et al. (2003), where the

220 ISO11466.3 method extracted 12% more Cu than the $\text{HNO}_3\text{-H}_2\text{O}_2$ method. This last method is
221 similar to the EPA 3050B used in the present work. This disagreement between EE of Cu by
222 the EPA 3050B and ISO 11466.3 reported by Pavlíčková and the EE obtained in the present
223 work could be explained by possible differences in composition of the samples studied in both
224 cases. Unfortunately, the composition of the samples studied by Pavlíčková was not provided
225 and thus, it was impossible to confirm this hypothesis.

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

232 On the other hand, the differences in the EE of Cu in the samples can be explained by the
233 different amounts of Cu associated with the silicate phase in each one. For example, the
234 silicate phase (quartz) content of samples S9 and S10 are very comparable as can be
235 confirmed by their similar X-ray diffraction patterns obtained before digestion (Figs. 3b and
236 3c). However, the EE of Cu in sample S10 by both partial methods is smaller (~ 70%) than
237 the corresponding value in sample S9 (~ 100%). Thus, the amount of Cu linked to the silicate
238 phase is probably larger in sample S10 than in sample S9. The presence of Cu in the
239 residues was corroborated by ED-XRF qualitative analysis (Fig. 4).

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

245 and 63.9% smaller than that obtained by EPA 3050B for samples S2, S5, S6 and S17,
246 respectively.

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

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

261 The EE of Pb by using both partial methods was less than 100% for all samples, except for
262 S10 treated with the ISO 11466.3 method. This result indicated that some fraction of Pb could
263 be associated to a silicate phase for all the studied samples, except for sample S10.
264 Specifically, the EE of Pb by both methods was similar in samples S5 and S9 (Fig. 2c). As in
265 the case of Cu, this behavior can be explained by the possible association of the non-
266 extracted fraction of Pb to the silicate phase (quartz). For the rest of the samples, the ISO
267 11466.3 method leached a higher amount of Pb than the EPA 3050B method (Fig. 2c). This
268 fact was particularly significant in samples S2, S6 and S10. The poor extractability of Pb by
269 the EPA 3050B method is explained if we assume that a part of this metal is present in those

270 samples as lead sulphide (PbS). The nitric acid oxidizes the PbS to a slightly soluble salt
271 (PbSO₄), which remains in the residues. In contrast, the presence of HCl (aq), as part of the
272 aqua regia used in the ISO 11466.3 method, favours the formation of a lead chloride complex,
273 which is highly soluble and is then easily extracted. The presence of sulphur (S) in the studied
274 sediments was confirmed by PIXE. See, for example, the PIXE spectra of sediments S2 and
275 S17 in Figs. 4e and 4f, respectively.

276 **3.2 Efficiency of the digestion method in the estimation of the Theoretical** 277 **Anthropogenic Fraction of metal**

278 The efficiency of the 3050B, ISO 11466.3 and Acetic acid methods in the estimation of the
279 TAF of Cu, Ni and Pb was evaluated on samples S5 and S9, which are representative
280 (Alonso-Hernández et al. 2006) of medium and high pollution level of metals in the northern
281 basin of the bay, respectively. S8 was selected as the non-polluted sample because it shows
282 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
283 (Alonso-Hernández et al. 2006; Pérez Santana et al. 2007), respectively in the zone.

284 The metal concentrations extracted by each method and the TAF of metals are shown in
285 Figure 6. For sample S5, the amount of Cu and Ni extracted by the Acetic acid method is
286 closer to the TAF than that extracted by the other two methods, which overestimated the TAF
287 of both metals. Contrastingly, in the same sample S5, the three partial leaching methods
288 underestimated the TAF for Pb. For sample S9, the Acetic acid method only estimated well
289 the TAF of Cu, while the TAF of Ni and Pb are better estimated by either of the other two
290 methods. Therefore, it is possible to suppose that the TAF of Cu in both samples, and the
291 TAF of Ni in S5, are mostly associated to the fraction of sediment easily interchangeable and
292 weakly absorbed (CaCO₃). Besides that, the TAF of Ni and Pb in S9 should be associated to
293 some less mobile fraction of sediment, for example, the organic matter provided by the
294 thermoelectric powerstation near the sampling site.

295 **4. Conclusions**

296 The EPA 3050B and ISO 11466.3 methods extract the total metal content or a fraction (10-
297 100%) of it, depending on the sample and the element. The non extracted fraction of Cu by
298 both methods seems to be associated to the silicate phase of the sediment, which is very
299 poorly attacked by the solvents used. The more complex behaviors observed for Ni and Pb
300 need additional explanations. Thus, the probable association of Ni to Fe/Mn oxides and
301 hydroxides, which are not completely dissolved by the aqua regia, could explain the smaller
302 amount of Ni extracted by the ISO method. On the other hand, the possible presence of Pb as
303 PbS could explain the higher EE of this metal by the ISO method due to the action of HCl
304 (aq), which favours the easy soluble lead chloride complex formation.

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

309 The results obtained in this work indicate the need of a detailed study of metal extraction
310 capability of the EPA 3050B and ISO 11466.3 partial digestion methods in specific sediments
311 of the ecosystem under study. Thus, the behaviors observed in the present work and the
312 possible explanations given might contribute to the best assessment of metal contamination
313 in other marine ecosystems.

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

319

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ACCEPTED MANUSCRIPT

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404 of Solid and Hazardous Wastes, USEPA: Cincinnati, OH, 1996.

405 Table 1. Accuracy \pm relative standard deviation of FAAS analysis as recovery percentage for
 406 three digestion triplicates

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

407 ^(*) For Ni, added concentrations were 150, 200 and 350 mg/L, respectively.

408

409

Table 2. Detection limits* 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

410

(*) expressed in mg.kg^{-1}




411 Table 3. Major element concentrations of sediment residues determined by EDX-SEM
 412 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

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

415 **FIGURE CAPTIONS**




416 **Figure 1.** Map of sampling points in the Cienfuegos Bay.

417 **Figure 2.** Comparison of the concentrations of Cu (a), Ni (b) and Pb (c) extracted from
418 sediments by the total digestion method (ISO 14869-1:2001- ) and the partial digestion
419 methods (EPA 3050B -  and ISO 11466.3 - ). The confidence interval calculated for $\alpha =$
420 0.05 and $n = 3$ are included for each measured concentration.

421 **Figure 3.** X-ray diffraction patterns of sediments S5 (3a), S9 (3b) and S10 (3c) before
422 digestion and of the residues of sediment S9 after digestion by EPA 3050B (3d) and ISO
423 11466.3 (3e) methods. Diffraction patterns of each sample are in different scales.

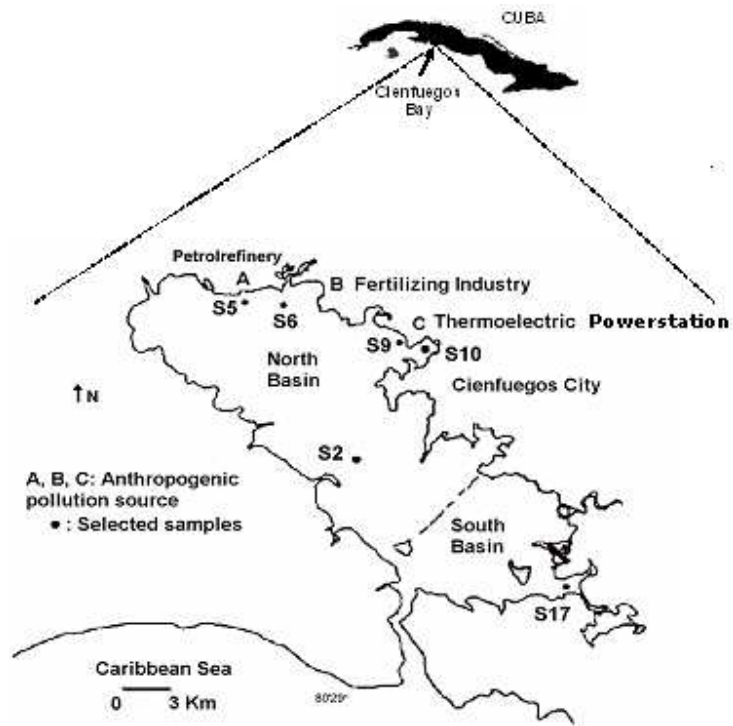
424 **Figure 4.** X-ray fluorescence spectrum of residues obtained from: sediment S9 treated by
425 EPA 3050B (4a); sediment S9 treated by ISO 11466.3 (4b); sediment S10 treated by EPA
426 3050B (4c) and sediment S10 treated by ISO 11466.3 (4d). The PIXE spectra of sediments
427 S2 (4e) and S17 (4f) were also included.

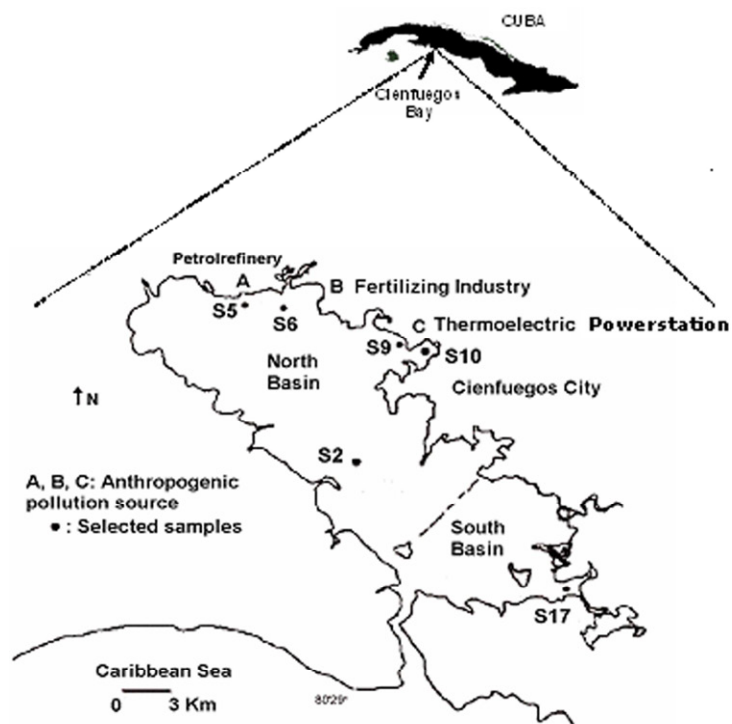
428 **Figure 5.** Shiny particles (inside circles in right side) observed in the mixed residues of
429 sample S9 after treatment by the EPA 3050B (5a) and ISO 11466.3 (5b) methods, and the
430 EDX-SEM spectra of the particles (left side).

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

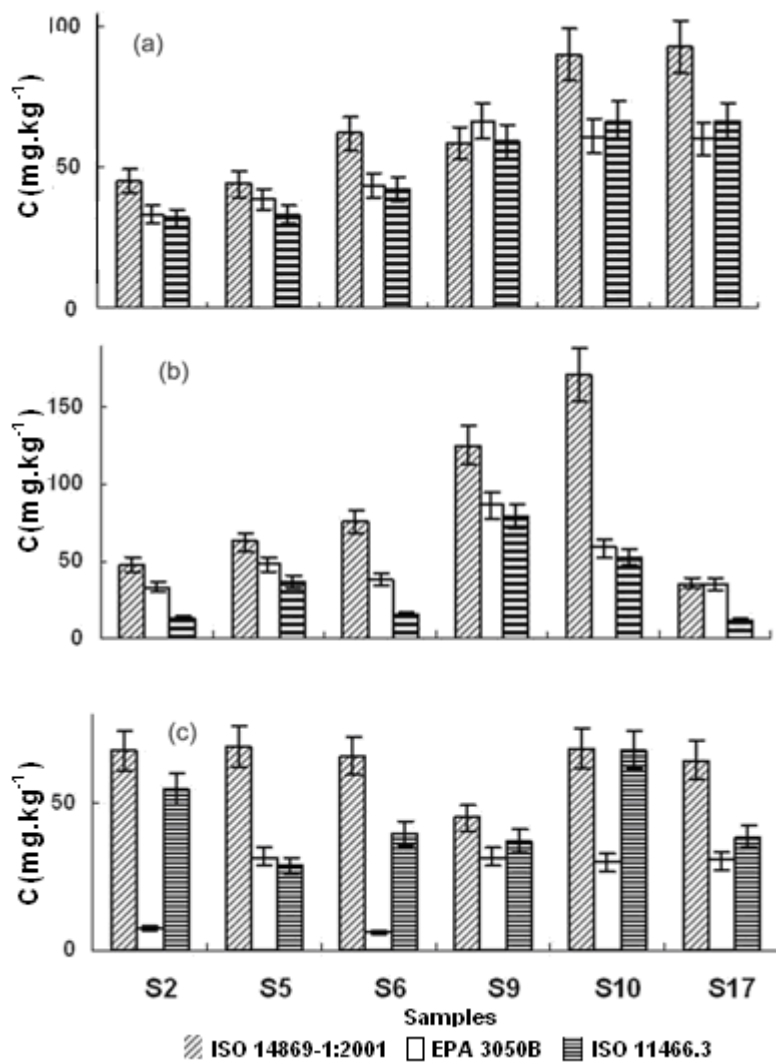
- Extraction of Cu, Ni and Pb by EPA 3050B and ISO 11466.
- 3 methods was compared.
- Similar amounts of Cu were extracted from sediments.
- In contrast, EPA extracted more Ni and less Pb than the ISO method.
- Some explanations were given using several techniques: XRD, PIXE, XRF and SEM-EDX.

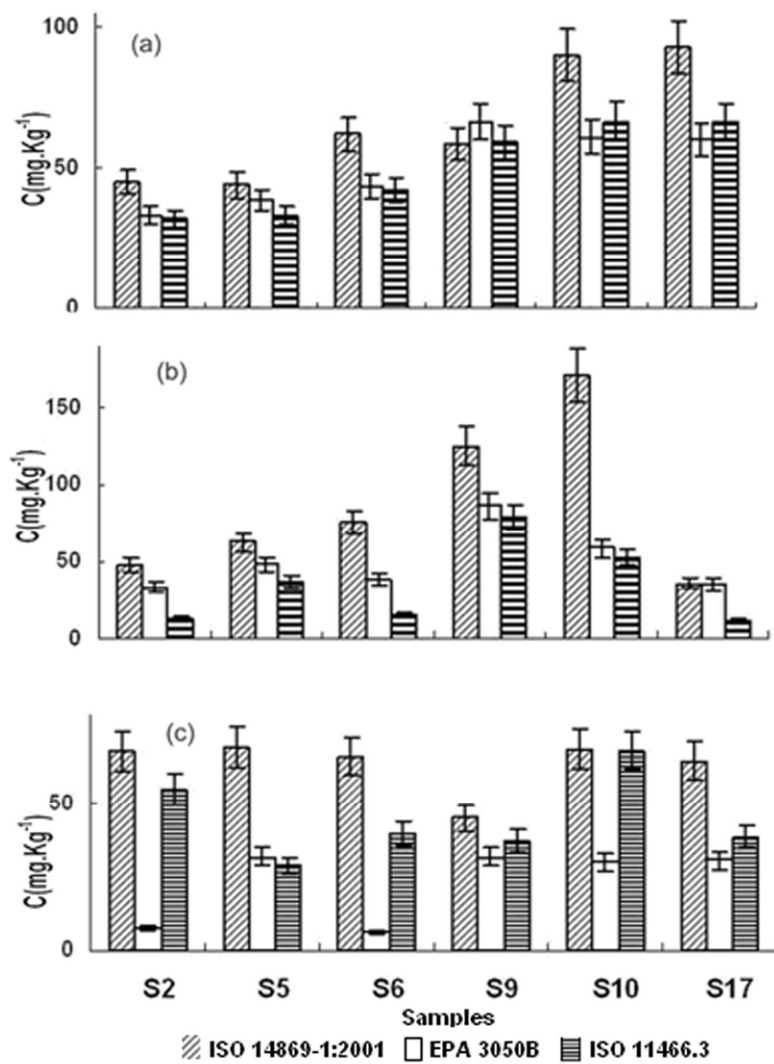
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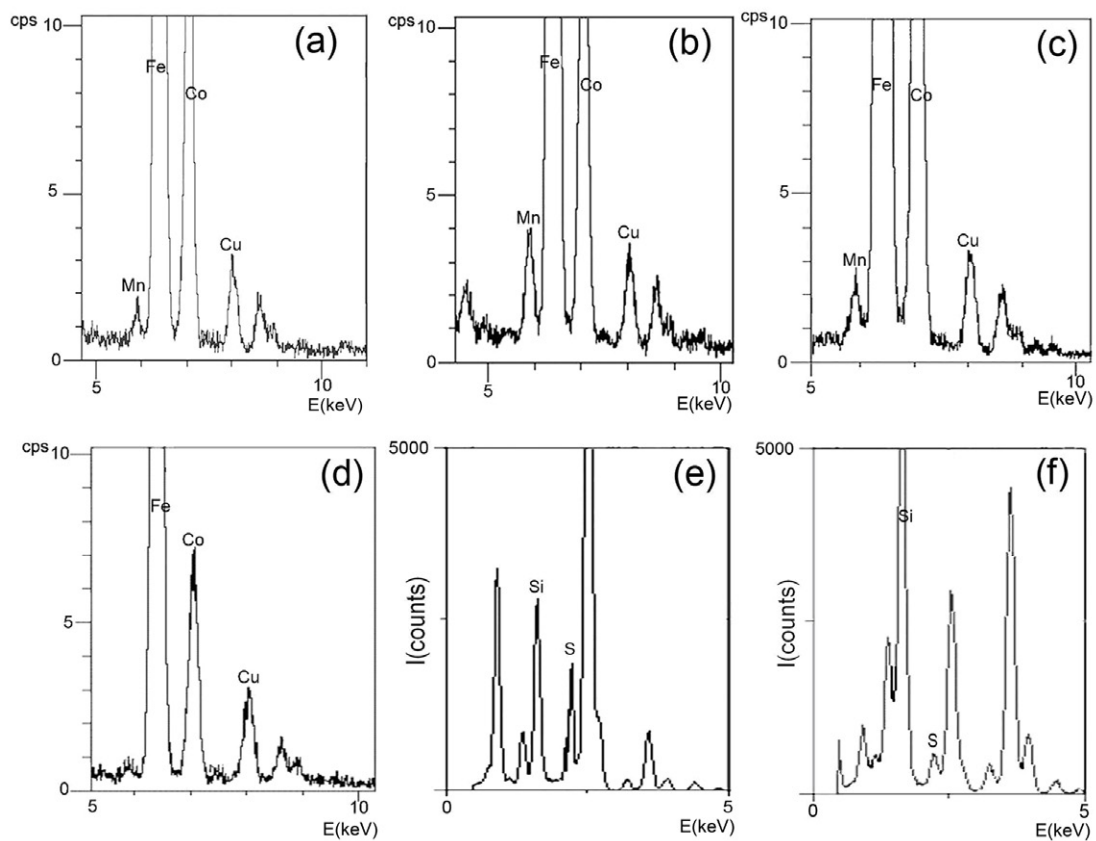


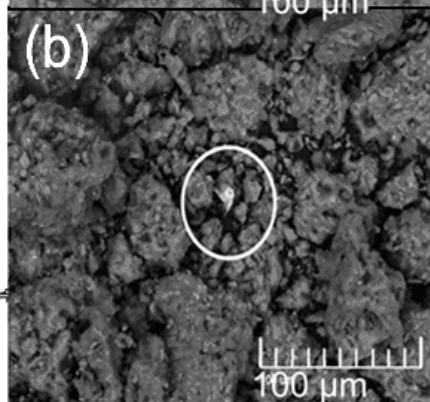
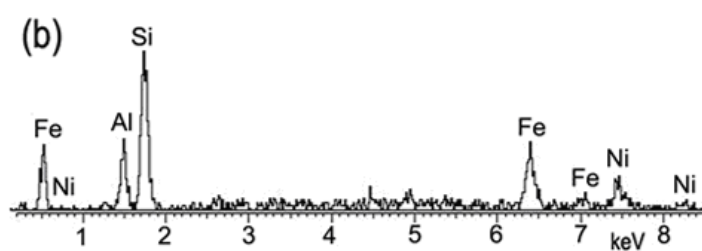
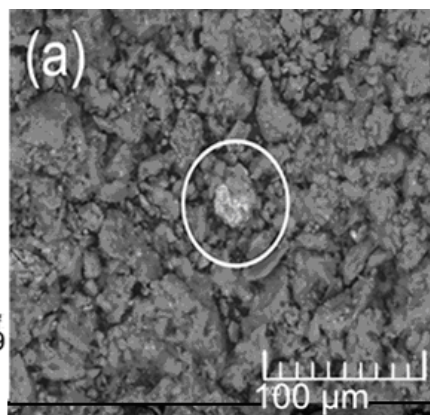
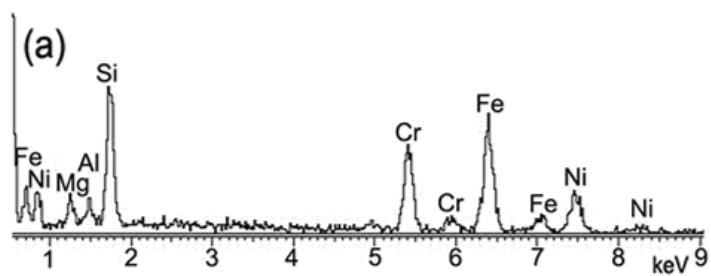


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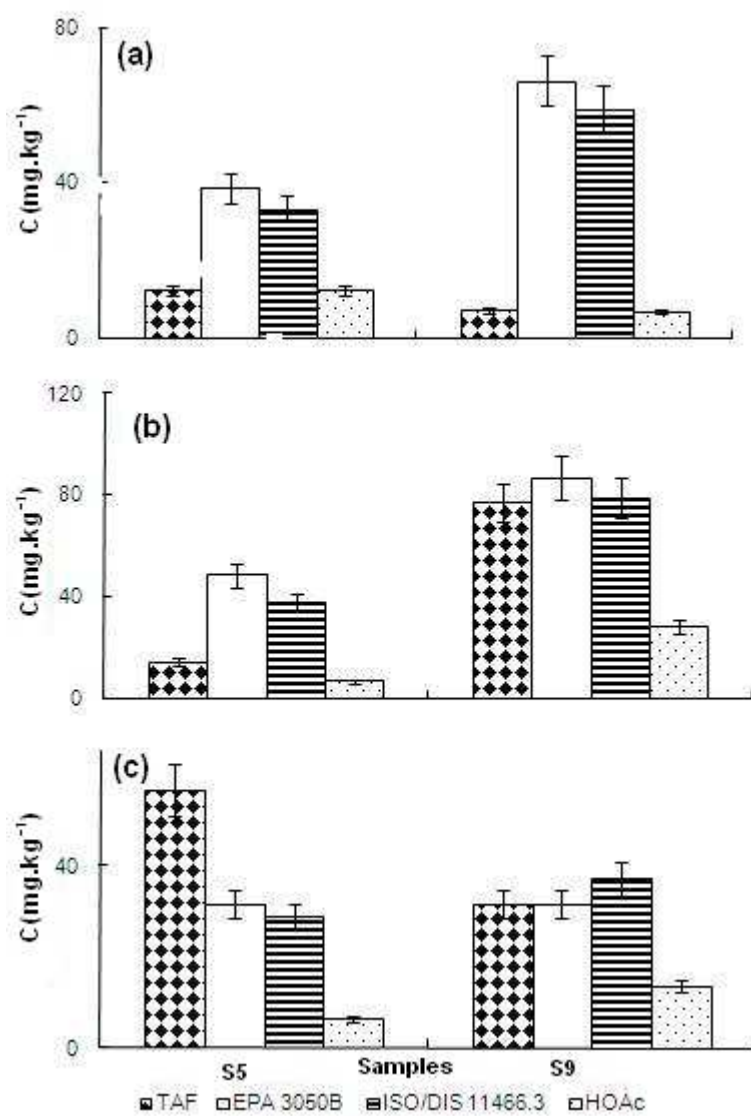


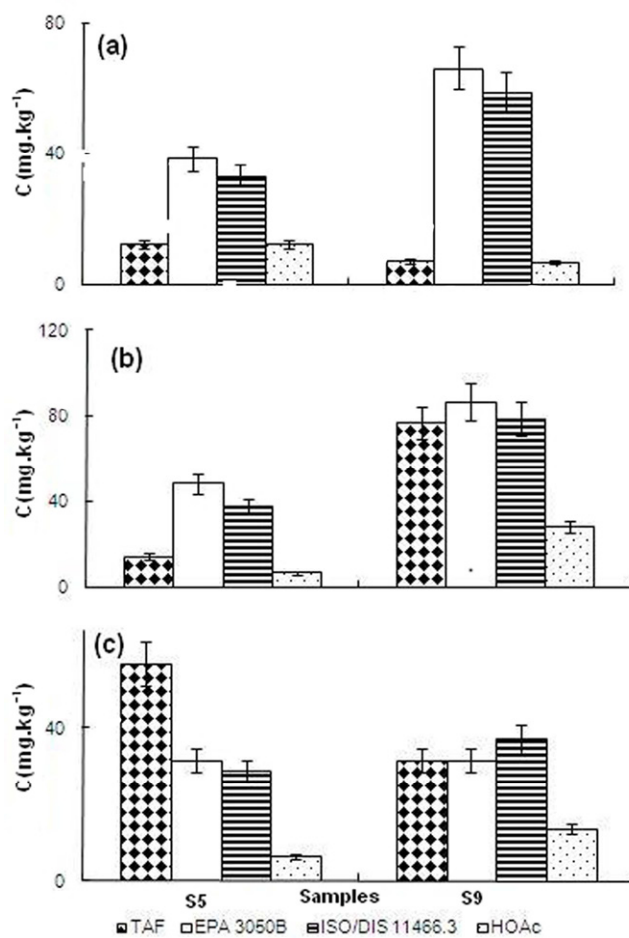


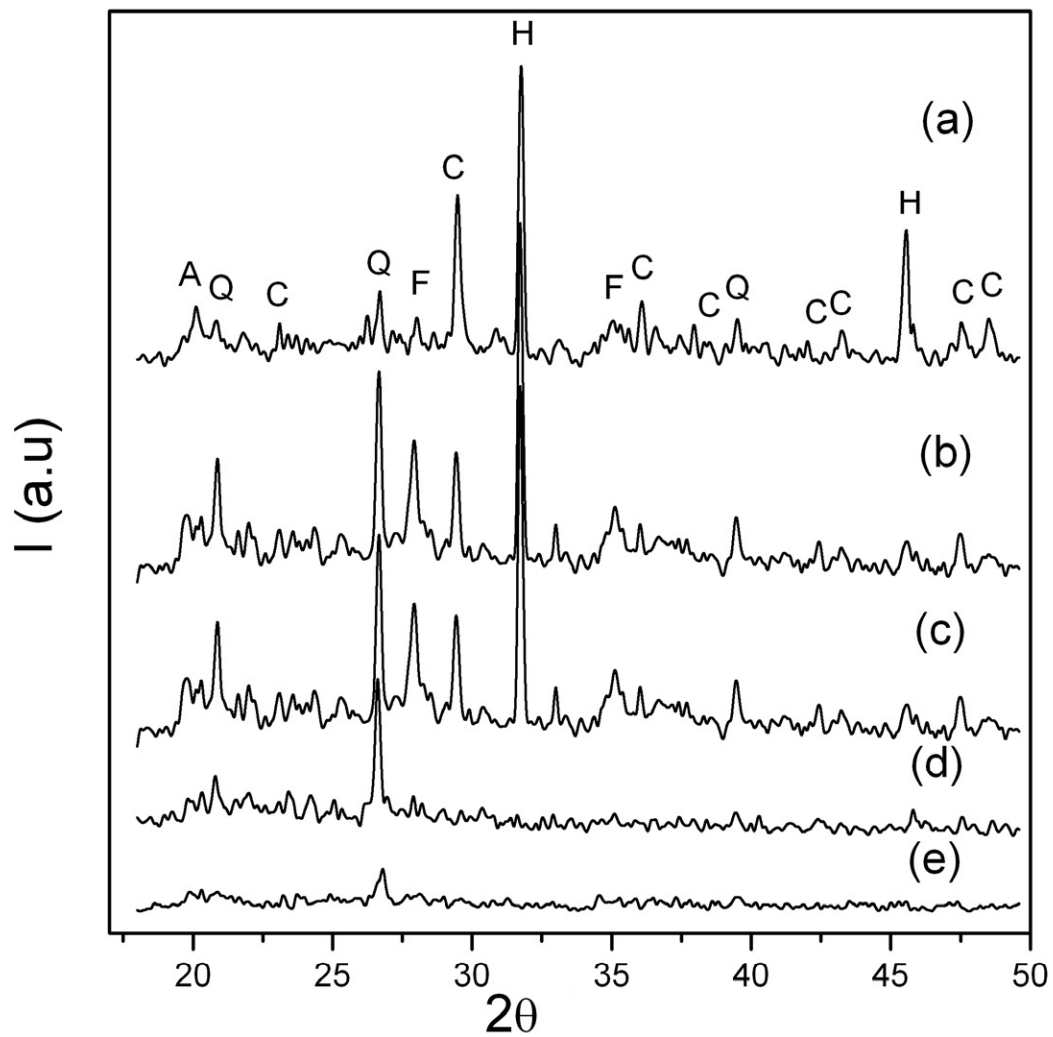




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A: arcille Q: quartz C: calcite F: feldspar H: halite