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Assessment of Direct Mercury Analyzer® to Quantify Mercury in Soils and Leaf Samples

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Este trabalho tem como objetivo avaliar um método para quantificação de Hg total em amostras de solo e folhas usando o analisador direto de mercúrio (DMA-80 TRICELL; Milestone Inc., Itália). Foram usados materiais de referência certificados de solo, de folhas de árvores e de sedimentos, e amostras coletadas para otimizar e validar o método. Dado que a técnica permite a análise direta de amostras por combustão, foram considerados aspectos como o efeito da variação da quantidade de amostra e de sua granulometria. A robustez do método foi avaliada mediante o teste de Youden, no qual foi observado que quatro dos sete parâmetros testados têm influência significativa nos resultados analíticos para análise de amostras de folhas, mas os resultados de amostras de solo não foram significativamente afetados por pequenas variações de qualquer um dos sete fatores. A recuperação para os materiais de referência variou de 102% para 104%, e a precisão (coeficiente de variação) foi menor que 5%.

This work aims to assess a method for total Hg quantification in soil and leaf samples by using a Direct Mercury Analyzer® (DMA-80 TRICELL; Milestone Inc., Italy). Standard Reference materials of tree leaves, river sediment and soil, and collected samples of soil and leaves were used to optimize and to validate the method. Given that the technique allows direct analysis of samples by combustion, aspects like effect of sample amount variation and sample granulometry were considered. Method's robustness was also assessed through the Youden test showing that for leaf sample analyses, among the seven tested factors, four were able to significantly affect the analytical results, but the soil samples results were not significantly affected by slight variations of any of the seven factors. Recoveries for reference materials ranged from 102% to 104%, and the precision (RSD) was lower than 5%.

Keywords: mercury, soils, leaves, direct analysis

Introduction

Mercury is of special interest because of its toxicity, widespread distribution in the environment, its tendency to bioconcentrate in aquatic food chain, and historical large-scale poisoning events.[1] Additionally, this element can be used as a geochemical indicator to differentiate between mineralized and non-mineralized zones and as a geochemical indicator of Au-rich zones.[2] Two of the most common techniques for total mercury quantification are cold vapor atomic absorption spectroscopy (CVAAS)[3,4] and cold vapor atomic fluorescence spectroscopy.[5] In both methods, mercury is determined as Hg(0). Traditionally, these analytical techniques require time consuming samples

preparation steps, which includes acid digestion in hot plate or in a microwave oven, Hg reduction (Hg(II) to Hg(0)) by the addition of a reductant such as tin(II) chloride and liquid-gas separation steps.[6,7] Nevertheless, some years ago commercial equipment appeared that allowed direct total mercury analysis in solid and liquid samples by combining sample combustion for thermal Hg reduction and Hg vaporization with cold vapor atomic absorption spectroscopy.[8] In comparison to the classical analytical method, this method appears as very interesting because it considerably reduces the time of analysis as it needs minimal sample preparation steps (such as grinding), it is totally mechanized after sample introduction, and it does not generate liquid chemical waste. The method is now recognized by the U.S. EPA[9] and has already been used to determine Hg concentration in geological

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and environmental samples.[10-14] Nevertheless, although commercial equipment allow adjustment of many variables, such as drying and ashing temperature, carrier gas type and flow, sample amount, etc., information about method optimization and validation are still scarce. The aim of this work was therefore to optimize and to validate the method for two types of solid samples (soils and vegetation) when using such an equipment.

Experimental

Analytical equipment

A Direct Mercury Analyzer® (DMA-80 TRICELL, Milestone, Italy) was used for soil and vegetal sample analyses. This equipment typically contains an automatic sampler, a quartz furnace, a cobalt-manganese oxide catalyst, a gold-coated sand amalgamator and an atomic absorption detection cell with three different path lengths (165, 120 and 4 mm). Method for solid sample analysis consists in placing a known amount of milled sample in a nickel or quartz boat (sample holder). The sample is introduced in the quartz furnace, where it is heated up to 200 °C (drying temperature) for 60 s and 650 °C (ashing temperature, maximum temperature allowed by the software of the equipment) for 105 s which allows Hg reduction and volatilization. Air or oxygen (99.99%) can be used as combustion and carrier gas. Mercury and combustion gases are flushed through the catalyst, where interferents like halogen compounds, nitrogen oxides and sulfur oxides are retained. Hg(0) is then selectively trapped in the amalgamator while combustion gases are removed from the detection cell. Mercury is then released from the amalgamator by heating at 850 °C for 3 s and carried to the detector, where the absorbance from the radiation emitted by a mercury lamp is measured at 253.7 nm for any of the three optic path lenghts. The path length used for detection in each sample is automatically selected depending on the Hg concentration and the amount of analyzed sample. The longest path length (more sensitive) is selected for low amounts of Hg (e.g., < 5 ng), and the shortest one is selected for high amounts of Hg (e.g., > 20 ng). Liquid sample analysis can be similarly performed, using quartz boats instead of nickel boats to prevent holder oxidation when liquid samples are acidified. According to the manufacturer recommendations up to 100 mg sample can be introduced in the sample holder for analysis and either oxygen or compressed air can be used as carrier gas. In this work the catalyst remained constant at 650 °C throughout the analysis procedure and air was used as carrier gas.

Calibration

The DMA calibration was performed from sextuplicate analysis of 5 to 70 µL aliquots of Hg standard solution (100 µg L^{-1}) prepared from a stock Hg standard solution (1.000 ± 0.003 mg mL^{-1}) (Tec-Lab® Hexis, Jundiaí, Brazil) diluted in deionized water (Direct-Q 5, Millipore, Billerica, USA). Calibration was performed in terms of amount of mercury and not in terms of concentration. The outliers of the calibration measurements were diagnosed and removed following the criterion of Jacknife's standardized residual test,[15] the homoscedasticity of the residuals was verified with the Levene test,[16,17] the linearity of the analytical curve was confirmed by the method proposed by Draper and Smith[18] and the limits of detection and quantification were determined by using 3 times and 10 times the standard deviation of the residuals from the linear regression, respectively, plus the linear regression coefficient value of the calibration equation.[19]

Samples

Standard reference materials (SRMs) of apple leaves (NIST 1515), peach leaves (NIST 1547), San Joaquin soil (NIST 2709) and river sediment (GBW-08301) were used for optimization and validation tests. Most of SRMs of soils have high Hg concentration (> 2 µg g^{-1}) and NIST 2709 is one with the lowest Hg concentration. As this work was focused primarily in validating a method for non-highly contaminated soil (Hg concentration < 200 ng g^{-1}), a SRM of river sediment with low mercury concentration was also analyzed. Other leaf and soil samples were also used in the optimization tests. They were collected in a forest area of the Brazilian Amazonia (Rio Branco, Acre). Each leaf sample consisted of a group of 20 leaves from the same tree, and each soil sample consisted of approximately 20 g of superficial soil (O-horizon and 0-5 cm). All samples were dried in a laminar flow hood for 48 h at room temperature. Leaf samples were ground in a knife mill to pass through a 1 mm sieve and soil samples were ground in a mechanical agate mill for 20 min. All samples were stored and manipulated with materials previously cleaned in 10% (v/v) HNO$_3$ solutions for 24 h and rinsed with deionized water. The boats were washed with deionized water, dried and heated at 650 °C for 3 min before each analysis for mercury quantification.

Optimization tests

It was first assessed whether the measurement of mercury concentration could be significantly affected by

Table 1. Levels used for each factor in the robustness experimental design

Level	Factors						
	1	2	3	4	5	6	7
Normal (1)	3.1 psi	1 day	50 mg	200 °C	650 °C	70 s	150 s
Modified (−1)	3.0 psi	0 day	60 mg	190 °C	620 °C	65 s	140 s

the sample amount to be analyzed and by the granulometry of the soil and leaf samples. Some of the aforementioned SRMs and some collected samples were used in order to assess the mass variation effect, between 20 and 100 mg. Only collected samples were used to assess the granulometry variation effect, since SRMs have fixed particle size distributions (approximately 0.075 mm).

Validation

Standard reference materials of apple leaves (NIST 1515), peach leaves (NIST 1547), San Joaquin soil (NIST 2709) and river sediment (GBW-08301) were used for accuracy and precision assessments. The robustness of the method for mercury quantification in soil and leaf samples was assessed through the Youden test.[20] The tests for soils and leaf samples consisted in a duplicate analysis of a $2^{(7-4)}$ fractional experimental design. The seven considered factors were: 1) Carrier gas pressure, 2) antecedence between decontamination and use of boats (sample holders), 3) sample mass, 4) drying temperature, 5) ashing temperature, 6) drying time and 7) ashing time. The levels used for each parameter or factor are shown in Table 1.

Results and discussion

Calibration

Analytical curves were constructed for the medium and the longest path lengths. Dynamic linear ranges of analytical curves were 0.5 to 5 ng of Hg and 1 to 7 ng of Hg, for the longest and the medium path lengths, respectively. The limit of detection of the method was 0.12 and 0.07 ng of Hg and the limit of quantification was 0.42 and 0.22 ng of Hg for the longest and the medium path lengths, respectively. The sensitivity of the analytical curve for the longest path length (0.085 ng^{-1}) was about twice the sensitivity of the analytical curve for the medium path length (0.045 ng^{-1}).

Sample mass effect

Mercury concentration in the tested soil samples did not show any significant variation when varying the

sample mass from 20 to 100 mg (Figure 1a). In this case, relative standard deviation (RSD) was < 3%. However, when varying sample mass of three leaf samples (two SRMs and one collected sample), results were randomly distributed around the mean only for analysis of less than 60 mg of sample (RSD < 2%, Figure 1b). In this condition, Hg concentrations in SRMs were within the certified range (Table 2). For masses between 60 and 100 mg Hg concentration gradually increased, showing a systematic error, which can lead to an overestimation of the Hg concentration. Some tests increasing the heating time of the samples in the DMA furnace were performed and the same systematic error was found. Vegetal samples have a
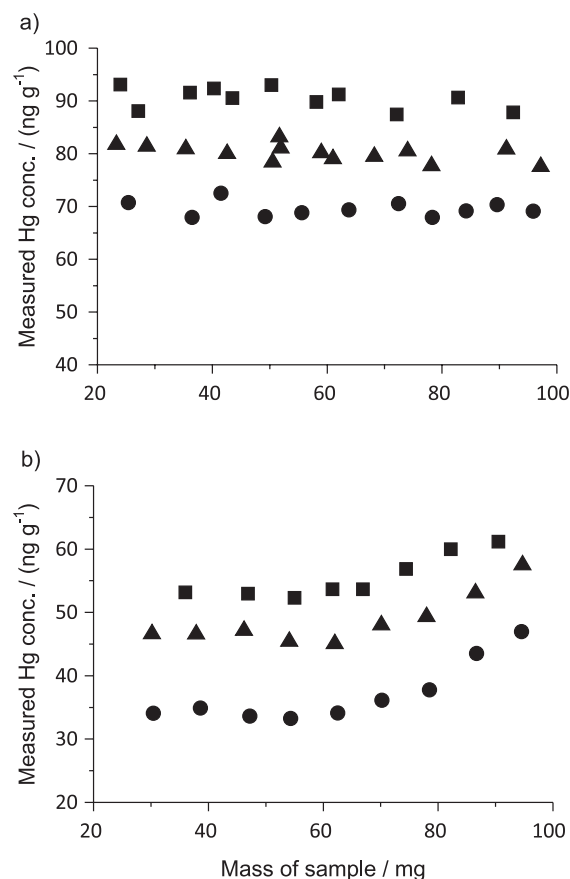

Figure 1. Measurements of mercury concentration as a function of sample mass of: a) three soil samples (■) (▲) (●), and b) SRM of peach leaves NIST 1547 (●), SRM of apple leaves NIST 1515 (▲), a leaf sample (*Theobroma cacao* L.) (■).

Table 2. Comparison of the certified concentration and determined concentration of mercury in SRMs peach leaves (NIST 1547), apple leaves (NIST 1515), San Joaquin soil (NIST 2709) and a river sediment (GBW-08301); (averages and confidence intervals at 95%)

Sample	Certified Hg concentration / (ng g^{-1})	Determined Hg concentration / (ng g^{-1})	n	RSD / %	Recovery / %
Peach leaves (NIST 1515)	44 ± 4	45.9 ±0.8	5	1.3	104
Apple leaves (NIST 1547)	31 ± 7	32 ± 1	5	1.5	103
San Joaquin soil (NIST 2709)	1400 ± 80	1450 ± 70	5	3.5	104
River Sediment (GBW-08301)	220 ± 40	225 ± 7	5	2.2	102

RSD: relative standard deviation; n: number of replicates.

high organic matter content (> 90%), when compared to the analyzed soil samples (< 18%). During heating of the leaf samples in the furnace, depending of the amount of sample, a sufficiently large amount of products of incomplete combustion of organic matter can probably be generated (and not efficiently removed) able to absorb in the UV-VIS, causing the overestimation of mercury concentration. To support this hypothesis the same experiment was therefore performed for the analysis of SRM of peach leaves in two other DMA-80 TRICELL equipments, but in which oxygen (and not compressed air) is used as combustion and carrier gas. In these cases the results did not show any shift of mercury concentration whatever the sample mass (20 to 100 mg). These results show that for high organic matter content, care must be taken about the amount of sample to be analyzed if using air instead of oxygen as combustion gas. All subsequent determinations of Hg were performed with the direct mercury analyzer configured with compressed air. Leaf sample analyses were performed with sample masses less than 60 mg and for soils with sample masses less than 100 mg.

Sample granulometry effect

Two soil and two leaf samples were sieved and analyzed to assess the effect of granulometry distribution profile on the measured mercury concentration. These four samples were divided in 4 or 5 granulometric fractions by using sieves of 0.35, 0.297, 0.150, 0.075 mm and mercury concentration was determined in each fraction. As shown in Table 3, Hg concentrations varied between fractions, with a tendency to higher concentrations in fractions with smaller particle sizes. With this first test we can only conclude that differences in Hg concentration between granulometric fractions can be associated with one of the following processes: 1) non-uniform distribution of mercury in particles with different size (naturally or during grinding or sample storage), as finer particles are expected to be more efficient in retaining trace elements[21] or 2) influence of the particle size distribution on the analytical

equipment efficiency (e.g., combustion efficiency). Two additional tests were applied to assess which of these two processes could be involved: 1) comparison of the mean Hg concentration of the unsieved sample with the weighted mean calculated from the mercury concentrations in each fraction and the percentage of the total mass of sample of each granulometric fraction (Table 3); and 2) the fraction 0.297-0.150 mm of each sample was re-ground and the concentration of mercury was determined again. Data before and after re-grinding is shown in Table 4. The first test did not show any significant difference between Hg concentrations (*t*-test, p > 0.05), which indicates that the samples were sufficiently homogeneous to obtain representative mercury concentrations of the entire sample, and the second test confirmed that differences of Hg concentration in fractions with different particle sizes are related with non-uniform distribution of mercury between them rather than to a lack of equipment efficiency in removing mercury from samples with lower grain size. In a previous work, Haynes *et al.*[22] did not find significant differences in Hg concentrations between homogenized and non-homogenized samples of fish muscle by using a DMA-80. In the present work only homogenized samples were tested in order to obtain representative results of each collected sample.

Robustness

The fractional experimental design used here allows to know statistically (*t*-test, p < 0.05), with a reduced number of experiments, if slightly (~5% in most cases) variations of the assessed parameters influence significantly the determination of mercury concentration. According to the results shown in Pareto's charts (Figure 2a), slight variations in four of the seven evaluated factors have a significant effect on the results of Hg concentration for leaf sample analyses. Gas carrier pressure was the most critical factor. On the other hand, for soil samples (Figure 2b), results were not significantly affected by slight variations of any of the seven factors. To compensate for the lesser

Table 3. Mercury concentration and granulometric mass fractions from two soil and two leaf samples. Hg concentration for sieved (weighted mean) and unsieved samples (mean ± standard deviation)

| Sample | Size particle distribution / mm | Sieved samples | | | Unsieved samples |
		Mass fraction / %	Hg conc. / (ng g^{-1})	Weighted mean / (ng g^{-1})	Hg conc. / (ng g^{-1})
Leaf sample 1	1-0.297	1.49	23 ± 2	28 ± 2	27.0 ± 0.4
	0.297-0.150	38.72	19.3 ± 0.8		
	0.150-0.075	34.16	25.1 ± 0.6		
	X < 0.075	25.64	44 ± 1		
Leaf sample 2	1-0.35	2.99	38.2 ± 0.8	56 ± 4	54.1 ± 0.7
	0.35-0.297	5.97	42 ± 1		
	0.297-0.150	55.30	42 ± 1		
	0.150-0.075	21.34	59 ± 2		
	X < 0.075	14.37	115 ± 3		
Soil sample1	> 0.297	7.34	55 ± 2	87 ± 2	87.4 ± 0.6
	0.297-0.150	31.75	49.3 ± 0.8		
	0.150-0.075	42.02	93.4 ± 0.1		
	X < 0.075	18.88	150.9 ± 1		
Soil sample 2	> 0.297	10.82	62 ± 2	89 ± 2	89 ± 2
	0.297-0.150	29.45	57.3 ± 0.9		
	0.150-0.075	48.51	97 ± 1		
	X < 0.075	11.19	159 ± 0.9		

Table 4. Mercury concentration in the 0.297-0.150 mm fraction of the samples before and after second grinding (mean ± standard deviation)

| Sample | Before 2nd grinding | | After 2nd grinding | | |
	Size particle distribution / mm	Hg conc. / (ng g^{-1})	Size particle distribution / mm	Mass fraction / %	Weighted mean / (ng g^{-1})
Leaf sample 1	0.297-0.150	19 ± 0.8	0.297-0.150	48.8	19.6 ± 0.3
			0.150-0.075	42.5	
			X < 0.075	8.6	
Leaf sample 2	0.297-0.150	42 ± 1	0.297-0.150	40.9	41 ± 0.8
			0.150-0.075	30.4	
			X < 0.075	28.7	
Soil sample 1	0.297-0.150	49.3 ± 0.8	0.297-0.150	70.2	50 ± 1
			0.150-0.075	20.5	
			X < 0.075	9.3	
Soil sample 2	0.297-0.150	57.3 ± 0.9	0.297-0.150	65.5	56 ± 1
			0.150-0.075	23.4	
			X < 0.075	11.1	

robustness found in the method for the analysis of leaf samples, the carrier gas pressure and drying temperature should be regularly monitored; the decontamination of the boats should be done on the same day of use for Hg determination and variations in the mass of sample should be minimized.

Accuracy and precision

Recoveries for the four SRMs ranged from 102% to 104%, and the precision (RSD) was generally found to be less than 5% (Table 2). Recoveries of 96-97%, 93-103%, 90-105%, 95-98% and precisions of 9-13%, 6-22%, 2-9%,
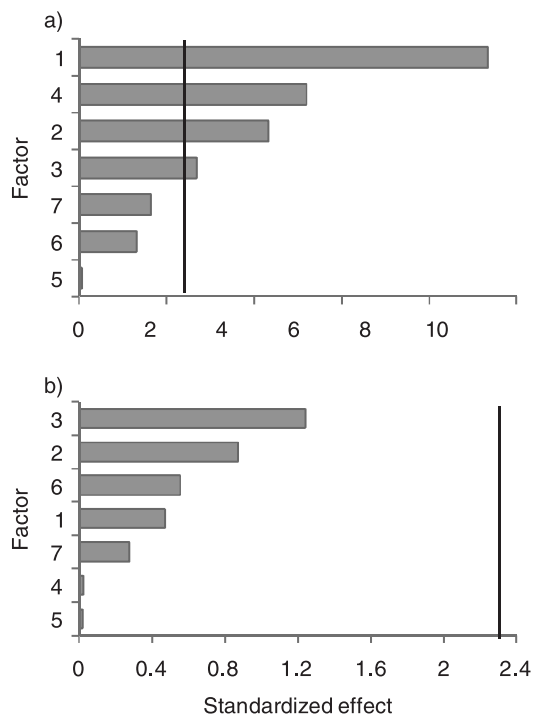
Figure 2. Pareto's charts of robustness test for a) leaf samples and b) soil samples. Factors: 1) Carrier gas pressure, 2) antecedence between decontamination and use of sample holders, 3) sample mass, 4) drying temperature, 5) ashing temperature, 6) drying time and 7) ashing time.

10-18% were previously reported for CVAAS analysis of geological and/or environmental samples with Hg concentration below 2 mg g^{-1} or mg mL^{-1}.[23-26] Although the accuracy of both methods (direct mercury analysis and CVAAS) allow obtaining results within the certified reference concentration of mercury, a better precision of the measurements can be observed by direct mercury analysis, likely due to the reduction of steps in sample preparation.

Conclusions

The analysis of soil and leaf samples by direct analysis of solid samples showed to be an analytical technique for total mercury quantification more precise than and as accurate as classical methods that includes sample acid digestion step. Furthermore, it is much less time consuming, considering that an autosampler with 40 boats is part of the equipment (~7 samples h^{-1}). For leaf samples, one of the most critical parameters of the direct analysis technique when using air as carrier and combustion gas, instead of oxygen, is the amount of analyzed sample, likely because of the high organic matter concentration. On the other hand, differences in particle grain distribution of soil samples do not significantly affect the experimental measurements. The analytical technique for soil samples, with higher mercury concentration, was shown to be more robust than for leaf sample analyses.

References

1. United Nation Environment Programme (UNEP); *Global Mercury Assessment 2013: Sources, Emissions, Releases and Environment Transport*; UNEP Chemicals Branch: Geneva, Switzerland, 2013.

2. Andrada, P.; Ruitenbeek, F. J. A.; Meer, F. D.; Fernández, R.; *Ore Geol. Rev.* **2012**, *45*, 61.

3. United States Environmental Protection Agency (US EPA); *Method 7471B*; U.S., 2007.

4. Zhu, L.; Lu, J.; Le, X. C.; *Microchim. Acta* **1993**, *111*, 207.

5. Bloom, N.; Fitzgerald, W. F.; *Anal. Chim. Acta* **1988**, *208*, 151.

6. Fostier, A. H.; Ferreira, J. R.; Andrade, M. O.; *Quim. Nova* **1995**, *18*, 425.

7. Gao, Y.; Shi, Z.; Long, Z.; Wu, P.; Zheng, C.; Hou, X.; *Microchem. J.* **2012**, *103*, 1.

8. Boylan, H. M.; Kingston, S.; *Am. Lab.* **1998**, *30*, 25.

9. United States Environmental Protection Agency (US EPA); *Method 7473, Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry*; February, 2007.

10. Kelly, J. G.; Han, F. X.; Su, Y.; Xia, Y.; Philips, V.; Shi, Z.; Monts, D. L.; Pichardo, S. T.; Xia, K.; *Water Air Soil Poll.* **2012**, *223*, 2361.

11. Rezende, P. S.; Moura, P. A. S.; Durão Jr., W. A.; Nascentes, C. C.; Windmöller, C. C.; Costa, L. M.; *J. Brazil. Chem. Soc.* **2011**, *22*, 910.

12. Cizdziel, J. V.; Tolbert, C.; Brown, G.; *Spectrochim. Acta B* **2010**, *65*, 176.

13. Roy, N. K.; Bose, S. S.; *Geostand. Geoanal. Res.* **2008**, *32*, 331.

14. Costley, C. T.; Mossop, K. F.; Dean, J. R.; Garden, L. M.; Marshall, J.; Carroll, J.; *Anal. Chim. Acta* **2000**, *405*, 179.

15. Belsley, D.; Kuh, E.; Welsch, R.; *Regressions Diagnostics: Identifying Influential Data and Sources of Colinearity*; Wiley: New York, 2005.

16. Levene, H.; *Robust Test for Equality of Variances;* Stanford University Press: Stanford, 1960.

17. Brown, M. B.; Forsythe, A. B.; *J. Am. Stat. Assoc.* **1974**, *69*, 364.

18. Draper, N.; Smith, H.; *Applied Regression Analysis*; Wiley: New York, 1988.

19. Miller, J. N; Miller, J. C.; *Statistics and Chemometrics for Analytical Chemistry,* 4th ed.; Pearson: Edinburgh, **2000**.

20. Youden, W. J.; Steiner, E. H.; *Statistical Manual of the Association of Official Analytical Chemists;* AOAC: Arlington, **1975**.

21. Durão Jr., W. A.; Palmieri, H. E. L.; Trindade, M. C.; Branco, O. E. A.; Carvalho Filho, C. A.; Fleming, P. M.; Da Silva, J. B. B.; Windmöller, C. C.; *J. Environ. Monitor.* **2009**, *11*, 1056.

22. Haynes, S.; Gragg, R. D.; Johnson, E.; Robinson, L.; Orazio, C. E.; *Water Air Soil Poll.* **2006**, *172*, 359.

23. Franklin, R. L.; Bevilacqua, J. E.; Favaro, D. I. T.; *Quim. Nova* **2012**, *35*, 45.

24. Collasiol, A.; Pozebon, D.; Maia, S. M.; *Anal. Chim. Acta* **2004**, *518*, 157.

25. Ribeiro, A. S.; Vieira, M. A.; Curtius, A. J.; *J. Braz. Chem. Soc.* **2004**, *15*, 825.

26. Segadea, S. R.; Tysonb, J. F.; *Spectrochim. Acta B* **2003**, *58*, 797.