# Novel Hybrid Flow Platform for On-line Simultaneous Dynamic Fractionation and Evaluation of Mercury Lability in Environmental Solids

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## ABSTRACT

A novel method has been developed for the simultaneous assessment of mobility and lability of mercury in environmental solid samples using an automatic hybrid flow system integrating flow-through dynamic sequential extraction and on-line chemical digestion prior to atomic fluorescence detection. A method for the automatic simultaneous assessment of mobility and lability of mercury in environmental solid samples has been developed for the first time. It has been implemented in a hybrid flow system integrating flow-through dynamic sequential extraction and on-line chemical digestion prior to atomic fluorescence detection. The method allows the determination of trace concentrations of labile mercury ($Hg_L$) and non-labile mercury ($Hg_{NL}$) in different bioaccessible phases of environmental solid samples thus providing expeditious data not only for Hg exposome studies but also for the selection of suitable environmental remediation techniques. The analytical procedure involves the sequential application of deionized water, 0.01 M $HNO_3$ solution, 1 M KOH solution and solution containing both $Na_2S$ and KOH (1 mol $L^{-1}$ each) to a solid sample packed in a column to release four Hg fractions according to their mobilities (i.e. water soluble, exchangeable, organic matter associated, and sulfide bound Hg) followed by the on-line determination of the concentrations of $Hg_L$ and $Hg_{NL}$ by flow programming. Apart from obtaining more comprehensive knowledge of risk exposure of Hg-laden solids, important advantages of the newly developed method compared to its batch-wise fractionation counterpart include (i) approximately 8-fold reduction in the time for acquisition of the dynamic extraction data, (ii) evaluation of the kinetics of release of Hg, (iii) Hg lability analysis, and (iv) minimization of matrix interferences and potential re-adsorption or transformation of the extracted Hg species. The method has been validated by analysing two reference materials (river sediment AGAL-10 and biosoil AGAL-12) with relatively high organic matter content.

*Keywords:* Mercury, Flow analysis, Dynamic fractionation, Mobility, Lability, Cold vapour generation, Sediment, Biosoil

## 1. Introduction

The inherent toxicity of various mercury species to living forms makes mercury and its compounds of significant health concern. Mercury can be bio-accumulated by terrestrial and aquatic organisms, while animals at higher trophic level and humans can be intoxicated by consuming tissues of those mercury polluted organisms [1]. A thorough understanding of the distribution and chemical forms of Hg in the environment is not only essential for its toxicity assessment, but also necessary for the selection of the most appropriate remediation approaches and their corresponding operational parameters. For instance, water soluble and exchangeable Hg species pose the highest risk to animals and humans, and their removal with several *in situ* approaches, such as flushing/water washing and electrokinetic techniques is more efficient than for the less mobile fractions [2]. In contrast, Hg species combined with sulfide are considered to be non-mobile and pose less health threat, yet their removal with the above mentioned techniques is less efficient. By adding KI into Hg contaminated soil, the removal of Hg can be much more efficient due to the formation of the stable negatively charged complex $[HgI_4]^{2-}$ [2].

Variables controlling the potential toxicity of Hg in solid settings can be divided into two major categories, 1) parameters controlling the release of Hg into the aquatic environment (mobility and thus bioaccessibility) and 2) parameters affecting Hg accessibility to biota (lability, i.e. potential bioavailability) [3]. In terms of mobility, Hg species in environmental solids, such as soil and sediment, can be classified as mobile, semi-mobile and non-mobile, depending on the phase of solid to which Hg is associated [4]. This can be assessed by chemical fractionation or partitioning of Hg according to its solubility in different reagents using sequential extraction procedures (SEPs) [5-7] followed by the determination of total Hg (T-Hg) in each fraction [8-21]. However, mobility data are mainly related to bioaccessibility [22]. Given that lability is a more decisive factor for the availability of Hg species to biota [7], study of labile Hg species is more meaningful for risk assessment/exposure. Labile Hg ($Hg_L$, or reactive Hg) has been defined to include those Hg species which can be readily reduced by $SnCl_2$ under acidic conditions [23] and thus includes the free $Hg^{2+}$ ion, metallic Hg ($Hg^0$) and weekly associated inorganic and organic Hg complexes [24]. Non-labile Hg ($Hg_{NL}$) consist of strongly bound to organic matter (OM) Hg, organic Hg (Org-Hg, such as methyl mercury) and Hg sulfide [24, 25]. Labile Hg has been determined by a number of physicochemical methods, such as acid extraction [26], stable isotope dilution techniques [27], voltammetric techniques [28], and the diffusive gradients in thin films technique (DGT) [29]. T-Hg in the mobile fraction has been often considered as $Hg_L$ in the literature [26, 30, 31]. This categorization seems questionable because $Hg_{NL}$ could be also present in the mobile fraction in the form of stable Hg complexes with dissolved organic matter [29]. Natural dissolved organic matter (DOM), such as humic acids (HA) and fulvic acids (FA), occurs in all natural sediments usually at concentrations much higher than that of Hg. These

substances, especially HA, form exceptionally strong complexes with Hg(II) due to their coordination with reduced sulfur (−S) or thiol (−SH) functional groups. Such complexation has been shown to limit Hg(II) availability for bacterial methylation and thus reducing its toxicity [32, 33]. As a consequence, this pool of Hg should be considered as non-labile [34].

DGT approach measures various Hg species that are able to pass through the diffusive gel and thus are likely to be captured by chelating resins that enable distinction between $Hg_L$ and $Hg_{NL}$ [29]. However, this operationally-defined technique only measures Hg in the mobile fraction of wet samples. It should be noted that remobilization and redistribution of Hg in solid samples can occur under certain conditions. For instance, HgS, which is a stable form of Hg, can be dissolved with the aid of organic matter [35]. Research has shown that DGT-induced depletion of Hg species in the pore water of sediment could be fully compensated by the remobilization of Hg from less mobile forms in the solid phase [36]. Therefore, $Hg_L$ in the semi-mobile fractions should not be obviated and measurement of $Hg_L$ and $Hg_{NL}$ in different phases of solid samples should be able to provide more insightful data for evaluation of worst-case scenarios in a risk assessment framework. In short, both mobility and lability are important determinants of Hg toxicity and bioavailability, but the two definitions have been always applied separately in the literature, thus information provided by each of them is still limited. In this study, we combine for the first time the fractionation and lability analysis of Hg in an integrated process for the simultaneous measurement of Hg mobility and lability in solid samples in an attempt to provide more comprehensive information on its potential bioavailability.

In reference to bioaccessibility methods for Hg, SEPs reported so far have been mostly performed under batch extraction conditions. Several inherent shortcomings have been, however recognized in conventional batch methods [37] including long duration, high labour intensity, re-adsorption of extracted Hg species [38] and/or species transformation [5, 11, 13, 16, 21, 39]. In addition, batch-wise SEPs cannot provide information on Hg desorption dynamics which offers a better understanding of Hg mobility and can be used to optimize the individual extraction steps. To tackle this issue, we have recently developed an on-line Hg fractionation method for subsequent determination of T-Hg in each fraction [40]. However, as described above, T-Hg results in each fraction are still not able to provide insights into the bioavailability of Hg. In this study, we propose an on-line dynamic holistic method combining the measurement of mobility and lability of Hg in troublesome environmental solids by conducting a specific multi-step SEP [14] with subsequent ultrasensitive lability analysis of Hg involving a facile on-line oxidation process. To the best of our knowledge, this is the first automatic flow analyser integrating complex, yet environmentally relevant SEP, for miniaturized dynamic extraction of Hg with on-line handling of the extracts aimed at identifying Hg mobility and lability concurrently and in real time.

## 2. Experimental

### *2.1. Reagents and apparatus*

All solutions were prepared in deionized water (18 MΩ cm, Millipore, Synergy 185). Individual standard solutions of $Hg^{2+}$ (1,000 mg $L^{-1}$), methylmercury (200 mg Hg $L^{-1}$) and ethylmercury (200 mg Hg $L^{-1}$), were prepared by dissolving 0.1354 g of $HgCl_2$ (Sigma Aldrich), 0.0252 g of methylmercury chloride (MeHgCl) (Aldric) and 0.0264 g of ethylmercury chloride (EtHgCl) (Chem Service), respectively, in 20 mL of 6 M HCl (Ajax Finechem) solution and diluted to 100 mL with deionized water. $NaBH_4$ (1 mg $L^{-1}$) solution for cold vapour generation (CVG) was prepared daily by dissolving 0.1 g of $NaBH_4$ (Scharlab S. L.) in 50 mL of 0.05 M NaOH (Chem-supply) solution, which was further diluted to 100 mL with a 0.05 M NaOH solution. A 20 mM $I_3^-$ solution was prepared by dissolving 0.5 g of sublimed $I_2$ (Asian Pacific Specialty Chemicals) in 100 mL of 1% KI (Chem-supply) solution. A 0.5 M HCl solution containing 1% $FeSO_4 \cdot 7H_2O$ (Chem-supply) and 0.5% ascorbic acid (AA) (Ajax Finechem) was prepared and used as the carrier stream in the CVG measurements. A 1% $K_2S_2O_8$ (J. T. Baker Chemical Co) solution was prepared by dissolving the solid chemical in deionized water. Two reference materials, namely, river sediment AGAL-10 and biosoil AGAL-12, both provided by National Measurement Institute (Australia), were used for method validation.

The SEP scheme reported by Wallschläger *et al.* [14] was successfully miniaturized and simplified previously by us by introducing on-line fractionation [40]. This automatic scheme was coupled in the present study with on-line Hg lability analysis. Briefly, deionized water, 0.01 M $HNO_3$ (Ajax Finechem) and 1 M KOH (Chem-supply) solutions were used as extractants for the 1$^{st}$, 2$^{nd}$ and 3$^{rd}$ fractions in this SEP scheme, respectively, while the extractant for the 4$^{th}$ fraction contained 1 M $Na_2S$ (Sigma-Aldrich) and 1 M KOH (Chem-supply). The four extractants aimed at releasing water soluble, exchangeable, organic matter associated, and sulfide bound Hg species, respectively. In the use of the fourth extractant, prepared by dissolving $Na_2S$ in KOH, it was observed that impurities, mainly $Na_2S_2O_3$ and $Na_2SO_3$, from the solid $Na_2S$ reagent generated relatively large quantities of elemental S during the acidification of the corresponding extract. This elemental S aggregated into visible particles which retained Hg species from the extract, thus causing biased results. Therefore, $Na_2S$ was purified prior to use by a $H_2S$ generation-absorption process as described previously [40]. The final concentration of $S^{2-}$ was determined by iodometric titration [41]. The purified $Na_2S$ solution could be stored for up to one month after which the generation of elemental S was observed during the acidification of the extract.

The experimental system for Hg fractionation and lability analysis was assembled by incorporating a FIAlab 3200 sequential injection (SI) analyser (FIAlab Instruments), which included

2 built-in syringe pumps, a ten-port selection valve (SV) and a built-in peristaltic pump (PP), with three auxiliary syringe pumps (MicroCSP-3000, FIAlab) and an atomic fluorescence spectrometer (AFS) (10.025 Millennium Merlin mercury analyser, PS Analytical, UK) (see Fig. 1). CVG was performed prior to AFS detection in the system as detailed below. The whole system was operated by FIAlab for Windows 5.0 software (FIAlab Instruments) and used for conducting the on-line sequential extraction, pre-treatment and on-line digestion of the extracts and Hg ($Hg_L$ and T-Hg) lability analysis. More details of the flow analyser are provided in the Supplementary Material.

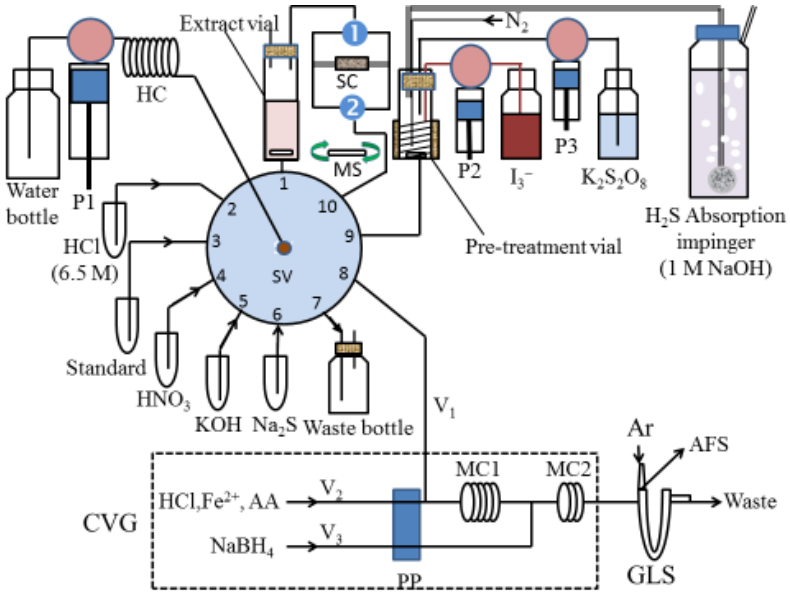



**Figure 1.** Schematic diagram of the experimental sequential injection – cold vapour generation – atomic fluorescence spectrometric (SI-CVG-AFS) system for on-line sequential extraction, digestion and automatic Hg lability analysis in environmental solids (P1 – P3, syringe pumps; PP: peristaltic pump, SV: selection valve, SC: sample column, ❶ and ❷: solenoid valves, HC: holding coil, MC1 and MC2: mixing coils, GLS: gas-liquid separator, MS: magnetic stirrer).

## *2.2. Operational procedure for automatic dynamic sequential extraction, Hg lability analysis and on-line detection of ultratraces of bioaccessible Hg*

The operational procedure coupled the previously reported by us on-line fractionation method [40] with a newly developed on-line lability analysis step. It involved the extraction of Hg from the solid sample packed in a micro-column (Supplementary Material) into aliquots (referred to as sub-fractions) of each of the four extractants used in the SEP (referred to as fraction) and its subsequent lability analysis by determining $Hg_L$ and T-Hg without and with the on-line digestion of $Hg_{NL}$, respectively. Consequently, the lability of the Hg species in each sub-fraction could be readily assessed. The dynamic extraction profiles for each of the first three extractants (fractions) were obtained by plotting the $Hg_L$ and T-Hg concentrations against the extractant volume. In the

determination of T-Hg, $Hg_{NL}$ was digested with $K_2S_2O_8$ at elevated temperature. Hg sulfide released with alkaline sodium sulfide as extractant was considered as non-labile [25]. Based on this definition, only T-Hg was determined here in the fourth fraction. The high concentration of sulfide in the sub-fractions of the fourth fraction completely depressed the fluorescent response of Hg due to the occurrence of highly stable Hg-S complexes in the extracats [42]. Therefore, sulfide was quantitatively removed with an *in situ* acidification-vaporization-oxidation technique as detailed previously [40].

For the Hg lability analysis a metered volume of 500 μL of the each of the sub-fractions of the first three fractions was used for the determination of $Hg_L$ without any further pre-treatment and another aliquot of 500 μL of each sub-fraction was utilized for the determination of T-Hg after digestion with $K_2S_2O_8$ in the reaction vial. In the determination of T-Hg in the sub-fractions of the fourth fraction, 500 μL of each sub-fraction were digested with $I_3^-$ and $K_2S_2O_8$ after removing acid volatile sulfide by the acidification-vaporization approach mentioned above. The operational procedure is described in detail in the Supplementary Material.

### *2.3. Hg calibration*

As mentioned above, $Hg_L$ in aqueous media has been defined as including those Hg species which can be easily reduced to $Hg^0$ by $SnCl_2$ under acidic conditions [23]. However, the reduction of organic Hg compounds (occurring at significantly higher levels in biosolids) by $SnCl_2$ in the presence of $Cd^{2+}$ and/or $Cu^{2+}$ as catalysts can lead to biased results for $Hg_L$ and $Hg_{NL}$. Therefore, $NaBH_4$ at the 1 mg $L^{-1}$ concentration was used as an alternative reductant to avoid the problem mentioned above, because it can selectively reduce $Hg_L$ to $Hg^0$ in the presence of Org-Hg [43]. The calibration of the SI-CVG-AFS system (Fig. 1) was performed with a series of $HgCl_2$ and MeHgCl standards (0, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 40 and 80 μg Hg $L^{-1}$), representing $Hg_L$ and $Hg_{NL}$, respectively. These standards were prepared in 0.4 M HCl solution as detailed in the Supplementary Material. $Hg^{2+}$ was determined by direct reduction with $NaBH_4$ while MeHgCl was determined after digestion with $K_2S_2O_8$. The slopes of the calibration curves for $Hg_L$ and $Hg_{NL}$ were found to be statistically indistinguishable as discussed below and thus a single calibration curve was deemed sufficient for determining the concentrations of both $Hg_L$ and T-Hg. Covalent Hg organic species, such as $MeHg^+$, are supposed to be thermodynamically more stable than Hg complexes with dissolved organic matter. As a result, quantification of overall $Hg_{NL}$ is better estimated using $MeHg^+$ as a standard.

### *2.4. Batch-wise sequential extraction*

The on-line dynamic SEP performed by the flow analyser, shown in Fig. 1, was compared with the batch-wise SEP procedure reported by Wallschläger *et al* [14]. In the latter procedure, 2 g of a solid sample placed in a 50 mL Cellstar plastic centrifuge tube (VWR International) were extracted

with 20 mL of the following extracting solutions: (1) water, (2) 0.01 M $HNO_3$, (3) 1 M KOH, and (4) 1 M $Na_2S$ in 1 M KOH. The solid sample was mixed with each individual reagent on an orbital shaker (Ratek Instruments) at 150 rpm for 24 h for obtaining the corresponding fraction. The residue after the extraction of each fraction was separated by centrifugation at 3,000 rpm for 20 min. $Hg_L$ and T-Hg in the supernatant of the first three fractions and T-Hg in the fourth fraction were determined by the newly developed on-line lability procedure described in the Supplementary Material.

### *2.5. Method validation*

To investigate the reliability of the on-line dynamic SEP/lability method two certified reference materials (CRMs) were analysed (AGAL-10 and AGAL-12). In every case, the solid residue in the Sample column (Fig. 1) after the SEP together with the frits and the Nylon membrane from the column were transferred to a 100 mL glass beaker. The mixture was digested with 15 mL of aqua regia at 230 °C on a hotplate for 40 min. The digest and water used to rinse the beaker (total of 50 mL) were transferred to a 50 mL polypropylene centrifuge tube and centrifuged at 3,000 rpm for 20 min. The residual Hg in the supernatant was determined by the newly developed SI-CVG-AFS method. Potential Hg adsorption by the surface of the plastic centrifuge tube was proven to be negligible [40]. The sum of T-Hg in the four fractions of the on-line SEP and the amount of residual Hg in the digest was compared to the certified value of the corresponding CRM provided by the National Measurement Institute and also with the amount of T-Hg (including the residual fraction), determined by the batch-wise SEP.

## 3. Results and discussion

### *3.1. Investigation of the experimental parameters of the on-line SEP*

Table S1 (Supplementary Material) lists the most relevant operational system parameters which were explored in this study together with their working ranges and selected values in the order in which the study was conducted. Each parameter was investigated as detailed in Supplementary Material and in the following sections:

### 3.2. *Analytical figures of merit of the on-line detection procedure for $Hg_L$ and T-Hg*

Under the selected experimental conditions (Table S1, Supplementary Material) the linear detection range for both $Hg^{2+}$ and MeHgCl spanned from 0 to 80 μg $L^{-1}$ with linear calibration equations given as $Y=49.012\times C_{Hg} + 1.336$ ($R$=0.9999) and $Y=49.009\times C_{Hg} + 1.356$ ($R$=0.9999), where Y is emission intensity expressed as peak height and $C_{Hg}$ is expressed in μg $L^{-1}$. A detection limit (3s of the blank signal, n=10) of 3 ng $L^{-1}$ was obtained for both Hg species and their limit of quantification was calculated as 10 ng $L^{-1}$. Intra-day relative standard deviation (RSD, n=10) of 2.2% for $Hg^{2+}$ and 3.1% for MeHgCl were attained for $C_{Hg}$ = 50 ng $L^{-1}$. Under the optimal conditions,

sample throughputs of 36 $h^{-1}$ and 30 $h^{-1}$ (without SEP) were achieved for Hg and MeHgCl, respectively. It was also established that $Hg^{2+}$ standards in the various extractants as described above had the same sensitivity as that of the calibration curve prepared in 0.4 M HCl, indicating the appropriateness of the HCl medium for the preparation of the standards. The statistically identical regression relationships for $Hg^{2+}$ and MeHg suggested that $Hg^{2+}$ standards alone could be used for the quantification of both $Hg_L$ and T-Hg.

### *3.3. Preservation of $Hg_L$ species in water soluble, exchangeable and organic matter associated fractions*

A potential problem in the determination of $Hg_L$ in the on-line extracts is the loss of Hg(II) via its reduction to $Hg^0$ by organic compounds in the leachate matrix prior to vapour generation and its subsequent purging by the $N_2$ gas flow on one hand and binding to the surface of the pre-treatment vial and plastic tubing of the flow manifold on the other [44]. To prevent this, preservative chemicals, such as $KMnO_4$, are often added to the sample solution to stabilize Hg(II) [45]. In the present study, the addition of $KMnO_4$ was expected to result in the potential oxidation of $Hg_{NL}$ thus compromising the Hg lability analysis. HCl is a potential alternative of $KMnO_4$ as a stabilizer because it forms Hg(II) chloride complexes, which are more difficult to reduce than non-complexed $Hg^{2+}$ [45]. Experiments involving the addition of 50 to 300 μL of 6.5 M HCl solution to 500 μL of the sub-fractions of the first three fractions confirmed the feasibility of the approach mentioned above and showed that the maximum fluorescence intensity for $Hg_L$ escalated with increasing the volume of 6.5 M HCl and levelled off at volumes above 200 μL. Therefore it was decided that for the reliable determination of $Hg_L$ in the first three fractions, 200 μL of 6.5 M HCl solution should be added to 500 μL of each sub-fraction. Under these conditions the recovery of $Hg_L$ in Hg(II)-spiked sub-fractions of all three fractions was better than 97%.

The presence of bioaccessible Fe(III) in the sub-fractions could lead to inflated results for $Hg_L$ because Fe(III) catalyses the reduction of Org-Hg by $NaBH_4$ [46]. Being capable of reducing Fe(III) to Fe(II) and eliminating its catalytic effect, ascorbic acid (AA) was added to the acidic reagent stream of the CVG unit (Fig. 1) so that it could prevent the catalytic reduction of Org-Hg during the vapour generation of $Hg_L$ with the additional advantage of eliminating the residual $K_2S_2O_8$ after the digestion step in the determination of T-Hg. The concentration of AA was varied between 0.5 and 2.0% and no difference in the maximum fluorescence intensity was observed. Therefore, 0.5% AA was selected for further studies.

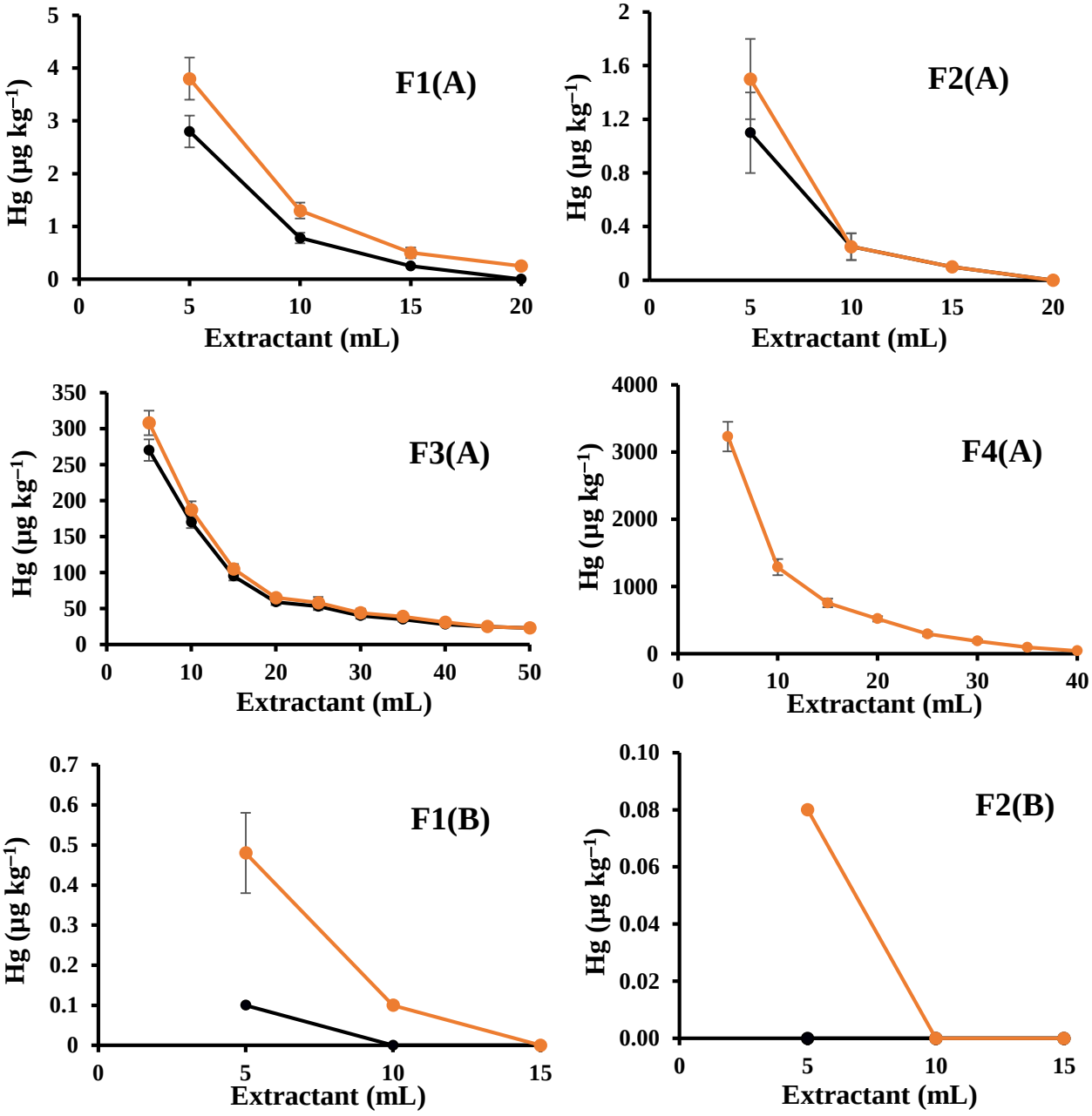
### *3.4. On-line fractionation and lability results*

An advantage of on-line SEP/lability method in flow systems is that unique kinetic information

of the extraction process can be obtained in real time [37]. Extractograms for $Hg_L$ and T-Hg in the sediment and biosoil CRMs AGAL-10 and AGAL-12 are shown in Fig. 2 where the results for T-Hg were obtained by us in a previous study where they were also used for successfully validating the on-line SEP procedure [40]. The results for AGAL-10 show that complete stripping of water soluble and exchangeable $Hg_L$ and T-Hg from the sediment reference material consumes 20 mL of each of the extractants, corresponding to an extraction time of 16.7 min each. This is a salient advantage compared to the batch-wise method in which 24 h were used for obtaining each fraction [14]. This also revealed that the Hg forms in the first two fractions were highly mobile and the use of extended extraction times, as recommended in the conventional SEP method, was unnecessary. The stripping of $Hg_L$ and T-Hg in the third and the fourth fractions consumed 60 mL and 40 mL of the corresponding extractants with extraction times of 50.0 min and 33.3 min, respectively, which were also much shorter than those used in the batch-wise SEP (24 h for each fraction) [14]. Similarly, complete extraction of $Hg_L$ and T-Hg from each of the four phases in the biosoil CRM AGAL-12 consumed much less time than in the batch-wise procedure. The shorter extraction times for the biosoil compared to the sediment in the on-line SEP method indicated that the extraction parameters were sample-specific. This illustrates how the newly developed SI-CVG-AFS method can automatically adjust the required volumes of extractants in accordance with the nature of the mercury species in the solid sample analysed.

Wallschläger *et al*. [14] pointed out that water soluble and exchangeable Hg were considered to be the most mobile and labile species, while Hg in the third and fourth fractions could be regarded as composed of less mobile species because of their high affinity for the natural organic matter and sulfide in environmental solids. Although the mobile fractions of Hg are often regarded as labile, it is evident from the results in Fig. 2 that non-labile Hg species, most likely Hg complexes with dissolved organic matter (DOM), are also present in those fractions. This is in agreement with literature data [14, 29, 32, 33]. Fulvic acids are the fraction of DOM with the lowest molecular mass and are soluble in acidic, neutral and alkaline media [14]. These substances are expected to account for the $Hg_{NL}$ in the water and acid extracts. Humic acids possess intermediate molecular mass and are only soluble in alkaline media. As a consequence, it is assumed that these substances account for the majority of $Hg_{NL}$ in the third fraction [14]. Compared to the river sediment CRM AGAL-10, the biosoil CRM AGAL-12 has a greater percentage of $Hg_{NL}$ in all of the first three fractions (F1 to F3) as a result of the higher content of DOM. In particular, the third fraction of AGAL-12 contains a greater proportion of $Hg_{NL}$ compared to that in first two fractions, most likely as Hg-DOM complexes, because KOH is especially effective in extracting DOM-bound Hg species [14]. This indicates the importance of the environmental conditions and sample type in the fate and transformation of Hg species [47].

DOM has been suggested to bind $Hg^{2+}$ predominantly via S-containing ligands [32, 48], while immobilization of Hg by sulfide includes the formation of HgS, Hg-polysulphides, and compounds where Hg is associated with Fe sulfides [14]. Fig. 2 shows that the majority of the bioaccessible Hg species in both CRMs were leached in the third and fourth fractions of the on-line SEP, thus they might not pose adverse effects to biota due to the high stability of their complexes with the DOM and sulfide. It is worth noting that a plethora of risk assessment methods endorsed by international and national regulators are based on conservative extraction conditions, whereby the mobility and lability are not estimated, and thus toxic effects in exposome studies are overestimated.



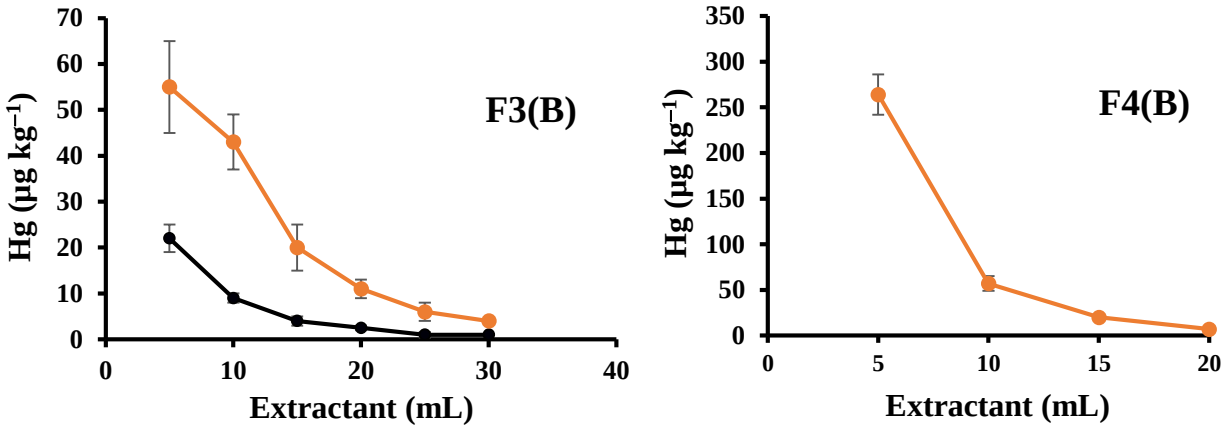



**Figure 2.** Extractograms of $Hg_L$ and T-Hg in AGAL-10 (A) and AGAL-12 (B). Optimal experimental conditions (Table S1, Supplementary Material) and extractant flow rate 20 µL $s^{-1}$; ● $Hg_L$; ● T-Hg; Error bars = ± SD. The results for T-Hg have been reported previously [40].

### *3.5. Method validation*

The newly developed automatic SEP/lability method was validated based on mass balance of the bioaccessible fractions ($1^{st}$+$2^{nd}$+$3^{rd}$+$4^{th}$ fraction) plus the non-bioaccessible Hg ($5^{th}$ fraction) obtained by acid digestion. The comparative results for AGAL 10 and AGAL 12 given in Table 1 show that the sum of T-Hg for all 5 fractions, i.e. 11.3 mg $kg^{-1}$ for AGAL-10 and 0.54 mg $kg^{-1}$ for AGAL-12, respectively, obtained with the newly developed on-line method are in good agreement with the corresponding certified values (i.e. 11.5 mg $kg^{-1}$ and 0.53 mg $kg^{-1}$ for AGAL-10 and AGAL-12, respectively), indicating that the analysis of bioaccessible Hg by the on-line SEP method was interference-free.

A salient merit of the new on-line method is the fact that the duration of the on-line SEP and lability analysis was significantly shortened as compared to the batch-wise SEP procedure [14] which dose not involve lability analysis. In particular, the entire automatic procedure for AGAL-12, including acid digestion, lasted only about 12 h. In addition, the on-line method used 200 mg of sample against 2 g in the original protocol [14], with no sample representativity issues for both CRMs analysed. The on-line extraction procedure also leached out lower concentration of organic matter than the batch-wise method. As a consequence, interference effects of organic matter on the Hg vapour stripping process were not observed.

$Hg_L$ and T-Hg in the CRMs extracted with the newly developed automatic hybrid flow analyser were compared against those of the batch-wise SEP method [14] with the extracts analysed by AFS-based flow-through lability analysis protocol used in this study (Table 1). The experimental results indicated that both $Hg_L$ and T-Hg concentrations in the first three fractions obtained by the on-line method were considerably higher than those obtained by the batch-wise method. This difference was

most likely due to the re-adsorption of extracted Hg in the batch-wise method onto newly generated solid surfaces. Re-distribution of extracted Hg by the remaining solid sample during the extraction of each fraction has been recognized as a major problem influencing the reliability of batch-wise SEP procedures [5, 49]. The results in Table 1 imply that the on-line extraction process is capable of minimizing re-adsorption of extracted Hg by continuously removing the bioaccessible fractions from the packed solid sample. As compared to the first three fractions, the results for the fourth fraction show that $Na_2S$ has the ability to inhibit the re-adsorption of the extracted Hg species. As a consequence, bioaccessibility of the most mobile forms of Hg, namely, those targeted in exposome investigations, may be underestimated by the batch-wise SEP while overestimating $Hg_{NL}$ pools [38].

The newly developed on-line SEP/lability method required only 14 h and 12 h for the analysis of CRM AGAL-10 and CRM AGAL-12, respectively, including system clean-up, re-loading of the sample column and any stand-by periods, which was significantly faster than the batch-wise SEP procedure (over 100 h per sample analysis). In fact, the AFS analysis of the $Hg_L$ bioaccessible pool was synchrobized with the automatic processing of the same sub-fraction for determination of T-Hg. It should be noted that the batch-wise procedure does not involve lability analysis.

**Table 1.** Average bioaccessible concentrations ± SD (n=3) of $Hg_L$ and T-Hg [40] for CRMs AGAL-10 and AGAL-12 as determined by the newly developed on-line SEP/lability method and the batch-wise SEP [14].

| | **Sediment standard reference material AGAL-10** | | | | **Biosoil standard reference material AGAL-12** | | | |
|---|---|---|---|---|---|---|---|---|
| Method | On-line | | Batch-wise | | On-line | | Batch-wise | |
| Species | $Hg_L$ | T-Hg | $Hg_L$ | T-Hg | $Hg_L$ | T-Hg | $Hg_L$ | T-Hg |
| F1 (μg $kg^{-1}$) | 3.8±1.5 | 5.9±1.7 | 1.1±0.2 | 2.4±0.4 | 0.10±0.08 | 0.6±0.2 | 0.24±0.04 | 0.66±0.05 |
| F2 (μg $kg^{-1}$) | 1.5±0.6 | 1.9±0.5 | 0.3±0.1 | 0.9±0.2 | ND | 0.08±0.03 | ND | ND |
| F3 (μg $kg^{-1}$) | (7.98±0.58)×$10^2$ | (8.85±0.96)×$10^2$ | (8.4±0.6)×$10^1$ | (2.35±0.32)×$10^2$ | (3.9±0.6)×$10^1$ | (1.39±0.22)×$10^2$ | (1.6±0.2)×$10^1$ | (3.8±0.4)×$10^1$ |
| F4 (μg $kg^{-1}$) | | (6.42±0.14)×$10^3$ | | (6.03±0.39)×$10^3$ | | (3.48±0.18)×$10^2$ | | (3.98±0.08)×$10^2$ |
| Total bioaccessible pools F1+F2+F3+F4 (μg $kg^{-1}$) | (8.00±0.61)×$10^2$ | (7.31±0.24)×$10^3$ | (8.5±0.6)×$10^1$ | (6.27±0.41)×$10^3$ | (3.91±0.61)×$10^1$ | (4.88±0.38)×$10^2$ | (1.6±0.2)×$10^1$ | (4.37±0.12)×$10^2$ |
| F5 (μg $kg^{-1}$) | | (3.96±0.12)×$10^3$ | | (4.07±0.03)×$10^3$ | | (5.5±0.4)×$10^1$ | | (5.9±0.5)×$10^1$ |
| F1+F2+F3+F4+F5 (μg kg | | (1.13±0.02)×$10^4$ | | (1.03±0.09)×$10^4$ | | (5.42±0.44)×$10^2$ | | (4.96±0.17)×$10^2$ |
| Certified value (μg $kg^{-1}$) | | (1.15±0.11)×$10^4$ | | (1.15±0.11)×$10^4$ | | (5.3±0.7)×$10^2$ | | (5.3±0.7)×$10^2$ |

* ND: Not detectable.

## 4. Conclusions

A new automatic hybrid flow method for the simultaneous evaluation of both the mobility and lability of Hg, present at trace level concentrations in solid samples, has been developed on the basis of an on-line Hg fractionation procedure [40] published earlier by us. The new method provides comprehensive leaching kinetic data of both labile and non-labile Hg which are more relevant to risk assessment of Hg in solid environmental compartments and the selection of potential chemical remediation approaches than the total leachable Hg concentration data provided by the previously reported method [40]. The volumes of extractants were automatically adjusted in accordance with the nature of the Hg forms in the solid samples analysed which makes the dynamic fractionation and lability analysis rapid and economical with respect to reagent consumption. The newly developed method was successfully applied to a sediment and biosoil CRMs and the results of the analysis of these materials revealed that their most mobile fractions of Hg (water soluble and exchangeable) and the less mobile fraction (stable organic matter associated) contained both labile and non-labile Hg forms. Such results also indicate that the method can provide more insightfull results than those of diffusive gradients in thin films in which the semi-mobile fraction of Hg cannot be measured. Further work is underway to explore the potential of the on-line bioaccessibility/lability concept for physiologically based extraction tests to investigate the binding of target elements to enzymes and organic compounds in gut fluids.

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# References


[1] S. Azimi, M.S. Moghaddam, Effect of mercury pollution on the urban environment and human health, Environ. Ecol. Res. 1(1) (2013) 12-20.
[2] F. He, J. Gao, E. Pierce, P.J. Strong, H. Wang, L. Liang, In situ remediation technologies for mercury-contaminated soil, Environ. Sci. Pollut. Res. 22 (2015) 8124–8147.
[3] M.M. Veiga, R.F. Baker, Protocols for environmental and health assessment of mercury released by artisanal and small-scale gold miners, Global Mercury Project, UNIDO, Vienna, Austria, 2004.
[4] R. Fernández-Martínez, J. Loredo, A. Ordóñez, M.I. Rucandio, Distribution and mobility of mercury in soils from an old mining area in Mieres, Asturias (Spain), Sci. Total Environ. 346(1-3) (2005) 200-212.
[5] N. Issaro, C. Abi-Ghanem, A. Bermond, Fractionation studies of mercury in soils and sediments: A review of the chemical reagents used for mercury extraction, Anal. Chim. Acta 631(1) (2009) 1-12.
[6] R. Fernández-Martínez, I. Rucandio, Assessment of a sequential extraction method to evaluate mercury mobility and geochemistry in solid environmental samples, Ecotoxic. Environ. Safety 97 (2013) 196-203.
[7] A.T. Reis, C.M. Davidson, C. Vale, E. Pereir, Overview and challenges of mercury fractionation and speciation in soils, TrAC 82 (2016) 109-117.
[8] R.T. Di Giulio, E.A. Ryan, Mercury in soils, sediments, and clams from a North Carolina Pleatland, Water Air Soil Pollut. 33(1) (1987) 205-219.
[9] N.W. Revis, T.R. Osborne, D. Sedgley, A. King, Quantitative method for determining the concentration of mercury(II) sulphide in soils and sediments, Analyst 114(7) (1989) 823-825.
[10] F.S. Kot, L.A. Matyushkina, Distribution of mercury in chemical fractions of contaminated urban soils of Middle Amur, Russia, J. Environ. Monit. 4 (2002) 803-808.
[11] N.S. Bloom, E. Preus, J. Katon, M. Hiltner, Selective extractions to assess the biogeochemically relevant fractionation of inorganic mercury in sediments and soils, Anal. Chim. Acta 479(2) (2003) 233-248.
[12] D.Y. Wang, C.L. Qing, T.Y. Guo, Y.J. Guo, Effects of humic acid on transport and transformation of mercury in soil-plant systems, Water Air Soil Poll. 95(1-4) (1997) 35-43.
[13] H. Biester, C. Scholz, Determination of mercury binding forms in contaminated soils: mercury pyrolysis versus sequential extractions, Environ. Sci. Technol. 31(1) (1996) 233–239.
[14] D. Wallschläger, M.V.M. Desai, M. Spengler, R.-D. Wilken, Mercury speciation in floodplain soils and sediments along a contaminated river transect, J. Environ. Qual. 27(5) (1998) 1034-1044.
[15] G. Liu, J. Cabrera, M. Allen, Y. Cai, Mercury characterization in a soil sample collected nearby the DOE Oak Ridge Reservation utilizing sequential extraction and thermal desorption method, Sci. Total Environ. 369 (2006) 384–392.
[16] R. Burt, M.A. Wilson, T.J. Keck, B.D. Dougherty, D.E. Strom, J.A. Lindahl, Trace element speciation in selected smelter-contaminated soils in Anaconda and Deer Lodge Valley, Montana, USA, Adv. Environ. Res. 8(1) (2003) 51-67.
[17] A.T. Reis, S.M. Rodrigues, C.M. Davidson, E. Pereira, A.C. Duarte, Extractability and mobility of mercury from agricultural soils surrounding industrial and mining contaminated areas, Chemosphere 81(11) (2010) 1369-1377.
[18] E.V. Ramasamy, A. Toms, C.M. Shylesh, K.K. Jayasooryan, M. Mahesh, Mercury fractionation in the sediments of Vembanad wetland, west coast of India, Environ. Geochem. Health 34 (2012) 575–586.
[19] C.M. Neculita, G.J. Zagury, L. Deschênes, Mercury speciation in highly contaminated soils from chlor-alkali pants uisng chemical extractions, J. Environ. Qual. 34(1) (2005) 255-262.
[20] D. Wang, X. Shi, S. Wei, Accumulation and transformation of atmospheric mercury in soil, Sci. Total Environ. 304(1-3) (2003) 209-214.
[21] A. Sahuquillo, G. Rauret, M. Bianchi, A. Rehnert, H. Muntau, Mercury determination in solid phases from application of the modified BCR-sequential extraction procedure: a valuable tool for assessing its mobility in sediments, Anal. Bioanal. Chem. 375 (2003) 578-583.
[22] H. Dočekalová, V. Kovaříková, B. Dočekal, Mobility and bioaccessibility of trace metals in soils assessed by conventional extraction procedures and passive diffusive samplers, Chem. Speciation Bioavailability 24(4) (2012) 261-265.
[23] R.P. Mason, W.F. Fitzgerald, G.M. Vandal, The sources and composition of mercury in Pacific Ocean rain, J. Atmos. Chem. 14(1) (1992) 489-500.

[24] M. Leermakers, C. Meuleman, W. Baeyens, Mercury speciation in the scheldt estuary, in: D.B. Porcella, J.W. Huckabee, B. Wheatley (Eds.) Third International Conference, Springer-Science + Business Media, B. V., Whistler, British Columbia, 1994, pp. 632-640.
[25] J.L. Ticknor, K.H. Kucharzyk, K.A. Porter, M.A. Deshusses, H. Hsu-Kim, Thiol-based selective extraction assay to comparatively assess bioavailable mercury in sediments, Environ. Eng. Sci. 32(7) (2015) 564-573.
[26] A.T. Reis, C.B. Lopes, C.M. Davidson, A.C. Duarte, E. Pereira, Extraction of available and labile fractions of mercury from contaminated soils: The role of operational parameters, Geoderma 259-260 (2015) 213-223.
[27] W. Shetaya, J.-H. Huang, S. Osterwalder, C. Alewell, Quantification of labile soil mercury by stable isotope dilution techniques, Geophysic. Res. Abstract. 18 (2016) EGU 2016-1568-1.
[28] F.H. do Nascimento, J.C. Masini, Complexation of Hg(II) by humic acid studied by square wave stripping voltammetry at screen-printed gold electrodes, Talanta 100 (2012) 57-63.
[29] P. Diviš, M. Leermakers, H. Dočekalová, Y. Gao, Mercury depth profiles in river and marine sediments measured by the diffusive gradients in thin films technique with two different specific resins, Anal. Bioanal. Chem. 382(7) (2005) 1715–1719.
[30] R. Fernández-Martínez, I. Rucandio, Total mercury, organic mercury and mercury fractionation in soil profiles from the Almadén mercury mine area, Environ. Sci. Process Impacts 16(2) (2014) 333-340.
[31] R. Fernández-Martínez, B. Belén Gómez-Mancebo, E.J. Peña, P. Galán, A. Matsuyama, F. García, I. Rucandio, Monitoring of mercury and other metals mobility by sequential fractionation in soils nearby an abandoned chlor-alkali plant in Managua (Nicaragua), Environ. Earth. Sci. 75 (2016) DOI: 10.1007/s12665-015-5171-3.
[32] B. Gu, Y. Bian, C.L. Miller, W. Dong, X. Jiang, L. Liang, Mercury reduction and complexation by natural organic matter in anoxic environments, Proc. Natl. Acad. Sci. USA. 108(4) (2011) 1479–1483.
[33] J.J. Alberts, M. Takács, M. Pattanayek, Influence of IHSS standard and reference materials on copper and mercury toxicity to vibrio fischeri, Acta Hydrochim. Hydrobiol. 28(7) (2000) 428-435.
[34] E. Ramalhosa, E. Pereira, C. Vale, M. Válega, P. Monterroso, A.C. Duarte, Mercury distribution in Douro estuary (Portugal), Mar. Pollut. Bull. 50(11) (2005) 1218-1222.
[35] M. Ravichandran, G.R. Aiken, M.M. Reddy, J.N. Ryan, Enhanced dissolution of cinnabar (mercuric sulfide) by dissolved organic matter isolated from the florida everglades, Environ. Sci. Technol. 32(21) (1998) 3305-3311.
[36] O. Clarisse, B. Dimock, H. Hintelmann, E.P. Best, Predicting net mercury methylation in sediments using diffusive gradient in thin films measurements., Environ. Sci. Technol. 45(4) (2011) 1506-1512.
[37] M. Rosende, M. Miró, Recent trends in automatic dynamic leaching tests for assessing bioaccessible forms of trace elements in solid substrates, Trends Anal. Chem. 45 (2013) 67-78.
[38] J.L. Gómez-Ariza, I. Giráldez, D. Sánchez-Rodas, E. Morales, Metal readsorption and redistribution during the analytical fractionation of trace elements in oxic estuarine sediments, Anal. Chim. Acta 399(3) (1999) 295-307.
[39] A.J. Renneberg, M.J. Dudas, Transformations of elemental mercury to inorganic and organic forms in mercury and hydrocarbon co-contaminated soils, Chemosphere 45(6-7) (2001) 1103-1109.
[40] Y. Zhang, M. Miró, S.D. Kolev, An automatic flow assembly for on-line dynamic fractionation of trace level concentrations of mercury in environmental solids with high organic load, Anal. Chim. Acta 975 (2017) 1-10.
[41] Z. Pawlak, A.S. Pawlak, Modification of iodometric determination of total and reactive sulfide in environmental samples, Talanta 48(2) (1999) 347-353.
[42] J.A. Jay, F.M.M. Morel, H.F. Hemond, Mercury speciation in the presence of polysulfides, Environ. Sci. Technol. 34(11) (2000) 2196–2200.
[43] S.R. Río Segade, J.F. F. Tyson, Determination of inorganic mercury and total mercury in biological and environmental samples by flow injection-cold vapor-atomic absorption spectrometry using sodium borohydride as the sole reducing agent, Spectrochim. Acta B 58(5) (2003) 797-807.
[44] J.H. Cragin, Increased mercury contamination of distilled and natural water samples caused by oxidizing preservatives, Anal. Chim. Acta 110(2) (1979) 313-319.
[45] J. Murphy, P. Jones, S.J. Hill, Determination of total mercury in environmental and biological samples by flow injection cold vapour atomic absorption spectrometry, Spectrochim. Acta B 51(14) (1996) 1867-1873.

[46] Y. Zhang, S.B. Adeloju, Speciation of mercury in fish samples by flow injection catalytic cold vapour atomic absorption spectrometry, Anal. Chim. Acta 721 (2012) 22-27.
[47] B. Leterme, P. Blanc, D. Jacques, A reactive transport model for mercury fate in soil—application to different anthropogenic pollution sources, Environ. Sci. Pollut. Res. 21(21) (2014) 12279–12293.
[48] D. Wallschläger, M.V.M. Desai, R.-D. Wilken, The role of humic substances in the aqueous mobilization of mercury from contaminated floodplain soils, Water Air Soil Pollut. 90(3) (1996) 507-520.
[49] M. Miró, E.H. Hansen, R. Chomchoei, W. Frenzel, Dynamic flow-through approaches for metal fractionation in environmentally relevant solid samples, Trens Anal. Chem. 24 (2005) 759-771.