Universidade de Aveiro Departamento de Química
Ano 2013

Ana Teresa Lemos Pereira Saúde Reis

Desafios na especiação e fracionamento de mercúrio em solos e sedimentos

Challenges in mercury speciation and fractionation in soil and sediment

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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Química, realizada sob a orientação científica da Doutora Maria Eduarda da Cunha Pereira, Professora auxiliar do Departamento de Química da Universidade de Aveiro (Portugal), da Doutora Christine Davidson, *Senior Lecturer* do Departamento de Química Pura e Aplicada da Universidade de Strathclyde (Glasgow, Escócia) e da Doutora Marta Otero, Investigadora do Departamento de Química e Física Aplicadas da Universidade de Léon (Espanha).

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palavras-chave Mercúrio, Especiação, Solo, Sedimento, Extração sequencial.

resumo

O interesse na especiação de mercúrio em matrizes ambientais sólidas tem vindo a aumentar nas últimas décadas, muito em parte porque a determinação da biodisponibilidade de um contaminante é um requerimento fundamental na avaliação de risco ambiental. Por sua vez, a biodisponibilidade de um elemento depende das formas em que este se encontra e da forma como cada espécie se relaciona com a matriz. Geralmente a especiação de um elemento é feita recorrendo a extrações químicas simples ou sequenciais, como por exemplo o protocolo proposto pelo BCR. No entanto, devido às suas características químicas, o mercúrio requer o desenvolvimento de procedimentos de especiação específicos, sendo esta necessidade veementemente enfatizada na literatura.

No sentido de dar resposta a esta questão, este trabalho de investigação teve como objetivo o estudo, desenvolvimento e validação de metodologias para a especiação de mercúrio em solos e sedimentos. Partindo de uma revisão detalhada da literatura, vários métodos foram escolhidos e testados em amostras de solo e sedimento de composição físico-química diferente e bem conhecida. Diversas soluções de extração foram consideradas, bem como a adequação de diferentes instrumentos analíticos para a quantificação de mercúrio nos extratos. Adicionalmente, foram efectuados estudos cinéticos com o objetivo de estabelecer o tempo de extração adequado para a liberação do mercúrio da matriz em cada fração, uma vez que este passo, a par com o tipo de solução de extração, é um dos parâmetros que mais varia entre procedimentos. Verificou-se que, nas zonas estudadas, apenas uma pequena percentagem de mercúrio está presente em formas potencialmente biodisponíveis. Os resultados indicam que a biodisponibilidade do mercúrio está relacionada com a composição química da amostra, sendo potenciada na presença de alumínio e manganês e inibida pela matéria orgânica e enxofre. Os resultados também indicam que o tamanho das partículas do solo ou sedimento tem influência no procedimento de extração. Solos arenosos, e portanto maioritariamente constituídos por partículas maiores, tendem a liberar o mercúrio mais rapidamente que solos argilosos, onde a compactação das partículas dificulta o “acesso” das soluções de extração à totalidade da amostra.

Embora os procedimentos de extração sequencial permitam uma melhor compreensão da distribuição do mercúrio num solo ou sedimento e o trabalho apresentado nesta tese possa contribuir para a otimização de alguns passos cruciais nestes procedimentos, a complexidade do processo de extração limita a sua aplicabilidade e robustez em trabalho de rotina.

No sentido de avaliar esta questão, foi organizado um exercício de comparação interlaboratorial, tendo como matrizes de teste solo, sedimento, peixe e cabelo humano. A par com a determinação de mercúrio total, foi proposto um conjunto de extrações simples com acetato de amónio 1 mol L^{-1}, HCl 0,1 mol L^{-1} e $CaCl_2$ a 0,1 mol L^{-1} para o solo. A extração da fracção organometálica foi também proposta para o solo, sedimento e peixe. Os resultados permitiram i) atualizar o conhecimento sobre técnicas que estão atualmente a ser utilizadas para a quantificação de mercúrio, os problemas associados e as fontes de erro; ii) avaliar a reprodutibilidade de procedimentos de extração química. Embora 74% dos participantes tenham tido uma performance satisfatória, foram detetados problemas na quantificação de mercúrio quando em concentrações mais baixas. O desenvolvimento ou optimização de técnicas analíticas para limites de quantificação mais baixos é, portanto, recomendável. Relativamente ao segundo ponto, apenas quatro participantes realizaram extrações. Este número é, por si, indicativo da relutância ou dificuldade dos laboratórios em realizarem extrações de mercúrio. Deste modo, espera-se que os resultados deste trabalho de doutoramento contribuam com alguns avanços necessários no campo da especiação de mercúrio em solos e sedimentos e que, ao mesmo tempo, tornem estas técnicas mais acessíveis à generalidade dos laboratórios.

Neste sentido, foi desenvolvido um método de especiação por termo-dessorção. Comparativamente com métodos de extração química, a especiação por termo-dessorção tem diversas vantagens: não requer qualquer tipo de reagente ou solução de extração; mais rápido; necessita de menor manipulação da amostra; é essencialmente controlado pelo software dos equipamentos, o que permite que seja realizado sob as mesmas condições operacionais em todos os laboratórios, possibilitando a inter-comparação dos resultados obtidos; as perdas de mercúrio são negligenciáveis; pode ser considerado " limpo", uma vez que não são produzidos resíduos.

De um modo geral, o trabalho apresentado nesta tese pretende contribuir para um maior conhecimento dos procedimentos analíticos envolvidos na especiação de mercúrio em solos e sedimentos, bem como para uma melhor compreensão dos fatores que controlam o comportamento deste elemento nessas matrizes e que podem também influenciar os procedimentos de extração.

keywords Mercury, Speciation, Fractionantion, Soil, Sediment, Sequential extraction.

abstract

This investigation focused on the development, test and validation of methodologies for mercury fractionation and speciation in soil and sediment. After an exhaustive review of the literature, several methods were chosen and tested in well characterised soil and sediment samples. Sequential extraction procedures that divide mercury fractions according to their mobility and potential availability in the environment were investigated. The efficiency of different solvents for fractionation of mercury was evaluated, as well as the adequacy of different analytical instruments for quantification of mercury in the extracts. Kinetic experiments to establish the equilibrium time for mercury release from soil or sediment were also performed. It was found that in the studied areas, only a very small percentage of mercury is present as mobile species and that mobility is associated to higher aluminium and manganese contents, and that high contents of organic matter and sulfur result in mercury tightly bound to the matrix. Sandy soils tend to release mercury faster that clayey soils, and therefore, texture of soil or sediment has a strong influence on the mobility of mercury. It was also understood that analytical techniques for quantification of mercury need to be further developed, with lower quantification limits, particularly for mercury quantification of less concentrated fractions: water-soluble e exchangeable.

Although the results provided a better understanding of the distribution of mercury in the sample, the complexity of the procedure limits its applicability and robustness.

A proficiency-testing scheme targeting total mercury determination in soil, sediment, fish and human hair was organised in order to evaluate the consistency of results obtained by different laboratories, applying their routine methods to the same test samples. Additionally, single extractions by 1 mol L^{-1} ammonium acetate solution, 0.1 mol L^{-1} HCl and 0.1 mol L^{-1} $CaCl_2$, as well as extraction of the organometallic fraction were proposed for soil; the last was also suggested for sediment and fish. This study was important to update the knowledge on analytical techniques that are being used for mercury quantification, the associated problems and sources of error, and to improve and standardize mercury extraction techniques, as well as to implement effective strategies for quality control in mercury determination.

A different, “non chemical-like” method for mercury species identification was developed, optimised and validated, based on the thermo-desorption of the different mercury species. Compared to conventional extraction procedures, this method has advantages: it requires little to no sample treatment; a complete identification of species present is obtained in less than two hours; mercury losses are almost neglectable; can be considered “clean”, as no residues are produced; the worldwide comparison of results obtained is easier and reliable, an important step towards the validation of the method. Therefore, the main deliverables of this PhD thesis are an improved knowledge on analytical procedures for identification and quantification of mercury species in soils and sediments, as well as a better understanding of the factors controlling the behaviour of mercury in these matrices.

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Preface and Outline

Healthy soil and sediment systems are essential for protection of groundwater and of the aquatic ecosystems, uptake of chemicals by plants and soil/sediment-dwelling organisms, the food chain, sustaining agricultural practices, the health of humans and animals that directly or indirectly benefit from these systems, and for maintaining the proper functioning of natural ecosystems. However, many soil and sediment systems have been contaminated, due to natural or anthropogenic causes, impairing their quality, and ultimately affecting human health and the overall environment.

Several efforts have been made to establish limit values for the concentration of potentially toxic elements (PTEs) in soil and sediment (CCME, 2007; Crommentuijn *et al.*, 2000; Fishwick, 2004), which are conservatively based on total concentrations, more specifically on the lowest concentration that has been reported to produce an undesired effect. However, the behaviour of PTEs largely depends on how an element interacts with the matrix, which determines its fate, transport, bioavailability, and toxicity to organisms. Hence, understanding the behaviour of PTEs in soil and sediment systems is an important task in hazard and risk assessment. As a result there is increasing interest in improving the understanding of element–solid phase associations in natural and polluted solid systems (Bacon *et al.*, 2008; Tomson *et al.*, 2003).

Mercury (Hg) is one of the most critical contaminants in the environment (Hissler *et al.*, 2006) and is present in water, soils, sediments and air usually at trace levels. However several human activities (e.g., mining, industry, sludge dumping) have increased its natural concentration and led to severely located contaminated environments (Hylander *et al.*, 2003).

In the environment, mercury undergoes a series of chemical transformations according to the bio-physico-chemical conditions of these systems, changing mercury speciation and interaction with environmental matrices. Therefore, understanding mercury speciation is key for risk assessment of mercury contaminated areas (Bollen *et al.*, 2008), since speciation largely affects its bioavailability, solubility, toxicological, and ecological effects (Biester *et al.*, 2002a; Clarkson, 2002; Lodenius, 1994).

Due to the aforementioned importance of preserving healthy soils and sediments, particular attention must be given to these systems, since they play an important role in the mercury cycle, acting both as a sink and source of this metal to biota, atmosphere and hydrological compartments (Oliveira *et al.*, 2007), through volatilisation or formation of soluble organic and inorganic compounds, with consequent dispersion of the contamination. Consequently, knowledge on the chemical forms of mercury present in soil and sediment is key to understand the real risk that these mercury-contaminated compartments represent to the overall environment. This is usually accomplished by the application of speciation or fractionation procedures (Bloom *et al.*, 2003b; Fernández-Martínez *et al.*, 2003; Han *et al.*, 2003; Issaro *et al.*, 2009; Reis *et al.*, 2012; Reis *et al.*, 2010; Revis *et al.*, 1989; Sakamoto *et al.*, 1992), used to subdivide the mercury content of samples into operational defined groups of more or less soluble species (Rubio *et al.*, 1996).

For the purpose of the work presented in this thesis, a review of relevant knowledge concerning mercury chemistry in the environment and the issue of speciation/fractionation, with special focus on mercury speciation and fractionation in soil and sediment will be given.

This thesis is organized in nine chapters, as listed bellow:

Chapter 1 - Introduction - reviews the background information necessary to the realization of this work.

Chapter 2 - Motivation and objectives - consists of the objectives and motivation behind this work.

Chapter 3 - The sampling and analytical methodologies, and quality control and quality assurance procedures used are described.

Chapter 4 – Extractability and mobility of mercury from agricultural soils surrounding industrial and mining contaminated areas - the extractability of mercury in soils with two different contamination sources (a chlor-alkali plant and mining

activities) is studied, by the application of a sequential extraction procedure - the Kingston method - that divides mercury species according to their mobility. The influence of soil properties on mercury fractionation is studied.

Chapter 5 - Focus on Kinetic extractions and is divided in two parts: I) Extraction of mercury water-soluble fraction from soils: an optimisation study; II) Desorption kinetics of mercury labile fractions from contaminated soils. The kinetics involved in the extractions were investigated, as well as water:extractant ratio, separation methods (centrifugation vs. filtration), and the analytical techniques used for mercury quantification in the extracts.

Chapter 6 - An international proficiency test as a tool to evaluate the current mercury determination status in organic and inorganic matrices - To assess the current status of mercury determination and evaluate the reproducibility of chemical extraction procedures, an international inter-laboratory study was organised. Soil, sediment, fish, and human hair were the chosen test materials. Together with total mercury quantification, extraction of the organometallic and exchangeable fractions was considered.

Chapter 7 - Development and validation of a simple thermo-desorption technique for mercury speciation in soils and sediments, refers to the development of a simpler speciation technique by thermo-desorption, using direct mercury analyser equipment. This has been presented as an alternative to lengthier chemical extraction procedures, besides many other advantages. The processes of validation and optimization are highlighted in this chapter. Application in “real” samples is also demonstrated.

Chapter 8 – A final discussion is provided, where an overview and a critical appraisal of results are provided.

Chapter 9. References.

The work presented in this thesis has partially been published in:

Reis A.T., Rodrigues S.M., Davidson C.M., Pereira E., Duarte A.C. (2010) Extractability and mobility of mercury from agricultural soils surrounding industrial and mining contaminated areas. Chemosphere. 81 (11) 1369-1377.
Link: http://dx.doi.org/10.1016/j.chemosphere.2010.09.030

Reis A.T., Coelho J.P., Rodrigues S.M., Rocha R., Davidson C.M., Duarte A.C., Pereira E. (2012) Development and validation of a simple thermo-desorption technique for mercury speciation in soils and sediments. Talanta. 99, 363-368.
Link: http://dx.doi.org/10.1016/j.talanta.2012.05.065

Reis A.T., Lopes C.B., Davidson C.M., Duarte A.C., Pereira E. (2014) Extraction of mercury water-soluble fraction from soils: An optimization study. Geoderma. 213, 255-260.
Link: http://dx.doi.org/10.1016/j.geoderma.2013.08.010

Three more manuscripts are ready for submission:

Reis A.T., Coelho J.P., Rucandio I., Davidson C.M., Duarte A.C., Pereira E. Improvements of a simple thermo-desorption technique for mercury speciation in soils and sediments. *To be submitted to Talanta.*

Reis A.T., Lopes C.B., Davidson C.M., Duarte A.C., Pereira E. Desorption kinetics of mercury labile fractions from polluted soils. *To be submitted to Geoderma.*

Reis A.T., Henriques B., Coelho C., Lopes C.B., Mieiro C., Tavares D., Ahmad I., Coelho J.P., Rocha L., Cruz N., Rocha R., Rodrigues S., Duarte A.C., Pereira E. An international proficiency test as tool to evaluate the current status of mercury determination in organic and inorganic matrices. *To be submitted to Trends in Analytical Chemistry.*

Introduction

1 INTRODUCTION

1.1 Physicochemical properties, applications, and sources of mercury

Mercury (Hg) is one of the most dangerous contaminants in the environment (Hissler *et al.*, 2006) and it differs from other metals in several aspects: it is the only metal liquid at room temperature, boils below 650 °C and it is quite chemically inert, having a higher ionization potential than any other electropositive element with the sole exception of hydrogen. Moreover, mercury is highly volatile and very dense. In its elemental state, mercury is a silver-white liquid, known as metallic mercury (Hg^0). Mercury is also present in the environment in two oxidized forms, mercuric ion (Hg^{2+}), and mercurous ion (Hg_2^{2+}). The latter, however, is not stable under environmental conditions and tends to disproportionate into Hg^0 and Hg^{2+}. The presence of ligands and metal ions often results in the formation of different inorganic and organic Hg^{2+} complexes, each with specific chemical characteristics, reactivity, toxicity and impact in the ecosystems and human health. In organomercury species the mercury atom is covalently bound to at least one carbon atom. Different organomercury species can be formed, including methylmercury, ethylmercury and phenylmercury, being the short chain alkyl mercury species, methylmercury (more correctly, monomethylmercury(II) cation - CH_3Hg^+) and dimethylmercury ($(CH_3)_2Hg$), the most hazardous compounds in terms of their toxicological effects. The mercuric ion has high affinity for Cl^-, OH^-, S^{2-} and S-containing function groups or organic ligands (Schuster, 1991). Additionally, mercury forms alloys ("amalgams") with many metals, particularly gold and silver (Horvat, 2005).

Mercury occurs naturally in the Earth's crust principally as the ore cinnabar (HgS) (Horvat, 2005). Therefore, natural sources of mercury include erosion or weathering of mineral deposits, but also volcanic and geothermal activity (Gochfeld, 2003; Gustin, 2003), association with hydrocarbons (Miedaner *et al.*, 2005), and volatilization from deep-sea hydrothermal vents (Crespo-Medina *et al.*, 2009).

Additionally, and as can be seen in **Figure** 1, mercury previously deposited on vegetation, land or water surfaces can be re-emitted through land use, biomass burning, meteorological conditions and exchange mechanisms of gaseous mercury at the air-water/top soil/snow-ice pack interfaces (Mason, 2009; Pirrone *et al.*, 2010).

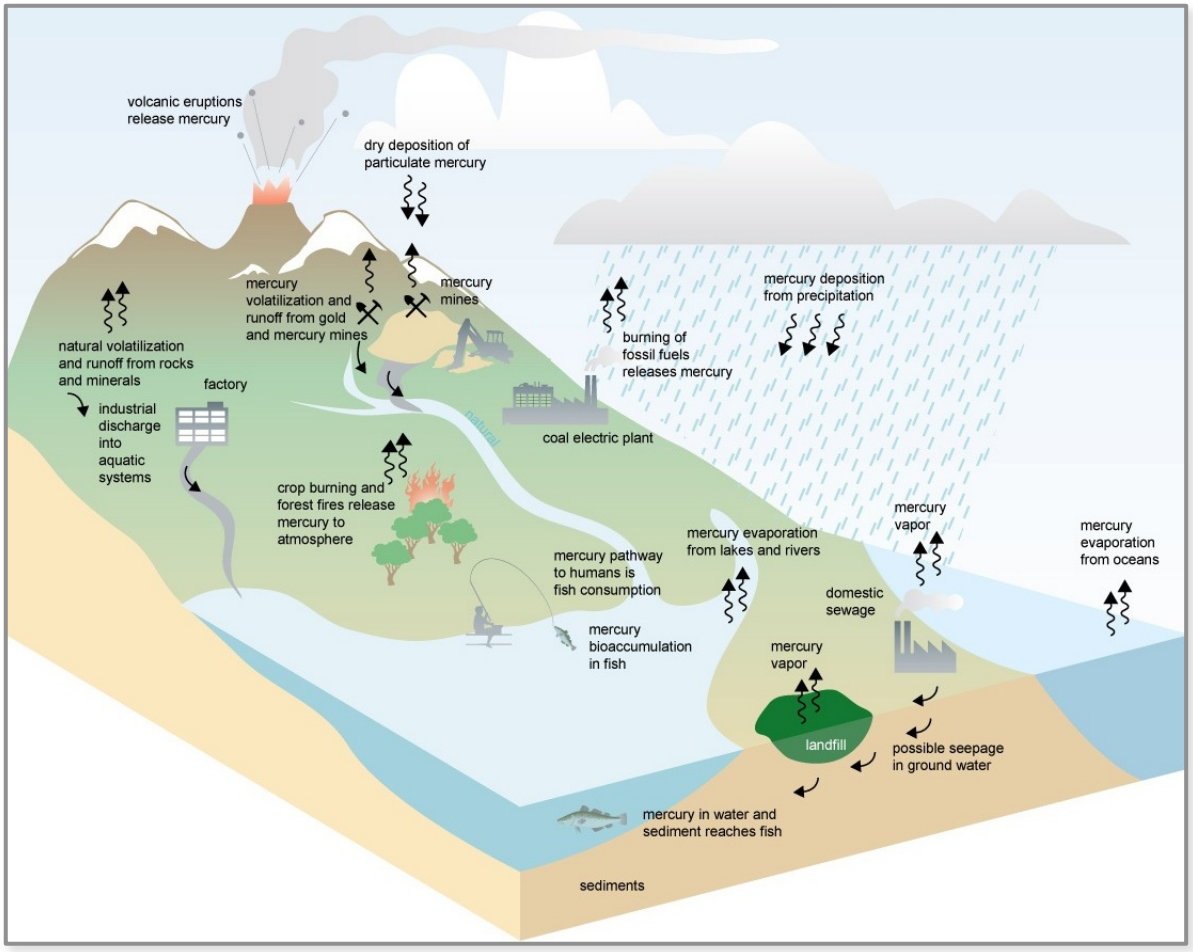

Figure 1. Mercury transport and cycle in the environment (Rekacewicz *et al.*, 2005).

Mercury's unique physical and chemical properties have had many commercially and medically valuable applications. The recovery and uses of mercury have been described since antiquity, possibly since the early 2nd millennium BC in Egypt, and the mining and recovery of cinnabar were described in the 4th century BC (Hylander *et al.*, 2003). Mercury has historically been used in gold mining operations to separate gold from other rocks and metals (Clarkson, 1998), a technique still worryingly used today in the artisanal gold mining sector (Malm *et al.*, 1995; Velásquez-López *et al.*, 2010). Mercury was also extensively used for therapeutic purposes, mainly due to its disinfectant properties (Clarkson, 1998). Human mercury exposure through dental amalgams used to fill dental caries is still a controversial issue (Bailer *et al.*, 2001), and considering that the amalgams contain approximately 50% of elemental mercury, they could be one of the primary exposures to mercury in the general population (Bates, 2011). Yet, the biggest controversy involving mercury use in medicine has to be thimerosal in childhood

vaccines. Thimerosal is an organomercury compound used as a preservative in vaccines since the 1930s and was linked to autism and neurodevelopment disorders (Dórea, 2010), a fact that the Institute of Medicine stated to lack empirical evidence (Immunization Safety Review Committee *et al.*, 2001). As a precautionary measurement, thimerosal-containing vaccines have been largely eliminated for administration to infants under 6 months of age in the developed world (Blaxill *et al.*, 2004), but many parents are still very reluctant to vaccine their children, a decision that has largely contributed to the reappearing of eradicated diseases like measles and pertussis.

Industrially, mercury has had and still has many applications. As a fluid of high density and uniform expansion properties, it has long been used in thermometers, barometers and to float the heavy lamps in lighthouses. It has been a common component of thermostats, due to its capacity to conduct electrical current. The use of mercury sulfate as a catalyst in the manufacture of acetaldehyde led to the health disasters in Japan in the 1950s and 60s when methylmercury compounds, unwittingly produced as a by-product, were discharged into bodies of water and accumulated into fish (Satoh, 2000). This situation prompted what was to become known as “Minamata Disease”, a condition caused by methylmercury poisoning and that results in several neurological disorders (Ekino *et al.*, 2007). One of the major uses of mercury has been in the manufacture of chlorine and caustic soda from brine, where metallic mercury is used as an electrode (Clarkson, 1998). This industry produced nearly 90% of European anthropogenic emissions to the atmosphere (Hylander *et al.*, 2003), and even though efforts were made to replace this process by cleaner technologies, widespread contamination of the ecosystems is still a significant problem, as many studies show (Biester *et al.*, 2002b; Reis *et al.*, 2009; Ullrich *et al.*, 2007).

Until the 1970s, mercury compounds were commonly used for agricultural purposes, mainly as fungicides on seed grain, resulting in poisoning by eating the dressed wheat grain. In Iraq, three epidemic poisonings have been famously reported: one in 1955–1956, another in 1959–1960, and the third and largest in 1971–1972 (Satoh, 2000).

Today mercury continues to be used in hundreds of different consumer products manufactured in all parts of the world. Recent applications include the production of batteries and fluorescent light bulbs, notebook computers, modern

telephones, new lighting technologies and anti-lock brakes in new cars. As programmes to recover mercury have started to work, much of the mercury used today comes from recycled sources, thus reducing demand for “virgin” mercury. The closures and conversions of mercury cell chlor-alkali plants have made large quantities of mercury available for resale and reuse (UNEP Chemicals, 2002).

The international effort to address the problems associated with environmental and health effects caused by mercury met a significant advance with governments agreeing to a global, legally-binding treaty to prevent mercury emissions and releases - the **Minamata Convention on Mercury**. The treaty has been four years in negotiation and was open for signature at a special meeting in Japan in October 2013. It includes a ban on new mercury mines, the phase-out of existing ones, the international regulation of the informal sector for artisanal and small-scale gold mining, and control measures on air emissions; the treaty also addresses the export, import and safe storage of waste mercury. Identifying populations at risk, boosting medical care and better training of health care professionals in identifying and treating mercury-related effects also forms part of the new agreement (UNEP, 2013).

1.2 Mercury in the environment: biogeochemical cycle and human toxicity

In general, mercury is present in water, soils and sediments at trace levels but several human activities and incautious handling have increased its natural concentrations and led to severely contaminated environments (Hylander *et al.*, 2003). Despite efforts to reduce mercury emissions, a recent study by Pirrone *et al.* (2010) estimates that the global mercury emission is still nearly 7527 tons per year and affects the atmospheric, terrestrial, aquatic, and biotic compartments. This situation is enhanced by the fact that some mercury species are particularly reactive in the environment, shifting rapidly between the four interconnected compartments (Pato, 2007), in the **mercury biogeochemical cycle**. The biogeochemical mercury pathways that occur in the environment are outlined in Figure 2 and, as can be seen from it, the cycle is very complex. This complexity is enhanced by the diversity of mercury species that can simultaneously exist in the environment. In sum, mercury can exist in the following main states, under natural conditions:

1. As metallic vapor and liquid/elemental mercury;

2. Bound in mercury containing minerals (solid);
3. As soluble ion complexes or bound in ionic compounds (inorganic and organic salts);
4. As gaseous or dissolved non-ionic organic compounds;
5. Bound to inorganic or organic particles/matter by ionic, electrophilic or lipophilic adsorption.

A description of the transformations and transportation of mercury forms within the four environmental compartments will be given next. For the purpose of this thesis mercury behaviour in soil and sediment will be explained in detail in section 1.3.

Mercury is emitted to the **atmosphere**, either from natural or anthropogenic sources. In the atmosphere, mercury consists almost entirely of elemental mercury (Hg^0), and small fractions of particulate mercury (Hg_p) and inorganic Hg^{2+}. The three species exhibit different transport characteristics. Hg^0 has a residence time of 6 months to 1 year and can be transported for very long distances (Selin, 2009). During this time, Hg^0 can be oxidized to inorganic Hg^{2+} compounds. In turn, these compounds can be similarly reduced back to elemental mercury (Chrystall *et al.*, 2009; Schroeder *et al.*, 1998). Particulate mercury species are likely to be deposited at intermediate distances, while Hg^{2+} species will be removed from atmosphere within a shorter distance from their source (Schroeder *et al.*, 1998), by wet or dry deposition. Therefore, the form mercury adopts in the atmosphere strongly influences its mobility and distribution potential, and this has consequences for the control of mercury emissions.

As shown in Figure 2, mercury enters the **aquatic compartment** through diverse ways, that include deposition of particles or ionic compounds from the atmosphere, runoff and erosion from the land surface, leaching from landfills, geothermal inputs, combustion and industrial discharges (Chrystall *et al.*, 2009).

Depending on the physicochemical conditions of the aquatic compartment (salinity, pH, redox potential, the presence of sulfate or sulfide, dissolved oxygen and organic matter content), mercury undergoes a series of chemical transformations - oxidation-reduction reactions, sorption-desorption processes on/from mineral surfaces and organic matter, and methylation-demethylation reactions (Beckvar *et al.*, 1996; Pereira, 1996).

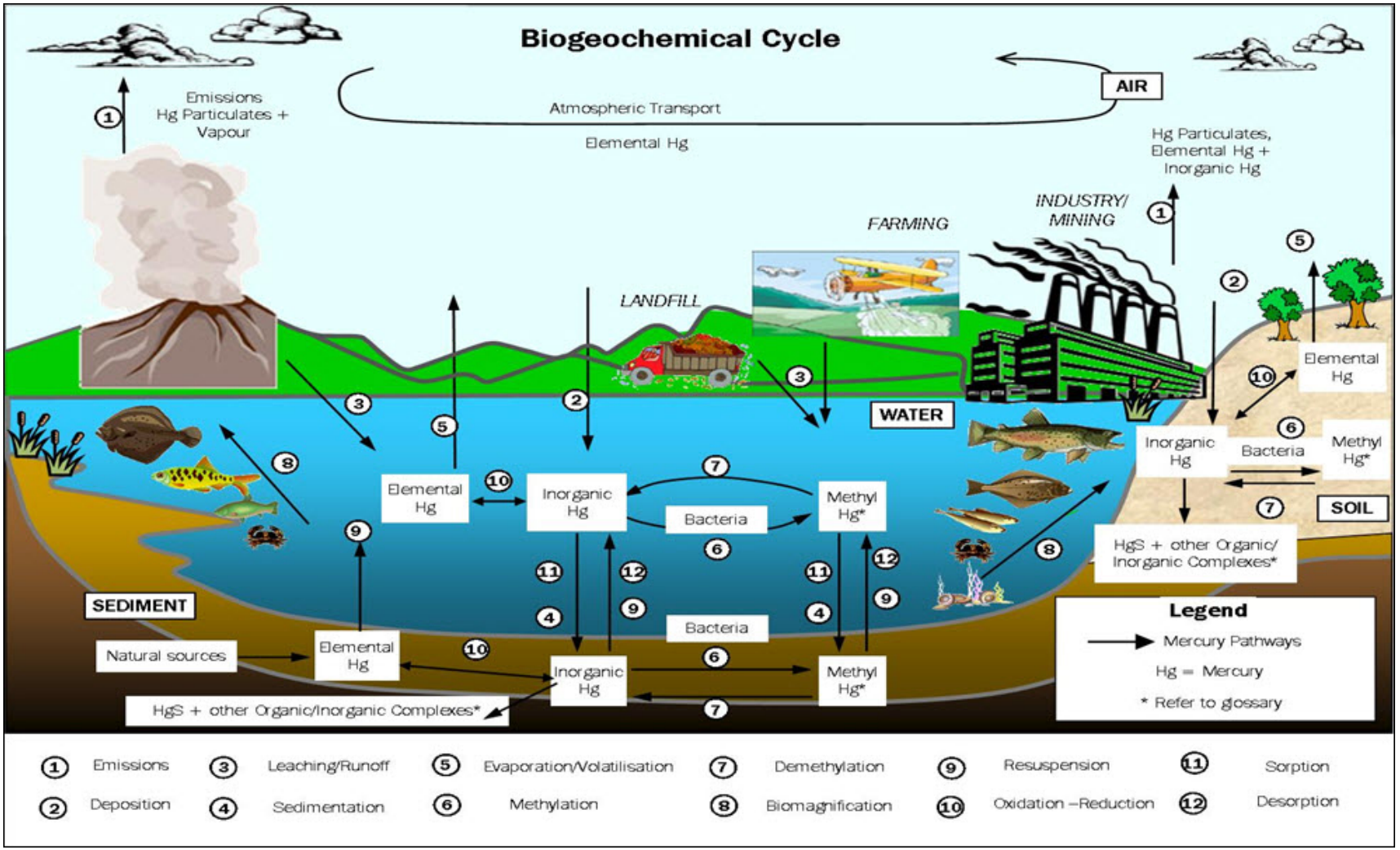

Figure 2. The mercury biogeochemical cycle (Chrystall *et al.*, 2009).

Methylation is one of the most biologically relevant processes in the mercury cycle and of particular concern, since organometallic mercury species are highly toxic and prone to bioaccumulate and biomagnify in organisms, and through the food chain (Coelho, 2009; Hissler *et al.*, 2006). Methylation is primarily assisted by bacteria (mainly sulfate-reducing and iron- reducing bacteria) under anaerobic conditions, but can also be mediated by chemical processes that do not involve living organisms (King *et al.*, 1999; Mason *et al.*, 2012). Mono and dimethylmercury are the main organometallic species formed in both sediment and water, but the high volatility of dimethylmercury, makes it unlikely to persist in aquatic environments (Beckvar *et al.*, 1996). In turn, dominant methylmercury forms will shift from hydroxides (CH_3HgOH) at low salinity, to chlorides (CH_3HgCl) at high salinity. Mercury losses from the aquatic compartment occur mainly through Hg^0 volatilization or sedimentation of mercury associated with suspended particles (Chrystall *et al.*, 2009). Mercury-particle association includes inorganic precipitates (HgS), associations with organic matter and mercury adsorbed to biological membranes or incorporated in organisms. This process of sedimentation results in higher mercury levels in sediments; therefore, these are considered a sink of mercury, at the same time that they become a potential source to interstitial waters and to biota, particularly to organisms that live in contact with the sediment (Coelho *et al.*, 2008a).

Mercury bioaccumulates in **biota** (aquatic plants, invertebrates, fish, and mammals), and can also be biomagnified along the trophic chain. These processes are affected by the mercury species present. Although inorganic mercury is the dominant form of mercury in the environment and is easily taken up, it is also depurated relatively quickly. On the contrary, methylmercury accumulates quickly and depurates very slowly, because it is readily transferred across biological membranes and tightly binds to sulfhydryl groups in the proteins of tissues. Therefore, it biomagnifies in higher trophic species (Mason *et al.*, 1995), and also increases with age in both fish and invertebrates (Beckvar *et al.*, 1996).

1.2.1 Mercury toxicity

Mercury, and especially methylmercury, is of major concern due to its adverse effects in living organisms. The already mentioned poisoning outbreak in Minamata

Bay (Japan) in the 1950's (Ekino *et al.*, 2007) is a good example, not only of the role of food webs on the bioaccumulation and biomagnification of mercury, but also of the neurological effects that mercury species have on biota and humans (Clarkson, 1998; Gochfeld, 2003). It should be mentioned that while mercury is highly toxic, the overall health consequences depend greatly on the species. Hg^0 is not well absorbed, but acute exposures may result in pulmonary and kidney problems, tremor, gingivitis and erythrism (Clarkson, 1998). Low levels of $HgCl_2$ and phenylmercuric acetate cause several teratogenic effects. Methylmercury can cross the blood-brain barrier and, at sufficient concentrations, may disrupt a range of neurological processes within the brain owing to its high affinity for proteins' thiol groups (Clarkson *et al.*, 2006). Characteristic symptoms of mercury poisoning include structural degeneration of the occipital cortex and the cerebellum, which leads to paraesthesia, ataxia, sensory impairment, memory loss, blurred vision, hearing impairment, olfactory and gustatory disturbances, clumsiness of the hands, and dysarthria (Clarkson *et al.*, 2006; Clarkson, 1998; Gochfeld, 2003). Mercury also has recognized mutagenic and teratogenic effects, and children born to mothers exposed to methylmercury showed extensive spongiosis of the cerebral cortex (Clarkson, 1998). Methylmercury is therefore the most toxic among mercury species.

1.3 Mercury in soil and sediment

Soil and sediment are naturally occurring materials that result from the weathering and erosion of rocks and are carried and deposited by wind, water, or ice. The difference between soils and sediments resides in the fact that the first are vertically weathering profiles that develop in place. Soils require time and a stable ground surface to develop. Sediments, on the other hand, form when particles transported by water or wind deposit at the bottom of a water body. It could be said that sediments are the result of movement, while soil profiles develop in the absence of movement.

The result is a very complex heterogeneous medium, which consists of the solid fraction (the soil/sediment matrix) containing minerals and organic matter, the fluid fraction (the soil/sediment solution and the soil/sediment air), and living material, which interact with each other and ions entering the system (Alloway, 1995). Soils and sediments play an important role in the biological cycle of mercury acting both

as a sink and source of this metal to biota, atmosphere and hydrological compartments (Oliveira *et al.*, 2007), from which mercury can be distributed back into circulation for many years after the initial deposition (UNEP Chemicals, 2002). In order to assess the dynamics of mercury within the soil and sediment system it is of paramount importance better to understand the relationships between mercury species and the matrix and how some soil and sediment characteristics can affect these processes.

In terrestrial ecosystems, mercury deposited into **soils** through wet deposition of Hg^{2+}, dry deposition of Hg^0 and deposition of particulate Hg, is subjected to a wide array of processes, including volatilization, dissolution and contamination of groundwater as well as chemical, physical and biological processes such as Hg^0 oxidation and Hg^{2+} reduction or methylation (Figure 2). Hg^0 is relatively non-reactive and, due to its volatile character, is easily liberated to atmosphere before oxidation. Therefore, Hg^{2+} is the main form present in soil. In general, Hg^{2+} in soil can occur in the following forms (Schuster, 1991):

1. In **dissolved form,** as free ion or soluble complexes;
2. **Non-specifically adsorbed** (weak electrostatic bond);
3. **Specifically adsorbed** (covalent bond);
4. **Chelated** (bound to organic substances);
5. **Precipitated** in mineral form (i.e. sulfide, carbonate, hydroxide, phosphate, etc.).

In soil and sediment, mercury is present in both the solution and solid fraction, and chemical, physical and biological processes at the solid-solution interface essentially control its speciation, behaviour and fate. In natural occurring conditions, mercury has tendency to associate to the matrix, therefore only trace amounts of soluble mercury species are found in soil and sediment solution. Yet, it is the activity of Hg^{2+} and Hg^{2+}-complexes in the solution that determines its availability to plants and organisms (Jing *et al.*, 2007). In turn, soil and sediment solution chemistry is controlled by the properties of the solid fraction and the kinetics of the reactions at the solid-solution interface, which include adsorption-desorption, precipitation-dissolution, and uptake-release (by plants or organisms) (Sauvé, 2002).

Mercury can be removed from solution by partitioning to inorganic and organic phases of soil or sediment. In the matrix, Hg^{2+} can be bound directly to the mineral

surface or to the organic matter present; the latter can, in turn, be associated to the mineral surface, resulting in an organomineral complex (Figure 3). Reactive sites for the sequestration of the metal occur on adsorption sites of organic matter (S-containing functional groups), and mineral surfaces (e.g. clays, oxides and hydroxides of aluminium, iron and manganese, and silicate minerals) (Sauvé, 2002). As previously said, mercury adsorption onto soil or sediment can occur as non-specific or specific adsorption. In the first case, cation exchange is involved, resulting in outer-sphere complexes. This process is reversible in nature, occurs rather quickly, and both organic and inorganic ligands are involved. In specific adsorption, stable complexes are formed. After some time, the tendency is that metals specifically adsorbed by the surface of colloids diffuse to the interior of particles, forming inner-sphere complexes and hindering subsequent desorption (Bradl, 2004).

1.3.1 Factors affecting adsorption of mercury to the soil and sediment

Many environmental factors can affect the adsorption-desorption processes. An understanding of mercury speciation and the related complex interactions is important to predict the fate and transport of the metal in soil and sediment systems. The availability of Hg^{2+} may vary considerably depending on the nature of the adsorption-desorption processes. In the particular case of mercury, adsorption on soil is influenced by soil pH, mercury speciation, presence of chloride ions, organic matter, soil composition and aging, and competitive inorganic ions (Bradl, 2004; Jing *et al.*, 2007; Yin *et al.*, 1997). Among these factors, soil pH and chloride concentration are the key parameters in determining the mercury speciation in soil solution (Biester *et al.*, 2002b; Miretzky *et al.*, 2005; Schuster, 1991; Stein *et al.*, 1996; Yin *et al.*, 1996).

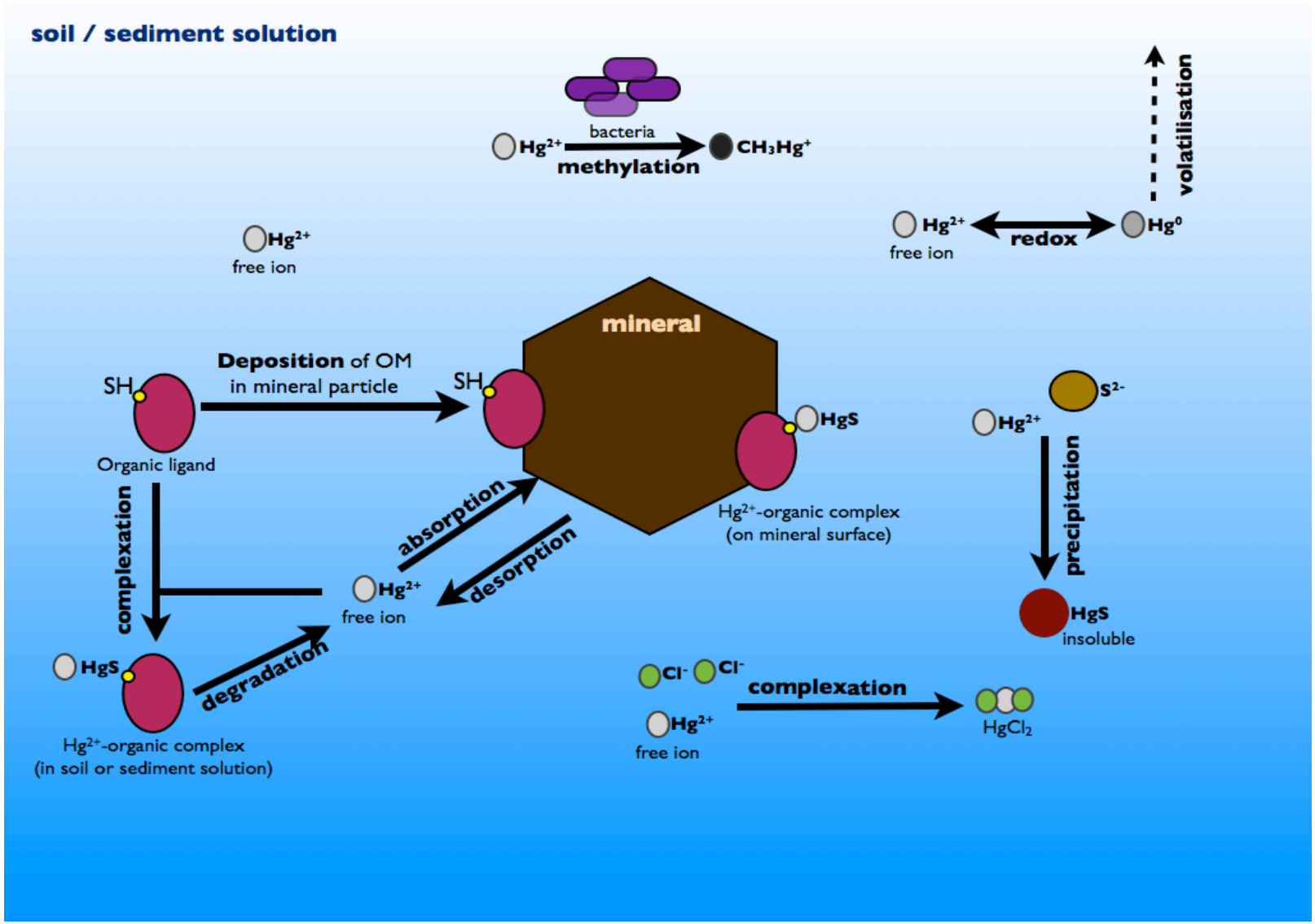

Figure 3. Mercury pathways in the soil/sediment matrix and solution (OM: organic matter; SH: thiol groups).

pH effect: According to Miretzky *et al.* (2005) and corroborated by Jing *et al.* (2007), adsorption of inorganic divalent mercury species to the solid matrix of (tropical) soil decreases with increasing pH, or at extremely low pH, with maximum adsorption occurring between pH 3.0 and 5.0, due to the strong Lewis acid nature of Hg^{2+}, and at pH 5.0 – 7.0 adsorption-desorption processes reach an equilibrium. Acidification to pH values below 3.0 can decrease mercury adsorption to the matrix, since the solid surface has a positive net charge that impedes adsorption of cationic species; adsorption decreases at pH > 7.0 mainly due to leaching of organic matter from the matrix, resulting in a decrease of adsorption sites in the solid fraction. This phenomenon can have a serious impact on the environment, since organic matter leached to the soil or sediment solution can enhance the concentration of dissolved Hg^{2+} complexes, increasing the mobility of mercury.

Chloride effect: Chloride reduces Hg^{2+} retention through the formation of soluble $HgCl_2$; this species is found in solution and has minimal affinity to the solid surface (Jing *et al.*, 2007). $HgCl_2$ and $Hg(OH)_2$ are the most abundant Hg^{2+}-complexes in soil and sediment solution, due to their high stability constants and the high concentrations of chloride and hydroxide ligands present in most natural systems. Chlorides occur in all natural soil and water systems and may be regarded as one of the most mobile and persistent complexing agents for mercury (Schuster, 1991). Thus, increasing chloride concentration increases mobility of mercury. The high mobility and solubility (74 g L^{-1}) of this mercury species represents a potential environmental risk, as $HgCl_2$ can easily be transported from the soil to groundwater, as observed by Bollen *et al.* (2008), or from sediment to the water column.

Organic matter: Mercury tends to bond preferentially to organic matter, both is soils and sediments, mainly due to its affinity to S-containing functional groups frequently found in organic molecules (Schuster, 1991; Yin *et al.*, 1997). Organic matter presence leads to the formation of organic mercury complexes and inhibits mercury biomethylation processes (Bloom *et al.*, 2003b). It must be mentioned that besides adsorption, other mechanisms can be involved in mercury association to organic matter and these are chelation and coprecipitation (Schuster, 1991).

The presence of **sulfur** is very important in the chemistry of mercury, as in the presence of sulfides mercury becomes tightly bound to them, forming the insoluble HgS (Boszke *et al.*, 2003). Because HgS is not reactive or mobile, the formation of

this compound allows mercury to be retained, becoming less available for methylation and less harmful to the environment.

The **soil texture and aging** plays an important role on metal retention. Generally, fine-grained soils show higher tendency to adsorb metals than coarse-grained soils, due to the larger surface area of the smaller particles like clays, iron and manganese oxyhydroxides, or humic acids, among other examples. Aging also has repercussions on metal retention, as inner-sphere complexes are formed as a function of time, acquiring a more stable and irreversible character.

Additionally, the presence of inorganic and organic ligands in the soil solution can affect the adsorption of metals to the solid phase (Sposito, 1983). The metal can be retained in the matrix if one the following two processes occur: **(i)** the metal has high affinity for the ligand and they form a soluble complex with high affinity for the adsorbent; **(ii)** the ligand has high affinity for the adsorbent, is adsorbed, and then the metal has affinity for the adsorbed-ligand "complex". On the other hand, if the metal has high affinity for the ligand and they form a soluble complex with low affinity for the adsorbent, metal mobility and possible availability will be promoted.

Sediment contamination is an important environmental concern because, like soils, they are both receptacle and source of contaminants, having the potential for spreading contamination to aquatic organisms and to the water system. Mercury in sediments derives essentially from the settlement of mercury rich suspended particulate matter from the atmosphere and water column. The importance of sediments as a source of mercury is exacerbated because sediments are a site for methylation and thus are perceived to be a source of methylmercury (MeHg) to the water column and to the aquatic food chain (Mason *et al.*, 2006). According to its biogeochemical cycle (Figure 2), mercury in the sediment may undergo several pathways and its behaviour is controlled by the presence of iron and manganese oxides and oxyhydroxides, sulfides, organic matter, pH, ionic strength and redox potential (Boszke *et al.*, 2003; Zhang *et al.*, 2013).

In the sediment, inorganic mercury (Hg^{2+}) can be reduced to Hg^0, which can be transferred to the water column. In oxic sediment layers, Hg^{2+} is mainly associated with iron and manganese oxides and oxyhydroxides; therefore mercury efflux from anoxic layers to the water column is blocked, as mercury is retained in the oxic layer by complexation (oxic sediment layer acts as a "barrier") (Coquery *et al.*, 1997). In

reducing sediment layers, Hg^{2+} may associate with sulfides and precipitate as HgS (Pato, 2007). Methylation can occur in anoxic sediment layers, where substantial amounts of methylmercury can be found in the pore water, but cannot freely diffuse to the water column, because of the oxic layer. In the oxic layer, methylmercury can be demethylated either by bacterial or catalytic action. Still it is known that mercury species formed in the anoxic layer can reach the water column and aquatic organisms. Potential pathways for the translocation of mercury from anoxic layers to overlying water and biota include resuspension of sediment, diffusion from interstitial water, and "transport" via the dietary intake of contaminated benthic organisms (Gagnon *et al.*, 1996; Mason *et al.*, 2006). Sediment resuspension can be caused by natural events (e.g., tidal currents, wind waves, storm events, and wave-current interaction) (Ogston *et al.*, 2004) and anthropogenic activities (e.g., dredging) (Schoellhamer, 1996).

1.4 Speciation and fractionation

It was previously mentioned that the implications of a metal in the environment are not merely dependent on its total concentration but mostly on the chemical species present that ultimately affect the metal's mobility, bioavailability, and transport through the environmental compartments. As a result, there is considerable interest in improving the understanding of speciation in natural and polluted systems (Bacon *et al.*, 2008).

1.4.1 Definition

The term "speciation" is defined in many different ways depending on the background of the scientist defining it. Ure (1991) defined chemical speciation as either "*the active process of identification and quantification of the different defined species forms or phases in which an element occurs in a material*" or "*the description of the amounts and kinds of species, forms or phases present in the material*". However, the International Union of Pure and Applied Chemistry (IUPAC) have later clarified the scope of speciation, which covers the following concepts (Quevauviller, 2000):

1. **chemical species**: specific form of an element defined according to its molecular, complex, electronic or nuclear structure;

2. **speciation analysis**: measurement of the amount of one or more individual chemical species in a sample;
3. **speciation of an element**: distribution of defined chemical species of an element in a system.

Ure also propose to subdivide speciation into three classes (Bacon *et al.*, 2008; Ure, 1991):

1. **Classical speciation** refers to specific chemical compounds or oxidation states of elements, e.g. cerussite ($PbCO_3$) vs. pyromorphite [$Pb_5(PO_4)_3Cl$]; Cr_{III} vs. Cr_{VI}.
2. **Functional speciation** refers to the observed role or behaviour of the element, and is characterized by terms such as 'plant available' or 'mobile' species.
3. **Operational speciation** refers to the situation where the reagent used to extract the sample defines the species, e.g. 'acetic acid soluble' or 'moderately reducible' species. Sequential chemical extraction is an example of operational speciation.

Therefore, speciation of an element is the distribution of that element amongst defined chemical species in a system. Nevertheless, in most cases it is not possible to determine the concentration of the different individual chemical species; in such cases, it is useful practice to do fractionation instead. The term "**fractionation**" was defined by IUPAC, and should be understood as the process of classification of an analyte or a group of analytes from a certain sample according to physical (e.g. size, solubility) or chemical (e.g. bonding, reactivity) properties.

1.4.2 Extractants and laboratory procedures

Interest in metal speciation in soils and sediments has been increasing in the last years because of recognition that toxicity, bioavailability, health hazard, risk assessment, and remediation of contaminated sites must be based on levels of specific chemical forms, rather than on total element levels (Pickering, 1995). This creates an analytical challenge due to 1) difficulties in isolating the compounds of interest from complex matrices such as soil and sediment; 2) changes caused in the

distribution of the various chemical species during extraction; 3) lack of appropriate certified reference materials and quality control procedures (Pickering, 1995). Quantification of the low concentration of analytes in extracts can sometimes represent another drawback, although the development of more sensitive analytical techniques has overcome this problem. Currently, various methods are available for assessing metal forms in a sample (e.g. X-ray absorption spectroscopy, X-ray diffraction, solid nuclear magnetic resonance, etc.), but the most commonly used are selective (single) or sequential chemical extraction procedures (Bloom *et al.*, 2003b; Fernández-Martínez *et al.*, 2003; Han *et al.*, 2003; Revis *et al.*, 1989; Sakamoto *et al.*, 1992). These extractions are used to subdivide the total metal content of the samples into operational defined groups according to the relative solubility of the species in, for example, salt or acid solutions (Rubio *et al.*, 1996).

Selective or single extractions are used to target only one fraction of interest and are frequently used for estimating the most potentially mobile fraction; for example, in the case of soils, the proportion available for plant uptake. A single extracting reagent is used to treat the sample and measurement is made on the amount of elements released from the matrix by that extractant (Abollino *et al.*, 2011). The choice of extractant depends on the aim of the study, as extractants can be divided into various groups, according to their extraction efficiency. According to Peijnenburg *et al.* (2007), extractants can be classified as:

Weak, soft or mild extractants: water or unbuffered salt solutions (e.g. $CaCl_2$, NH_4Ac, $Ca(NO_3)_2$, $BaCl_2$), which are frequently used to predict the plant-available fraction (Han *et al.*, 2006; Jing *et al.*, 2008; Renneberg *et al.*, 2001; Wang *et al.*, 2003);

Reductive extractants: sodium ascorbate, hydroxylamine-HCl (Han *et al.*, 2006);

Weak acids: dilute solutions of acetic, malic and citric acid; are secreted as metabolic products through plant roots, hence they are believed to simulate natural conditions (Rubio *et al.*, 1996; Sakamoto *et al.*, 1992; Ure *et al.*, 2002).

Chelating agents, like DPTA or EDTA. Despite concerns of being over-aggressive for this purpose, they are sometimes employed for estimating plant-available fraction of elements (Jing *et al.*, 2008). The Standards, Measurements and Testing (SMT) Program (formerly BCR) developed and validated a single extraction

protocol (0.05 mol L^{-1} ammonium EDTA, 1 hour, room temperature) (Beckvar *et al.*, 1996);

Combined salt-acid extractants: ammonium oxalate-oxalic acid, sodium acetate-acetic acid, among others (Neculita *et al.*, 2005);

Diluted strong acids, as for example, 0.01 mol L^{-1} HNO_3 (Wallschlaeger *et al.*, 1998) or 0.5 mol L^{-1} HCl (Sutherland *et al.*, 2008);

Concentrated strong acids: Acids at high concentrations (e.g. concentrated HNO_3 or *aqua regia*), mostly used for extraction of the least labile and residual fractions (Sahuquillo *et al.*, 2003; Wallschlaeger *et al.*, 1998).

In sequential extraction procedures, a sequence of reagents is applied to the same sample to sub-divide the total metal content. The procedure typically contains 3-8 treatments of the solid phase, with the "vigour" of the treatment generally increasing through the steps, from initial mild conditions (e.g. shaking with water, a salt solution or dilute acetic acid) to the use of much harsher reagents (e.g. hot mineral acid) (Bacon *et al.*, 2008). The fractions extracted early in the process are more labile due to be being weakly bound to the solid fraction and have greater potential mobility and toxicity.

One of the first sequential extraction procedures developed was the **Tessier scheme**, designed by Tessier *et al.* in 1979 (Tessier *et al.*, 1979) for the partitioning of elements into five operationally defined fractions. Most of the other procedures derive from it and several adaptions consisting of schemes with more steps, different extractants, time of agitation, pH, among other operational conditions can now be found in literature.

The Tessier scheme was extensively applied during many years, but in order to harmonize fractionation procedures and ensure comparability, The Measurement and Testing Program of the European Union later developed the **BCR protocol** (BCR EUR 14763 EN), a harmonised three-step sequential extraction procedure; the BCR protocol was revised in the late 1990s: step 1. water-soluble, exchangeable, and acid-soluble; step 2. reducible; step 3. oxidisable. An additional step consisting of the analysis of the residual fraction is also advisable (Rauret *et al.*, 1999; Sahuquillo *et al.*, 1999). The element fractions defined by this method were operationally defined rather than target mineral phases, such as soluble and exchangeable cations; iron and manganese oxyhydroxides; organic matter and

sulfides (Bacon *et al.*, 2008). Although a large number of different protocols have been reported, the Tessier and BCR schemes and their adaptations remain amongst the most widely used. A comprehensive review of sequential extraction schemes for metal partitioning in environmental solid samples was provided by Filgueiras *et al.* (2002). A summary of the most common target phases in sequential extraction procedures and respective mobility in the environment is given in Figure 4. In this figure, there are also given examples of the most applied extractants for each fraction.

1.4.3 Limitations and uncertainties of sequential extraction procedures

There are several recognized limitations in sequential extraction procedures (Bacon *et al.*, 2008; Filgueiras *et al.*, 2002; Peijnenburg *et al.*, 2007). Lack of extractant selectivity, re-adsorption of previously extracted species, effects of sample pretreatment, incomplete extraction, heterogeneity of natural matrices, and presentation, interpretation, and comparison of data, are regarded as the most limiting factors, which will be addressed concisely in the next paragraphs. The use of the remaining solid matrix for the next step may have influence on further steps of chemical extraction, since substrate composition has been altered.

In sequential extraction, extractants are chosen to divide the potentially toxic elements content into fractions, corresponding to well defined mineral phases; however, several examples can be found in literature where it is proven that the selectivity and leaching capacity of the most widely used extractants constitutes a major problem. For example, Ahnstrom and Parker (Ahnstrom *et al.*, 1999) reported substantial amounts of trace elements bound to organic matter when hydroxylamine-hydrochloride in nitric acid medium was used to extract the reducible fraction; as a consequence, this fraction may be overestimated at the expense of the oxidisable fraction. Ammonium salts of strong acids, such as NH_4Cl or NH_4Ac, can lower the pH and encourage the hydrolysis of clays through their complexing action, overestimating the exchangeable fraction (Filgueiras *et al.*, 2002). Premature extraction of organically bound metals has been noted in both the Tessier and the BCR procedures, and presumably occurs because analytes can be liberated by exchange processes as well as following destruction of the organic matter.

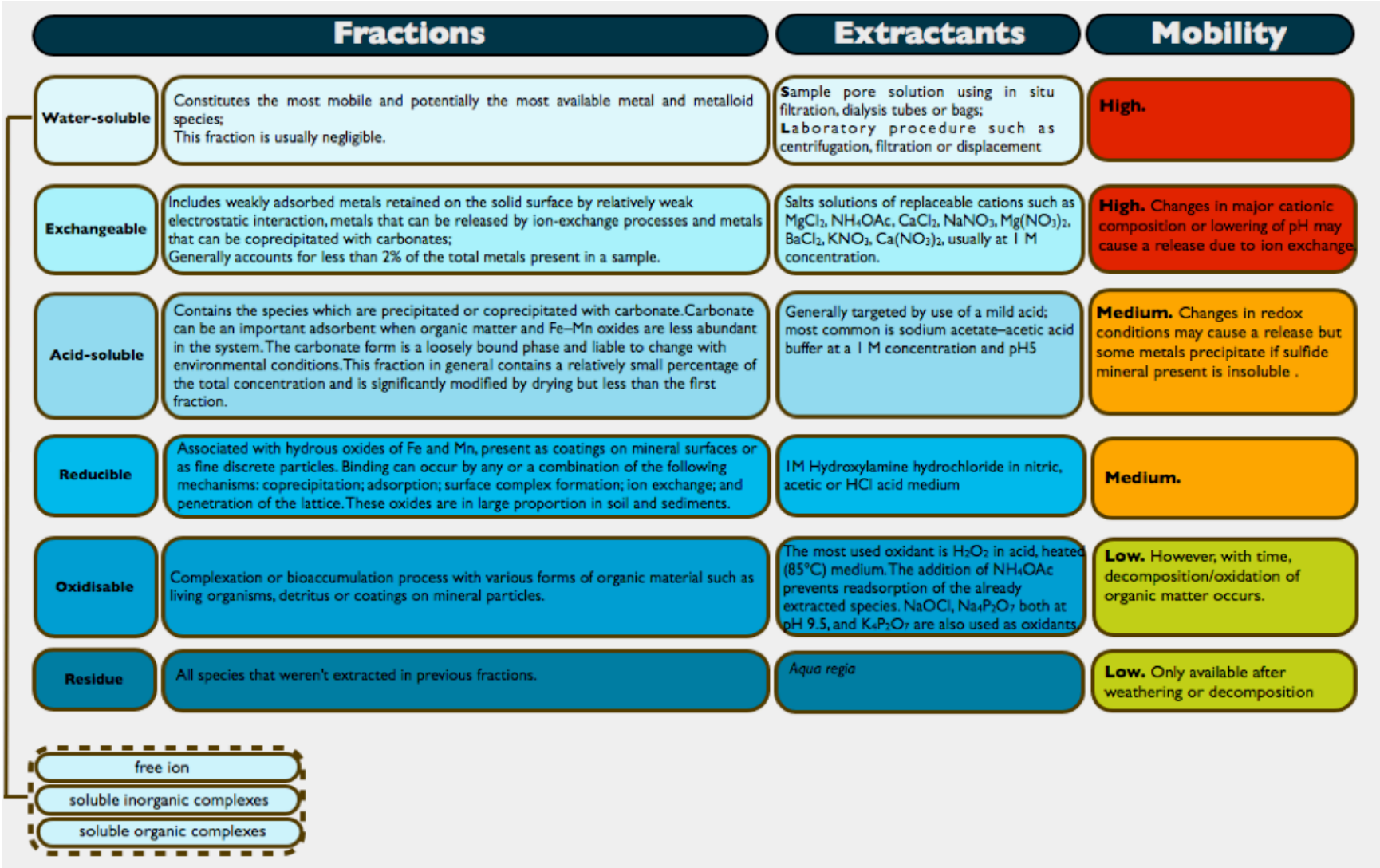

Figure 4. Operationally-defined phases targeted in most SEP, common extractants and respective mobility (Filgueiras *et al.*, 2002; Issaro *et al.*, 2009; Rao *et al.*, 2008; Rauret, 1998).

Re-adsorption and subsequent metal redistribution among the remaining solid fractions is also regarded as other problem to be considered when performing sequential extractions. Re-adsorption can lead to significant underestimation of the metal present in one fraction. This tends to be a consequence of the inability of the extractant to maintain the dissolved species in the soluble phase, a fact that was highlighted in the work of Gomez-Ariza *et al.* (1999) when using 1.0 mol L^{-1} $MgCl_2$. The same authors also noted that the degree of re-adsorption was dependent on the geochemical characteristics of the sample.

Sample pretreatment is usually required in soil and sediment analysis. Ideally, one should not disturb the original metal distribution. Drying, for example, has been linked to acceleration of the crystallization of solids such as iron and manganese oxides and, at the same time, promotes iron, manganese and sulfur oxidation, causing an increase in metals bound to them, to the detriment of more labile phases (exchangeable and carbonatic). Still, preservation of soil and sediment samples is necessary and recommended, keeping in mind that this may affect speciation. Often samples are air-dryed, in order to facilitate their handling, homogenization and representative sub-sampling. This process also reduces the heterogeneity inherent to natural soil and sediment samples.

If data from one study is to be compared with those from another study then consistency of methodologies and extraction conditions becomes important. Difficulties in the comparison of sequential extraction results for speciation or fractionation relate particularly to inconsistencies between different extraction protocols (Bacon *et al.*, 2008). Different experiments have elucidated the effects of extraction time, solid-to-liquid ratio, alternate solvents, matrix, particle size and crystallinity on results obtained and have shown that sequential and selective extractions for identification of specific metal fractions should be used with caution (Bloom *et al.*, 2003c; Kim *et al.*, 2003; Sladek *et al.*, 2003).

An on-going limitation of the use of sequential extraction procedures is the quality control and quality assurance of the experiments when the methods are applied by different laboratories and to different solid matrices (Bacon *et al.*, 2008). In addition, only a few reference materials for checking the performance of methods and laboratories in the case of extractable trace metal contents were produced so far (Quevauviller, 1998; Quevauviller *et al.*, 1997). A third constraint is that the range of

elements investigated in sequential extraction studies is usually limited and “less common” elements such as mercury are often not included.

Nevertheless, and despite the drawbacks aforementioned, information provided by sequential extractions is important. If applied correctly, sequential extraction procedures provide valuable information about current and potential metal mobility and bioavailability. Moreover, sequential extraction has proven to be useful to distinguish between anthropogenic and geogenic sources of metal species in soil and sediment (Filgueiras *et al.*, 2002; Gleyzes *et al.*, 2002).

1.4.4 Mercury speciation and fractionation methods: review

Common selective extraction procedures are applied to cadmium, copper, chromium, iron, lead, manganese, nickel, and zinc but are not appropriate for mercury. The particular chemistry of mercury requires the development of specific extraction schemes dedicated to this element (Bacon *et al.*, 2008). Due to the numerous and diverse species of each element, with unique physical and chemical properties, the fractionation of this element is very difficult and complex. Consequently, research dedicated to mercury speciation/fractionation has gained attention in recent years (Bloom *et al.*, 2000; Bloom *et al.*, 2003c; Fernández-Martínez *et al.*, 2005b; Gray *et al.*, 2006; Kim *et al.*, 2003; Millán *et al.*, 2006; Sánchez *et al.*, 2005; Sladek *et al.*, 2003). As an example, Figure 5 presents the number of publications per year concerning mercury speciation in soils and sediments, from 1994 to 2013. The increased interest in this theme in last 20 years is visible.

Several protocols can be found in the literature regarding mercury speciation and fractionation, as reviewed by Issaro *et al*. (2009). At present there is not a consensual protocol regarding mercury sequential extraction (Issaro *et al.*, 2009), but three main lines can be identified in mercury speciation/fractionation methodologies: 1) chemical extraction; 2) X-ray absorption techniques; and 3) thermo-desorption.

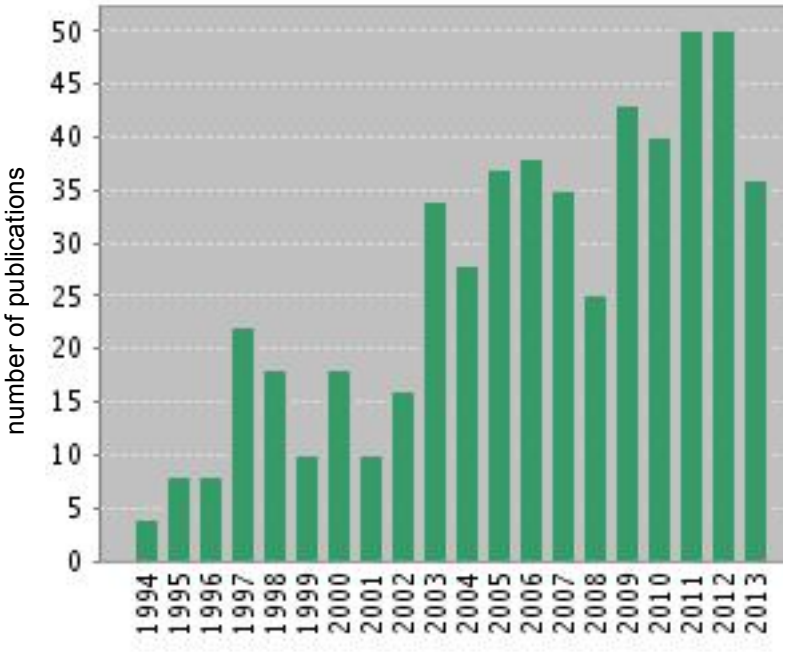

Figure 5. Number of publications relating to mercury speciation in soils and sediments, from 1994-2013. (Source: Web of Knowledge, retrieved on 20th October 2013)

Single or sequential chemical extraction procedures are the most used. Single extractions mainly aim at determination of the organometallic fraction (Canário *et al.*, 2005; Domagalski, 2001; Leermakers *et al.*, 2003; Nevado *et al.*, 2008), by acid or alkaline extraction combined with solvent extraction, distillation, or solid-phase microextraction. While organometallic fraction has been the main focus of interest in mercury speciation, due to its extremely toxicity, in fact it usually represents less than 3% of total mercury in soils and sediments (Canário *et al.*, 2007; Chen *et al.*, 2012; Kongchum *et al.*, 2006; Rimondi *et al.*, 2012). Elemental Hg^0 has too been determined by single extraction, using a combination of strong acids such as H_2SO_4 and HNO_3 and heat (Bargagli *et al.*, 2007). Procedures vary in temperature and time of heating, therefore data interpretation and comparison is equivocal. At the same time, the treatment may also remove other volatile species, such as $HgCl_2$, overestimating Hg^0.

Sequential extractions have been extensively applied in mercury fractionation. The lack of standardised procedures though, has resulted in a diversity of extractants and protocols for the determination of each fraction. Table 1 summarises some of those procedures, extractants used and respective target fractions. In general, all procedures begin with extraction of the more labile fractions: water-soluble and/or exchangeable fractions using, respectively, distilled water and salt

solutions that remove mercury by ion-exchange (e.g. NH_4Ac, $MgCl_2$, $CaCl_2$). In the next fraction, oxidisable reagents, such as NaOH, KOH, HNO_3 or H_2O_2, are applied to extract mercury bound to organic matter. In the last steps, the unreactive species that are strongly bound to the matrix are extracted with strong acids, including HNO_3, HF and *aqua regia*. All mentioned procedures are operationally defined, as no extractant is species specific; some Hg species are extracted over multiple steps and a substantial amount can still be found in the residual fraction (Biester *et al.*, 1997a; Gómez Ariza *et al.*, 2000; Kim *et al.*, 2003; Reis *et al.*, 2010).

Difficulties in the comparison of sequential extraction results for mercury fractionation relate particularly to inconsistencies between different extraction protocols (Bacon *et al.*, 2008), as is well demonstrated in Table 1. It must be underlined that a limitation to the use of sequential extraction procedures in general is the lack of certified reference materials in mercury speciation/fractionation for checking the performance both of method and the laboratory; these procedures are also time-consuming, and involve many steps, that altogether limit the procedure robustness (Gómez-Ariza *et al.*, 2005).

Therefore, establishing easy-to-use protocols is key to successful assessment of risk and contaminant-soil/sediment interaction in contaminated areas.

As an alternative, X-ray absorption fine structure spectroscopy (EXAFS) (Kim *et al.*, 2000; Kim *et al.*, 2004) and X-ray absorption near edge structure (XANES) (Kim *et al.*, 2003) can be applied to identify Hg species in soils and sediments. These techniques are however expensive and require samples with high mercury concentration (> 100 mg kg^{-1}) (Kim *et al.*, 2000), which strongly limits their applicability.

A third approach consists of the thermo-desorption speciation for identification and quantification. Developed by Biester *et al*. (1997b), it consists of the thermal release of mercury species at different temperatures. The main advantage is that this technique is species-specific. Thermo-desorption procedures will be further explained in Chapter 7.

Table 1. Sequential extraction methods for mercury fractionation found in the literature (adapted from Issaro *et al*. (2009)).

Authors	Reagents	Compounds extracted
Renneberg and Dudas (2001)	1. Deionized water	1. Water-soluble
	2. 1 mol L^{-1} $MgCl_2$	2. Exchangeable compounds
	3. 0.2 mol L^{-1} de NaOH	3. Organic acids I bound mercury
	4. 0.005 mol L^{-1} de NaOH	4. Organiques acids II bound mercury
	5. 0.005 mol L^{-1} de CH_3COOH	5. Organic basic
	6. 3% H_2O_2 (pH 2)	6. Residual organic matter I
	7. 30% H_2O_2 (pH 2)	7. Residual organic matter II
	8. $HNO_3/K_2S_2O_8$	8. Residual
Biester and Scholz (1997)	1. Deionized water	1. Water-soluble
	2. 1 mol L^{-1} NH_4Ac	2. Exchangeable compounds
	3. 1 mol L^{-1} NH_4OH	3. Fulvic and humic
	4. 0.02 mol L^{-1} HNO_3/ 30% H_2O_2/1 mol L^{-1} NH_4Ac	4. Organic sulfur
	5. Aqua regia	5. Residual
Bloom and Katon (2000)	1. Deionized water	1. Water-soluble
	2. HCl/CH_3COOH (pH 2)	2. Human stomach acid soluble
	3. 1 mol L^{-1} KOH	3. Humic
	4.12 mol L^{-1} HNO_3	4. Complex-compounds
	5. Aqua regia	5. Residual and HgS
Neculita *et al.* (2005)	1. Deionized water	1. Water-soluble
	2. 0.5 mol L^{-1} NH_4Ac-EDTA+$CaCl_2$	2. Exchangeable compounds
	3. 0.2 mol L^{-1} NaOH+CH_3COOH (4% v/v)	3. Organic compounds
	4. HNO3+H2SO4+HClO4	4. Residual compounds
Wang *et al.* (2003)	1. 0.1mol L^{-1} $CaCl_2$ (pH 7)	1. Active Hg (include soluble Hg and exchangeable Hg)
	2. 1 mol L^{-1} HCl + 1% $CuSO_4$	2. HCl-dissoluble Hg
	3. 1% KOH	3. Organic bound Hg
	4. 2 mol L^{-1} HNO_3	4. Hg^0 form
	5. Aqua regia	5. Residual Hg

Table 1. Continuation.

Authors	Reagents	Compounds extracted
Wallschlaeger *et al*. (1998)	1. Deionized water	1. Water soluble
	2. 0.01 mol L^{-1} HNO_3 (pH 2)	2. Organic extracted/acid
	3. 1 mol L^{-1} KOH	3. Organic extracted/base
	4. Na_2S	4. HgS
	5. Concentrated HNO_3	5. Residual
Wang *et al*. (1997)	1. 1 mol L^{-1} $CaCl_2$	1. Available Hg
	2. HCl/0.1 mol L^{-1} $KBrO_3$-KBr	2. Hg bound to organic matter
	3. $H_2SO_4/HNO_3/KMnO_4$	3. Residual Hg
Miller *et al*. (1995)	1. 0.01 mol L^{-1} K_2SO_4+0.01 mol L^{-1} KCl, Toluene	1. Organic and soluble compounds
	2. 0.2 mol L^{-1} HNO_3	2. Acid soluble
	3. 1:3 HNO_3+H_2O	3. HNO3 soluble
	4. 1:6:17 $HCl+HNO_3+H_2O$	4. Residual
Sahuquillo *et al*. (2003) (modified BCR-SEP)	1. 0.11 mol L^{-1} CH_3COOH	1. Exchangeable, Water soluble, and carbonates
	2. 0.5 mol L^{-1} NH_2OH/HCl (pH 1.5)	2. Fractions bound Hg
	3. 8.8 mol L^{-1} H_2O_2 (pH 1.5)	3. Reducible Hg
	4. 1 mol L^{-1} CH_3COONH_4 (pH 2)	4. Oxidizable Hg
	5. Aqua regia/HF	5. Residual
Panyametheekul (2004)	1. Heated at 180°C	1. Hg^0
	2. Deionized water	2. Water soluble Hg
	3. 0.5 mol L^{-1} $MgCl_2$	3. Exchangeable Hg
	4. 0.5 mol L^{-1} HCl	4. Strongly bound Hg
	5. 0.02 mol L^{-1} $HNO_3/30\%H_2O_2/Al(CH_3COO)_3$	5. Organic Hg
	6. Na_2S	6. HgS
	7. HgT – extracted Hg in all fractions above	7. Residual

Table 1. Continuation.

Authors	Reagents	Compounds extracted
Han *et al* (2006)	1. 1 mol L^{-1} NH_4Ac	1. Soluble and exchangeable Hg
	2. 1 mol L^{-1} $NH_2OH{\cdot}HCl$	2. Easily reducible oxides bound Hg
	3. 0.01 mol L^{-1} HNO_3/H_2O_2 30%	3. Hg bound to organic matter
	4. 0.2 mol L^{-1} $(NH_4)_2C_2O_4$/0.2 mol L^{-1} $H_2C_2O_4$	4. Hg bound to amorphous iron oxides
	5. 0.04 mol L^{-1} $NH_2OH{\cdot}HCl$ in 25% HNO_3	5. Hg bound to crystalline iron oxides
	6. 4 mol L^{-1} HNO_3	6. Residual non-HgS
	7. Na_2S (saturated)	7. HgS
Lechler *et al.* (1997)	1. 0.5 mol L^{-1} $MgCl_2$	1. Exchangeable compounds
	2. 0.5 mol L^{-1} HCl	2. Strongly bound-Hg
	3. 0.2 mol L^{-1} NaOH/ 4% CH_3COOH	3. Organic
	4. Aqua regia	4. Residual
Barrocas *et al.* (1998)	1. 1 mol L^{-1} NH_4Ac	1. Exchangeable compounds
	2. 1 mol L^{-1} Ammonium hydroxide	2. Hg bound to Humic substances
	3. 12 mol L^{-1} HNO_3	3. Organic matter
	4. Saturated Na_2S	4. Hg bound to sulfide
	5. Aqua regia	5. Residual
Burt *et al.* (2003)	1. Deionized water	1. Water-soluble
	2. 0.1 mol L^{-1} $NaNO_3$	2. Exchangeable compounds
	3. 1 mol L^{-1} CH_3COONa/CH_3COOH (pH 5)	3. Adsorbed/ bound to carbonates
	4. 1 mol L^{-1} Na_2OH-HCl/ 25% CH_3COOH	4. Bound to Fe and Mn oxide
	5. 0.02 mol L^{-1} HNO_3 / 30% H_2O_2 / 3.2 mol L^{-1} NH_4Ac	5. Organic matter and sulfur
	6. 16 mol L^{-1} HNO_3 / 12 mol L^{-1} HCl	6. Residual
Boszke *et al.* (2006)	1. Chloroform/ 0.01 mol L^{-1} $Na_2S_2O_3$	1. Organic bound Hg
	2. Deionized water	2. Water-soluble Hg
	3. 0.5 mol L^{-1} HCl	3. Acid soluble Hg
	4. 0.2 mol L^{-1} NaOH	4. Hg associated with humic matter
	5. Heated at 150°C/*Aqua regia*	5. Hg^0
	6. Aqua regia	6. Residual Hg

1.5 Analytical techniques used in mercury quantification

As the mercury problem is still very much present these days, analytical techniques that are both selective and sensitive, and capable of detecting both trace and high amounts of this element are fundamental. A variety of techniques exist and are currently used in mercury quantification in various matrices, in different areas (environmental, food products, clinical, etc.). These methods include: atomic absorption spectrometry (AAS), mainly as cold-vapor atomic absorption spectrometry (CV-AAS), cold-vapor atomic fluorescence spectrometry (CV-AFS), atomic emission spectrometry (AES) and its coupled techniques like inductively coupled plasma - atomic emission spectrometry (ICP-AES), microwave induced plasma - atomic emission spectrometry (MIP-AES), and direct current plasma - atomic emission spectrometry (DCP-AES), X-ray fluorescence (XRF), electron probe micro-analysis (EPMA), proton induced X-ray emission (PIXE), inductively coupled plasma - mass spectrometry (ICP-MS), and chromatography, among other methods (Brown *et al.*, 1995; Clevenger *et al.*, 1997). Radiochemical methods, like neutron activation analysis (Delft *et al.*, 1988), although rapid and sensitive for trace concentrations, are less commonly applied, as are electrochemical methods (polarography, amperometry, voltammetry, etc.).

Cold-vapor atomic absorption spectroscopy (CV-AAS) and cold-vapor atomic fluorescence spectroscopy (CV-AFS) are the most widely used methods in mercury determination (Clevenger *et al.*, 1997), because, due to the high vapor pressure of mercury, they allow direct determination of this element without the need of an atomizer. Prior to analysis, mercury has to be released “liberated” from the matrix; in case of solid samples, a digestion process in required. In the next step, mercury present in the sample solution as Hg^{2+} is reduced to Hg^{0} using tin chloride ($SnCl_2$) or sodium borohydride ($NaBH_4$). The mercury vapor is then purged from the solution by aid of a gas stream, such as air, nitrogen or argon, and introduced into the optical path of an atomic absorption spectrometer. Absorption at λ=253.7 nm is then measured with the use of mercury vapor lamps or hollow cathode lamps as the light source.

The digestion process is usually the most labor-intensive part of the analytical work and can also be responsible for mercury losses. Direct mercury analysers are

an alternative method that takes advantage of mercury's high volatility, enabling the quantification of mercury in solid and liquid samples of organic or inorganic composition, without requiring time-consuming sample preparation or digestion methods (Costley *et al.*, 2000). The method, as described by Costley *et al.* (2000), consists of thermal decomposition of the sample, followed by gold amalgamation and detection through atomic absorption spectroscopy. A thorough explanation of the direct mercury analyser can be found in section 3.4.1.

During recent years new analytical techniques have become available that have contributed significantly to the understanding of mercury speciation in natural systems. In particular, these include ultra sensitive and specific analytical equipment and contamination-free methodologies. These improvements eventually allow for the determination of total and major species of mercury to be made. Before these progresses, extractants which released a large portions of the element were preferred, but the development of analytical techniques with lower quantification limits has allowed that milder extractants, such as 0.01 mol L^{-1} $CaCl_2$ and 1.0 mol L^{-1} NH_4NO_3, can be used, mimicking more "real" available or reactive element pools.

Therefore, analytical methods must be selected depending on the nature of the sample and, in particular, the concentration levels of mercury present (Horvat, 2005).

Motivation and objectives

2 MOTIVATION AND OBJECTIVES

Today we are witnessing a growing interest in metal speciation, mostly because of the need to establish ready and accessible metal-specific tools and data sets in order to make informed, science-based decisions in risk assessment and remediation strategies.

However, in the case of mercury, its particular chemistry requires the development of specific extraction schemes, specifically dedicated to this element (Bacon *et al.*, 2008), as selective and sequential extraction procedures commonly used for other elements (e.g. cadmium, chromium, cobalt, copper, iron, lead, manganese, nickel, and zinc) are not appropriate for mercury. The literature vehemently stresses the need to develop methods specific for mercury, as well as adequate quality control procedures and associated reference materials. Despite several attempts to develop such methods, at present there is still not a consensual protocol regarding mercury sequential extraction (Issaro *et al.*, 2009). Although some steps have already been taken towards developing a robust and reproducible methodology for mercury speciation in soils and sediments, the complex chemistry of this element, in addition to the intricacy of soil and sediment chemistry and the interaction of the contaminant with soil or sediment matrix, has not yet allowed fulfilling this objective.

Therefore, the need to improve knowledge in this area has prompted the study here presented.

2.1 Objectives of the PhD work

This PhD program focused on the evaluation and validation of methodologies for mercury fractionation and speciation in solid matrices. The objectives have been assembled so that this research will be useful for a most effective implementation of risk assessment methodologies in mercury contaminated sites and for a better understanding and prevention of risks arising from practices such as sewage sludge, fertilizers or pesticides application in agricultural soils.

The scientific objectives of this study include:

I. Testing and evaluation of single and sequential extraction procedures for the fractionation of mercury contents in soil and sediment samples.

II. Identification of relevant factors and matrix effects that contribute for the fractionation of the metal in both sediment and soil samples;

III. Identification of possible sources of error and variability in the results obtained;

IV. Preparation of reference materials to be tested in the scope of an international inter-laboratory exercise that will test the performance of selected sequential extraction procedures.

The research had the following approach:

I. Review the different procedures described in past studies for fractionation and speciation of mercury in soils and sediments, as well as reagents used for mercury extraction;

II. Assess differences among the different procedures available from the literature and select those that provide most relevant information on mercury fractionation;

III. Application the selected speciation and fractionation procedures in well characterizes soil and sediment samples;

IV. Combine the extraction studies with a comprehensive characterisation/ analysis of the samples;

V. Analysis of the suitability of the materials tested to be used in the future development of reference materials for validation of extraction schemes;

VI. Organization of an international proficiency-testing exercise aimed at testing the performance of proposed single or sequential mercury extraction procedures, using the sediment and soil reference materials previously prepared.

Sampling and methodologies

3 SAMPLING AND METHODOLOGIES

3.1 Sampling sites

Sampling sites were chosen based on previous studies and exploratory surveys, therefore following a judgmental sampling approach.

Part of the soil samples was collected in the vicinity of the industrial complex of Estarreja, North-Western coast of Portugal (Figure 6). This complex dates back to 1950 (Inácio *et al.*, 1998) and is home to a large chlor-alkali plant which used to produce chlorine and caustic soda by the mercury cell process, where liquid elemental mercury is utilized as a cathode in the electrolysis of a saturated brine solution (Ullrich *et al.*, 2007). As many studies show (Biester *et al.*, 2002b; Lacerda *et al.*, 1998; Reis *et al.*, 2009) mercury-cell chlor-alkali plants have been identified as major sources of mercury to the environment. Although the plant started to change the production process in 1994 and completely ceased the use of mercury in 2002 (Ospar Commission, 2006), mercury emitted from the existing plant still remains significant in the surrounding environment (Reis *et al.*, 2009). Until 1975 the liquid effluents from this plant, containing many different types of contaminants (Batista *et al.*, 2002), including mercury, were discharged directly into man-made effluent streams. Consequently, the pollutants were transported for several kilometres through the agricultural fields surrounding the chlor-alkali plant (Costa *et al.*, 2001). Although after 1975 impermeable pipes were constructed, and the streams are no longer used for effluent transport, these are still present in fields. Soil samples were therefore collected from fields within a radius of < 1 km from the industrial complex of Estarreja. These fields are used mainly for agricultural and cattle grazing purposes.

Another set of soil samples was collected at the Caveira sulfide mine, which is located in Grândola (South-West Portugal) and is part of the Iberian Pyrite Belt (IPB, Figure 6). The IPB is a well-known mining district of worldwide significance, due to its unusual concentration of large and medium sized mineral deposits, including ores of copper, iron, lead, sulfur and zinc. Antimony, arsenic, cadmium, cobalt, gold, mercury, selenium and silver can also be found in soils from the IPB (Barriga, 1990). Past mining activities at the Caveira mine included pyrite (FeS_2) and Cu extraction. From 1936 until the 1970´s Caveira massive sulfides were exploited for sulfur. Although the mine is now closed, soil metal contamination and acid mine drainage

still pose severe environmental problems at the site. Large volumes of waste were produced by the mining activities and various types of tailings deposited in the area (the amount of waste stored on the site is estimated to be higher than 2 Mt) (Cardoso Fonseca *et al.*, 2000). Rainwater circulates and percolates easily over and through these tailing materials causing significant erosion and transport of tailings debris to areas nearby and downstream. Soil samples were collected from fields within a radius of < 2 km from the mine. Ryegrass (*Lolium perenne*) was the predominant plant species at these fields.

Non-contaminated soil samples, collected at Gandra (North Portugal, Figure 6), were occasionally used as reference.

Sediment samples were collected at the Laranjo Bay, the most contaminated area of Ria de Aveiro, a coastal lagoon located along the Atlantic Ocean, on the northwest coast of Portugal (Figure 6). With an extensive area of wetlands (83 km^2-high tide and 66 km^2-low tide), it is a mesotidal system, where tides are semi-diurnal and propagate from the mouth to the inner lagoon areas. The Ria de Aveiro is one of the most mercury-contaminated systems in Europe, due to the continuous mercury discharges from the abovementioned chlor-alkali plant (Pereira *et al.*, 1998).

Occasionally, soil samples from Spanish mine areas were used. The Almadén mining district is responsible for one-third of the total world Hg production and is considered one the most Hg-contaminated places on Earth, due to its numerous mercury ore deposits, which have in common a simple mineralogy that includes dominant cinnabar (HgS) and minor pyrite (FeS_2) (Higueras *et al.*, 2006). Asturias was also a site of abundant mining activities due to its mercury deposits, in the form of cinnabar, metacinnabar and occasionally native mercury, and the abandoned solid waste and industrial installations are still present in agricultural and pastoral fields (Loredo *et al.*, 1999).

A general characterisation of sampling locations is given in Table 2.

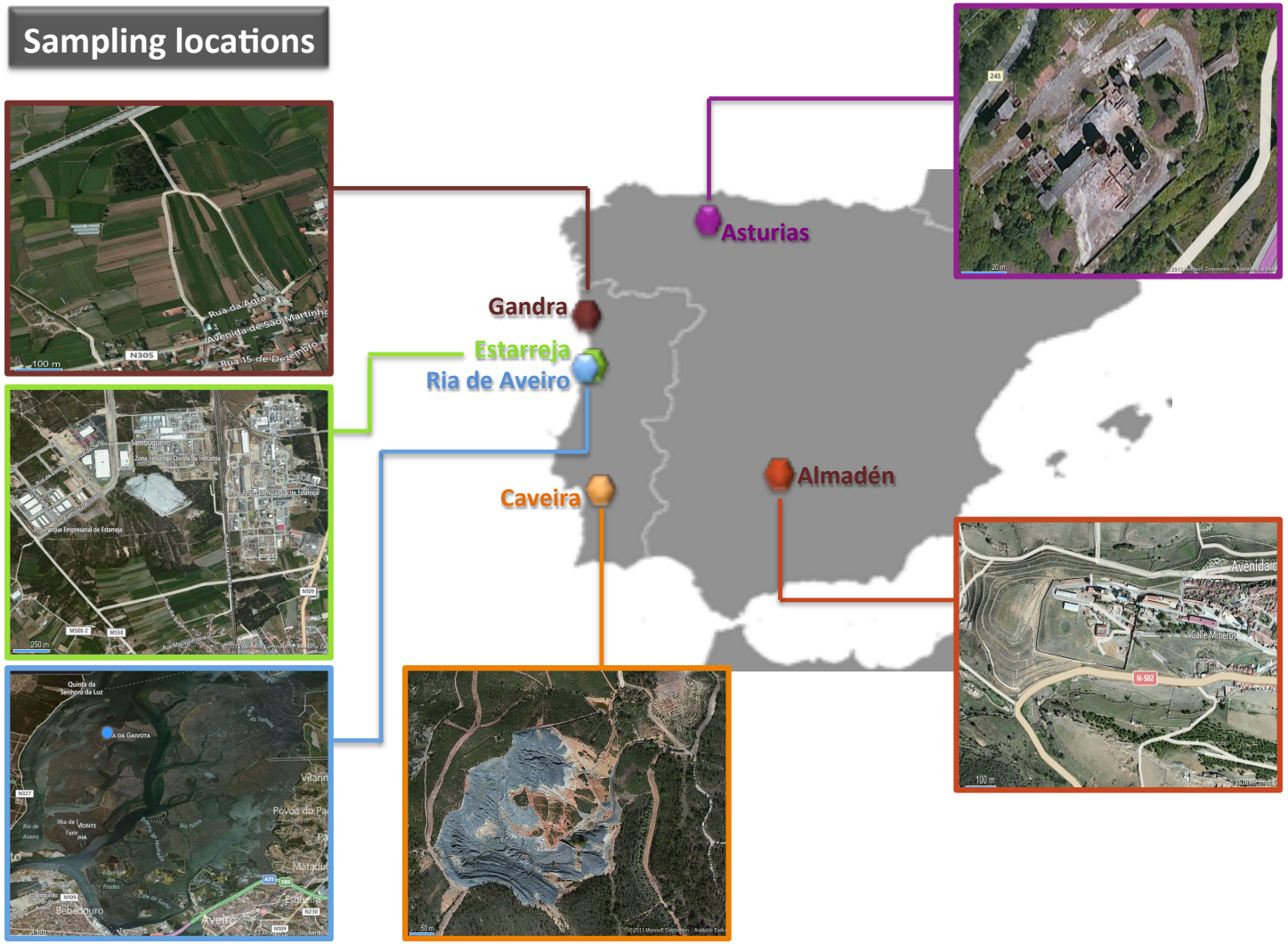

Figure 6. Soil and sediment sampling sites in Portugal and Spain.

3.2 Sampling and sample treatment

Sample handling and storage can profoundly affect analysis results, particularly when measuring bioavailability and chemical speciation. In this case sample handling and storage become critical (Sheppard *et al.*, 2006). Thus, all efforts were made in order to assure samples preserved their integrity. Manipulation in the field, sample handling, transport and treatment in the laboratory were reduced to only absolute necessary guaranteeing that cross-contamination risk and mercury losses were reduced to the minimum.

Soil sampling was performed using a plastic spatula, to a depth of 15 cm, while for sediments a 50 cm deep, 7 cm diameter corer was used (Figure 7). Samples were placed in polyethylene bags during transport to the laboratory, where they were pre-treated within one hour. During warmer months, samples were transported in lunch coolers. All material used during sampling was washed with distilled water between samples. Once in the laboratory, samples were air dried in a cool room, to constant weight (Figure 8). Stones, shells, roots, etc. were removed and clumps were crushed and homogenised during the drying stage. The dried samples were sieved to < 2 mm (soils) and < 1 mm (sediments) to obtain appropriate particle size distribution. Nylon sieves were used in order to avoid metal contamination. All subsequent analyses were performed on these fractions, which were stored in polyethylene bags, sealed and with headspace reduced to a minimum to reduce loss of volatile mercury. Analyses were performed as soon as possible. Immediately before extractions or analyses were performed, samples were manually remixed to improve homogeneity.

Additionally, once extracts were separated from the solid material, acidification to pH<2 with concentrated HNO_3 was done, and extracts were stored at 4 °C and analysed within 48 hours. This procedure is recommended to guarantee a better conservation of extractants (Ianni *et al.*, 2001).

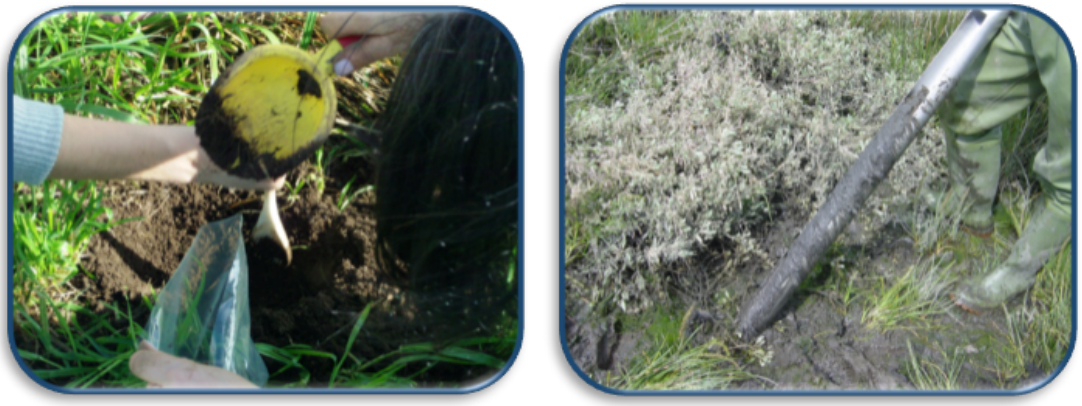

Figure 7. Soil (left) and sediment sampling (right).

Figure 8. Samples being air dried.

3.3 Analytical methodologies

Note: Determination of particle size distribution and organic matter content was performed differently in soil and sediment samples, applying the most commonly used methodologies for each matrix.

3.3.1 Fine fraction content

The percentage of fine fraction (< 63 µm) in sediments was determined in sediment samples and was calculated gravimetrically through wet sieving of approximately 5 g of sediment through a 63 µm nylon mesh, under a gentle water flux (Pereira, 1996). The fraction retained in the mesh is dried at 120 °C in a forced-

air oven, until constant weight, and the percentage of fine fraction calculated by difference from total weight. This determination was run in triplicate.

3.3.2 Organic matter content

Total carbon (TotC) content in soils was measured on an Elemental Analysis instrument (LECO CNH-2000), according to ISO 10694:1995. For the determination of organic carbon content (OrgC), an excess of 4 mol L^{-1} hydrochloric acid (HCl) was added to a crucible containing a weighed quantity of soil. The crucibles were left to stand for 4 hours and then were dried for 16 hours at 60–70 °C. The analysis of carbon content after the removal of carbonates was performed using the same procedure as total carbon determinations.

In sediments, the organic matter content was estimated by loss on ignition (LOI), placing the sediments at 500 ºC during 4 hours in a muffle. The results were expressed as percentage.

3.3.3 Particle size distribution

The particle size distribution and clay contents of the soil samples were determined using a Coulter LS230 laser diffraction particle size analyser. The classification of soils followed the USDA Texture Classes: sand fraction (0.050 < % < 2 mm), silt fraction (0.002 < % < 0.050 mm), and clay fraction (% <0.002 mm). Classification of samples was achieved by using the Talwin 42® classification software program.

3.3.4 pH

The soil and sediment pH was determined according to the ISO 10390:1994 method, using a WTW pH meter-538. A suspension of soil was made up in five times its volume of 0.01 mol L^{-1} $CaCl_2$ in water. The suspension was then shaked vigorously, for 5 minutes and let rest for about 2 hours. The pH-meter was adjusted with pH=7.01 and pH=4.01 buffer solutions. Care was taken to assure that the temperature of buffer solutions and samples did not differ by more than 1 °C. Just

before measurement, the suspension was thoroughly shaked and the pH measured in the settling suspension, after stabilization was reached. Two replicates were done for each sample.

3.3.5 Other elements quantification

The pseudo-total contents of aluminium (Al), iron (Fe), manganese (Mn) and sulfur (S) were extracted by *aqua regia* (according to ISO 11466:1995) and analysed by ICP–MS (ICP–MS THERMO X Series, Peltier Nebulizing Camera, Burgener Nebulizer; CETAC AS510 auto-sampler; the CeO+/Ce+ ratio was optimized at <2%; Internal standard: In). The instrument was tuned using a 10 µg kg^{-1} multi-element tuning solution. The operational conditions used are summarized as follow: RF power: 1400 W; plasma gas flow (argon): 13 L min^{-1}; auxiliary gas (argon): 0.90 L min^{-1}; nebulizer flow (argon): 0.95 min^{-1}.

Amorphous iron (Fe_ox) and aluminuim oxides (Al_ox) were determined by the extraction of 2.5 g of soil with 50 mL of 0.1 mol L^{-1} oxalic acid (buffered to pH 3 by ammonium oxalate) and shaken mechanically in the dark for 2 h. Aluminium and iron contents in the filtered extracts were measured by ICP–MS. Two replicate extractions were performed for each sample. Two extraction blanks were included in each batch of 20 bottles. The filtered extracts were analysed by ICP–MS, according to ISO 17294–1:2005 and ISO 17294–2:2003, with operational conditions as previously described.

Table 2. General characterisation of sampling locations.

Estarreja soil
Anthropogenic mercury
Organic carbon 2-4%
Variable texture
Acid-almost neutral pH (4.8-6.0)

Ria de Aveiro sediment
Anthropogenic mercury
LOI (%) 22-41%
Fine particle % (<0.63µm) 20-34%
Almost neutral pH 6.1-6,9

Caveira soil
Geogenic mercury
Organic carbon 0.5-5.0%
Texture varies between loam, sandy loam, and silt loam.
Acid pH (2.9-5.3)

Almadén soil
Geogenic mercury
Organic matter 9.9%
Loam
pH 5.4

Asturias soil
Geogenic mercury
Organic matter 9.9%
pH 6.5

3.4 Mercury quantification

3.4.1 Direct Mercury Analyser

Total mercury contents in soils, sediments and some extracted solutions were determined by thermal decomposition atomic absorption spectroscopy (AAS) with gold amalgamation (LECO model AMA-254), a rapid and simple total mercury determination method that requires little sample handling prior to analysis. Solid or liquid samples are placed in a nickel boat that is inserted in a quartz combustion catalytic tube. The sample is initially dried at 120 °C, prior to combustion at 750 °C (150 s) in an oxygen atmosphere. The mercury vapour produced is trapped on the surface of a gold amalgamator. After a pre-specified time interval (120–150 s), the amalgamator is heated to 900 °C to quantitatively release the mercury which is transported to a heated cuvette (120 °C) and then quantified by atomic absorption spectroscopy, using a silicon diode detector, at 253.6 nm (Figure 9) (Costley *et al.*, 2000).

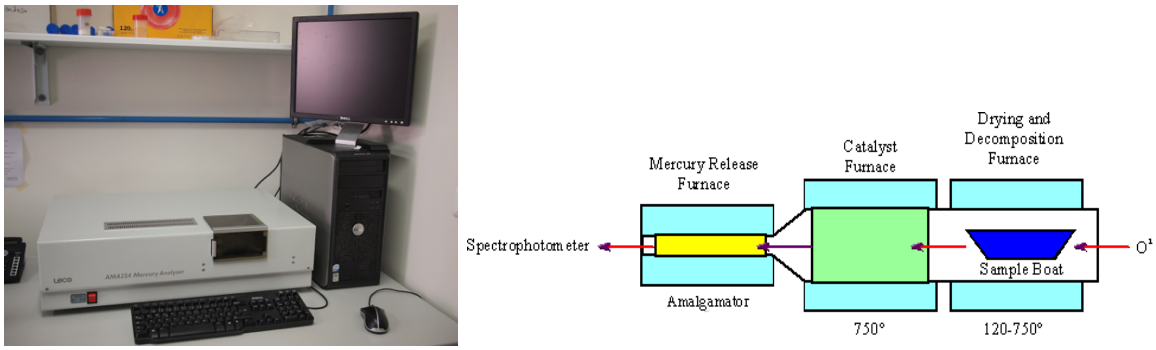

Figure 9. LECO AMA-254 (left) and representative scheme (right).

The two automatic mercury analysers (LECO AMA-254) used have different internal calibration ranges: one equipment has calibration ranges from 0.1 to 30 ng Hg and 100 to 500 ng Hg; the second equipment has a more sensitive optic cell and calibration ranges from 0.1 to 8.0 ng Hg and 10 – 200 ng Hg. A limit of quantification of 0.05 ng Hg was established for both equipments.

3.4.2 Cold Vapour Atomic Fluorescence Spectroscopy

Total mercury concentration in extracted solutions was measured by cold vapour atomic fluorescence spectroscopy (CV-AFS; PSA model Merlin 10.023 equipped with a detector PSA model 10.003) using tin(II) chloride as a reducing agent. Prior to analysis, 50 mL of sample plus 500 µL of a saturated solution of potassium persulfate were irradiated with a UV lamp (1000 W) for 30 minutes, to guarantee that all mercury was available for quantification. Following irradiation, the excess oxidant was reduced with 37.5 µL of 12% hydroxylamine solution (w/v) (Mucci *et al.*, 1995).

Standard mercury solutions were prepared by stepwise dilution with 2 % HNO_3 from a standard stock solution (Merck) containing 998±2 mg L^{-1} of mercury as $Hg(NO_3)_2$. All standards were freshly prepared prior to use and the equipment was calibrated daily. The detection limit of the CV-AFS technique for total mercury was 2.3 ng L^{-1}. Blanks were run with samples, and their contribution corrected when necessary; additionally, at least one mercury standard was tested every three samples to check for instrument drift.

3.5 Quality control and quality assurance

In analytical work, the quality of the results is vital as upon it depends the delivery of reliable information. It includes determination of precision and accuracy. These are directly related to ‘‘fitness of use’’ of the data and they determine the degree of total variability (uncertainty or error) that can be tolerated in the data. Therefore, implementation of quality control (QC) methods is extremely important.

3.5.1 Precision and accuracy

Precision was assessed through the repeatability of replicate analysis, which, in turn, was assessed through the relative standard deviation (RSD, Equation 1). Acceptance criterion for sample analysis was established as RSD below 10% for three replicate results, above which samples were re-analysed. In cases of very

heterogeneous samples (e.g. soils), with higher RSD, at least 10 replicate analyses were performed to assure a reliable result.

Equation 1. $$RSD = \frac{SD}{mean} \times 100$$

To determine the **accuracy**, certified reference materials (CRM) of similar matrix to the samples were analysed and the concentration obtained was compared to the certified value, through the determination of recovery (Equation 2). Certified reference materials used are depicted in Figure 10.

Equation 2 $$\text{Recovery} = \frac{\text{Hg}_{\text{obtained}}}{\text{Hg}_{\text{certified}}} \times 100$$

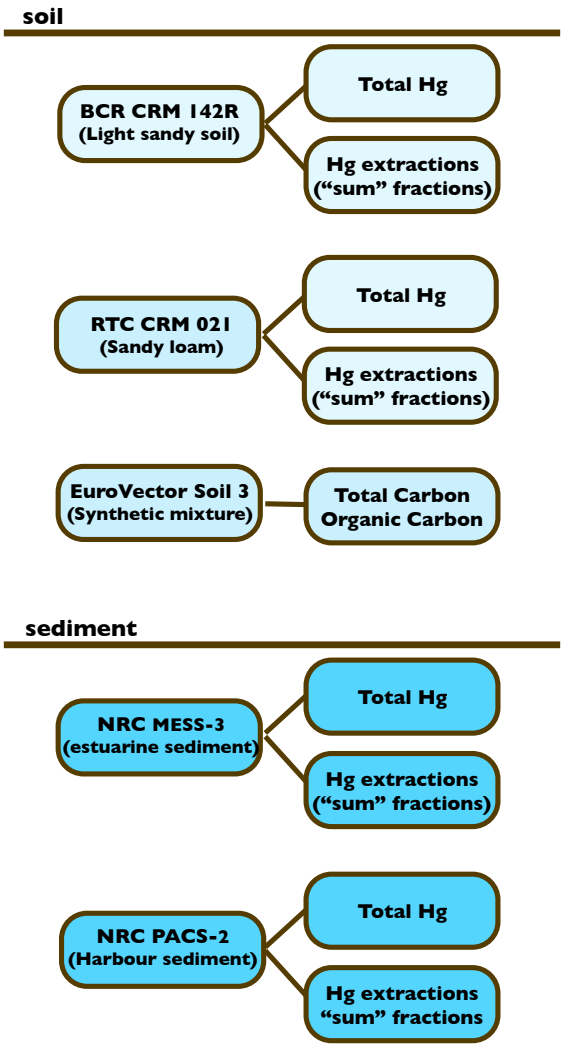

Figure 10. Certified reference materials used in the analysis' quality control.

3.5.2 Quality control for AMA-254

The internal calibration of the automatic mercury analyser (LECO AMA-254) was checked on a daily basis by analysing certified reference materials (CRM) of similar matrix to the samples (Figure 10). The accuracy of the equipment was assessed by the analysis of BCR CRM 142R and RTC CRM 021 for soils and NRC MESS-3 and PACS-2 for sediment analysis. Mean mercury concentrations and recoveries obtained are described in Table 3 for the four CRM. Correction of results was performed according to the daily recoveries obtained for CRM. This procedure corrects daily variation of the equipment's response and loss of accuracy due to deterioration of the catalytic tube. The replacement of the catalytic tube is expensive and, hence, only performed when the value determined for a CRM is no longer within the certified confidence interval. Also, given that the different internal calibration curves of the AMA-254 provide different recovery efficiencies, and the fact that during a working day samples may fall in the range of different calibration curves, this result correction is essential for sample comparison. To control memory effects, blanks were analysed between samples (Coelho, 2009).

3.5.3 Quality control for cold-vapour atomic fluorescence spectroscopy

QC/QA for CV-AFS included blank runs between samples and alternate mercury standards tested every three samples to check for instrument drift. If the standard showed a relative standard variation (RSD) above 10%, when compared with previous measurements, the calibration curve was repeated. The precision of the sample measurement, expressed as the RSD, was lower than 9% (n=6).

Ultra-pure water obtained from a Millipore apparatus (resistivity = 18 MΩ cm) and mercury-free HNO_3 (Emsure) were used throughout. Distilled water used in the extraction procedure was tested and found to be sufficiently low in mercury (less than 10 ng L^{-1}) before use.

3.5.4 Other procedures used for quality assurance

All solutions were prepared from reagent-grade chemicals and were tested and found to be sufficiently low in mercury (less than 10 ng/l) before use. Analytical procedures were conducted using ultra-clean glassware to avoid contaminating sample extracts; glassware was soaked in Derquin 5%, for at least 24 hours, followed by acid bath (HNO_3 25%), for a minimum of 24 hours.

During sampling and all procedures, care was taken to avoid cross-contamination of the samples and mercury losses by volatility.

Three replicates of each sample were taken for sequential extraction. Each set of samples extracted included one blank, to check if both material and reagents were mercury free, and a certified reference material of adequate matrix and mercury concentration.

Quality control for the determination of total carbon in LECO CNH-2000 was performed through the analysis of certified reference material Synthetic Mix for Soil #3 from EuroVector, for which a mean recovery of 114% was obtained.

Table 3. Mean concentrations and recoveries obtained in CRM analysis.

Equipment	CRM		certified value	average	SD	average %Rec
LECO AMA-254 I	BCR CRM 142R	Hg (mg kg^{-1})	0.067±0.011	0.062	0.005 (n=84)	**92.5**
	RTC CRM 021	Hg (mg kg^{-1})	4.7±0.4	4.51	0.19 (n=11)	**96.0**
LECO AMA-254 II	BCR CRM 142R	Hg (mg kg^{-1})	0.067±0.011	0.064	0.006 (n=73)	**95.5**
	RTC CRM 021	Hg (mg kg^{-1})	4.7±0.4	4.65	0.24 (n=24)	**98.9**
	NRC MESS-3	Hg (mg kg^{-1})	0.091±0.009	0.089	0.004 (n=18)	**97.8**
	NRC PACS-2	Hg (mg kg^{-1})	3.04±0.20	3.01	0.11 (n=24)	**99.0**
LECO CNH-2000	EuroVector Soil 3	C (%)	4.4*	5.02	0.11 (n=16)	**114**

*standard deviation not provided.

Extractability and mobility of mercury from agricultural soils surrounding industrial and mining areas

4 EXTRACTABILITY AND MOBILITY OF MERCURY FROM AGRICULTURAL SOILS SURROUNDING INDUSTRIAL AND MINING CONTAMINATED AREAS

Highlights

- Information concerning the mobility of mercury species in soil; mercury fractions were classified as mobile, semi-mobile and non-mobile.

- In all samples mercury was mainly present in the semi-mobile phase (between 63% and 97%).

- The presence of mercury in the mobile phase could be related to manganese and aluminium soil contents. Organic matter and sulfur contents contributed to mercury retention in the soil matrix.

Abstract

This study focused on a comparison of the extractability of mercury in soils with two different contamination sources (a chlor-alkali plant and mining activities) and on the evaluation of the influence of specific soil properties on the behaviour of the contaminant. The method applied here did not target the identification of individual species, but instead provided information concerning the mobility of mercury species in soil. Mercury fractions were classified as mobile, semi-mobile and non-mobile.

The fractionation study revealed that in all samples mercury was mainly present in the semi-mobile phase (between 63% and 97%). The highest mercury mobility (2.7 mg kg^{-1}) was found in soils from the industrial area. Mining soils exhibited higher percentage of non-mobile mercury, up to 35%, due to their elevated sulfur content. Results of factor analysis indicate that the presence of mercury in the mobile phase could be related to manganese and aluminium soil contents. A positive relation between mercury in the semi-mobile fraction and the aluminium content was also observed. In contrast, organic matter and sulfur contents contributed to mercury retention in the soil matrix reducing the mobility of the metal.

Despite known limitations of sequential extraction procedures, the methodology applied in this study for the fractionation of mercury in contaminated soil samples provided relevant information on mercury's relative mobility.

Keywords: Mercury; mobility; sequential extraction; soils.

4.1 Introduction

This chapter focuses on a method for sequential extraction of mercury in soils and sediments validated by Han *et al.* (Han *et al.*, 2003), generally known as the "Kingston method". There are several recognized limitations associated with sequential chemical extraction procedures that have been mentioned in section 1.4.3; however, when the main target is to evaluate the mobility of mercury in any given sample it is still realistic that the application of this procedure provides valuable information. The Kingston method is based on the sequential extraction of different operationally defined fractions and provides detailed information about the potential mobility of mercury in the samples. Mercury mobility is defined in terms of the mercury leached in the following three fractions: mobile (M), semi-mobile (SM), and non-mobile (NM) (Fernández-Martínez *et al.*, 2005a; Han *et al.*, 2003), with toxicity decreasing in that order. The operationally-defined mercury fractions are summarized in Table 4.

Table 4. Operationally-defined mercury fractions (adapted from (Han *et al.*, 2003)).

Operationally-defined Hg fractions	Individual mercury species
Mobile mercury fraction	MeHgCl EtHgCl $HgCl_2$ $Hg(OH)_2$ $Hg(NO_3)_2$ $HgSO_4$ HgO Hg^{2+} complexes
Semi-mobile mercury fraction	Hg^0 or Hg^0–Metal (amalgam) Hg^{2+} complexes Hg_2Cl_2 (minor)
Non-mobile mercury fraction	Hg_2Cl_2 (major) HgS HgSe

The main objective of this work was to assess mercury extractability and mobility in agricultural soils from two locations, with different sources of mercury contamination (industrial and mining activities). The study also focused on the

evaluation of the influence of specific soil properties on the distribution and behaviour of the contaminant. Improved understanding of these relationships will allow more effective prediction of how changes in environmental conditions and soil characteristics (e.g. due to processes associated with climate change) may affect the mobility of mercury in contaminated soils, its potential availability to plants and toxicity to organisms.

4.2 Materials and methods

4.2.1 Sampling and methodology

Samples 1, 2, 4, 6, 8, 10, and 12 were collected in an agricultural field close to the former effluent streams of the Industrial Complex of Estarreja (Ullrich *et al.*, 2007). Caveira mine samples (3, 5, 7, 9, 11, 13, and 14) were collected in the surroundings of the mine pit. The description of these locations is given in section 3.1

Soil samples were analysed for the following parameters, according to the methodologies presented in sections 3.2 and 3.3:

- Total mercury content;
- pH;
- Total carbon (TotC) and organic carbon (OrgC);
- Iron (Fe), aluminium (Al), manganese (Mn) and sulfur (S);
- Particle size distribution.

General quality control and quality assurance procedures applied in this work are described in section 3.5. As there are no certified reference materials for mercury fractionation in soil, the sequential extraction was controlled by applying the procedure to RTC CRM 021 (sandy loam). Although this reference material is not certified for the mercury fractions targeted by the Kingston method, the sum of the three fractions was compared to the certified value for total mercury (4.7 mg kg^{-1}) – Equation 3. The mean results found for the 8 replicate samples analysed were 0.0199 mg kg^{-1} and 4.5 mg kg^{-1}, for mobile and semi-mobile fractions, respectively. Mercury levels for the non-mobile and residual fractions were below the detection limit (0.05 ng). The mean sum (4.5 mg kg^{-1}) was within the confidence interval (4.5 –

5.1 mg kg^{-1}) and, as a recovery of 96% was obtained, the extraction efficiency was found acceptable.

Equation 3. $$\frac{\text{Hg mobile+ Hg semi-mobile+Hg non-mobile+Hg residual}}{\text{certified total Hg}} \times 100$$

4.2.2 Sequential extraction procedure

The study of mercury fractionation was performed by the application of the "Kingston method" as described by Han *et al.* (2003) and Fernández-Martínez *et al.* (2005a).

Extraction of the mobile fraction (M)

Extraction of mobile and organometallic mercury species involves the use of a solution of 2% (v/v) HCl+10% (v/v) ethanol.

A sample (1.0–2.0 g) was weighed and added to a 10 mL centrifuge tube with 2.5 mL of the extract solution. The sample and the extract solution were mixed well by vigorous shaking for 2 minutes. The pH was checked and, when necessary, concentrated HCl was added drop-wise until the pH of the mixture was between 1.5 and 3. The sample was then sonicated at room temperature (not at 60±2 °C, as referred in Han *et al.* (2003)) for 7 minutes, and centrifuged (3200 rpm, 5 minutes) to separate the supernatant from the soil matrix. The supernatants were collected using a Pasteur pipette and transferred to a vial. This extraction was repeated three more times. The residue was then rinsed by adding 2.5 mL of DDI water, shaken for 2 minutes and centrifuged. All the extraction supernatants and the water rinse were combined. This final solution was kept at 4 °C and analysed within 48 hours.

Extraction of the semi-mobile fraction (SM)

Before proceeding to the extraction of the semi-mobile phase, the residue was tested for the presence of chloride ions because the presence of chloride can promote the solubility of non-mobile mercury species (e.g., HgS) into the semi-mobile extract solution and consequently must be avoided. Because all samples revealed the presence of chlorine, a procedure was undertaken to remove them, according to Fernández-Martínez *et al.* (2005a). This consists of washing the residue

with 5 mL distilled water, until the addition of 0.1 mol L^{-1} $AgNO_3$ causes no turbidity. This procedure should not be applied more than 3 times, which was never necessary in any of the samples analysed.

For the extraction of semi-mobile species, a solution of 1:2 (v/v) HNO_3:distilled water is required. A 5 mL aliquot of this solution was added to the residue and mixed by shaking it vigorously. The mixture was heated to 95±2 °C for 20 minutes in a sand bath. To avoid losses of volatile mercury species, glass spheres replaced the tubes' caps during the heating step, providing both sufficient cover and reflux. After cooling to room temperature, samples were centrifuged (3200 rpm, 5 minutes), the supernatant was collected, and the extraction was repeated. The remaining soil residue was washed with 5 mL distilled water. The rinse water was combined with both supernatants and the solution stored at 4 °C until analysis.

Extraction of the non-mobile fraction (NM)

The procedure for the extraction of the non-mobile phase was similar to the one used for the semi-mobile phase except that the extraction solution was 1:6:7 (v/v/v) HCl:HNO_3:distilled water. The remaining residue (RES) was dried at 40 °C and analysed for mercury content.

4.2.3 Statistical analysis

Statistical analysis was performed using SPSS Statistics 17.0. The relation between the variables was evaluated by factor analysis, considering the correlation matrix. Factors were extracted by Principal Components Method, followed by Varimax rotation. Retained factors presented *eigenvalues* greater than 1; this observation was confirmed by Scree Plot analysis.

4.3 Results

4.3.1 Total mercury and soil characteristics

Results obtained for the determination of total mercury in the fourteen samples are shown in Table 5. Total mercury concentration ranges between 1.0 and 91 mg

kg^{-1} for Estarreja samples and 1.1 and 98 mg kg^{-1} for soils of Caveira. The soil properties are also shown in Table 6.

Soil pH in Caveira varied between 3.6 and 5.3. Although all soils analysed were acidic, an unusually low pH value was observed in sample 11 (pH 3.6). The Caveira area is known to be affected by acid mine drainage (Cardoso Fonseca *et al.*, 2000) which may explain the low pH. Acid mine drainage is formed when pyrite (FeS_2) and other metal sulfides are exposed to oxygen and water and subjected to oxidising conditions resulting in the production of sulfuric acid (low pH), sulfates and dissolved metal ions (Ziemkiewicz *et al.*, 1997).

Total carbon % values varied between 1.6 and 5.1% while organic carbon % varied in the range 1.6-4.3%. A considerable fraction of the total carbon content is in the form of organic carbon, in the entire dataset.

Variable soil textures were obtained for these soils: loamy sand, sandy loam, loam and silt loam with clay percentages between 3.2 and 17%. In general, soils from Caveira showed higher clay content than soils from Estarreja.

The “active” forms of aluminium and iron (which occur as amorphous hydroxides and are bound to organic matter) were extracted as oxalates from soil samples and measured in an ammonium oxalate-oxalic acid extract. A large variability between soil samples was observed with respect to amorphous aluminium oxides (Al_{ox}) and amorphous iron oxides (Fe_{ox}) (which varied in the range 21.2-79.6 mmol kg^{-1} and 12.1-183.4 mmol kg^{-1}, respectively). In general, Al_{ox} were present in relatively higher concentrations in samples from Estarreja while the highest contents of Fe_{ox} were found in samples from Caveira. The iron amorphous oxides contents of these soils, particularly at the Caveira area, are relatively higher than those from a study of Portuguese agricultural acid soils which reported a Fe_{ox} range of 1.3-82.7 mmol kg^{-1} and a median of 17.2 mmol kg^{-1} (Horta *et al.*, 2007). The contents of Al_{ox} observed in Estarreja were also higher than those observed by Horta and Torrent (2007). Manganese concentrations and sulfur % were higher in Caveira soils than in those from Estarreja.

These soil samples cover a wide range of mercury contamination and allow testing of the Kingston method both in soils with very different mercury concentrations and in soils with different origins and characteristics.

Table 5. Mercury concentration (mean±standard deviation, mg kg^{-1}) in each extracted fraction.

Sample		Mobile	Semi-mobile	Non-mobile	Residual	Fraction's sum	Total Hg	Recovery %
Estarreja soils	**1**	1.2 ± 0.2	85.9 ± 0.1	1.83 ± 0.01	0.261 ± 0.003	89	91	98
	2	0.010 ± 0.001	0.86 ± 0.3	0.0026 ± 0.0003	0.038 ± 0.001	0.91	1.0	91
	4	0.18 ± 0.03	15.5 ± 0.6	0.075 ± 0.007	0.124 ± 0.003	16	17	93
	6	0.55 ± 0.06	30.4 ± 1.2	0.46 ± 0.08	0.035 ± 0.002	31	38	83
	8	1.07 ± 0.06	67.6 ± 3.5	1.3 ± 0.2	0.060 ± 0.004	70	78	90
	10	0.91 ± 0.01	75.4 ± 5.8	2.9 ± 0.2	0.082± 0.003	79	77	103
	12	2.7 ± 0.1	46.1 ± 4.1	0.95 ± 0.2	0.054 ± 0.012	51	70	73
Caveira soils	**3**	0.19 ± 0.01	44.9 ± 3.3	15.1 ± 3.8	0.42 ± 0.01	61	97	62
	5	0.31 ± 0.02	10.6 ± 0.7	1.31 ± 0.05	0.78 ± 0.06	13	16	83
	7	0.10 ± 0.01	19.3 ± 1.2	10.8 ± 0.3	0.51 ± 0.04	31	31	99
	9	2.3 ± 0.2	32.6 ± 3.5	0.54 ± 0.18	1.9 ± 0.3	37	37	101
	11	0.12 ± 0.03	44.2 ± 2.1	7.5 ± 1.1	0.84 ± 0.08	53	60	88
	13	0.38 ± 0.03	72.0 ± 1.8	1.2 ± 0.1	2.9 ± 0.4	77	98	78
	14	0.0079 ± 0.0009	0.90 ± 0.06	0.17 ± 0.09	0.029 ± 0.009	1.1	1.1	101

Table 6. General characterization of soil samples.

Sample		pH	TotC %	OrgC %	Mn mg/kg d.w.	S total %	Fe total %	Al total %	Al_ox %	Fe_ox %	Sand* %	Silt* %	Clay* %
Estarreja	**1**	5.5	2.6	2.5	184	<0.05	2.0	1.2	0.21	0.38	18	70	12
	2	4.8	2.1	1.7	146	0.11	1.1	0.99	0.084	0.12	19	71	10
	4	4.9	2.8	2.2	185	<0.05	0.93	0.88	0.17	0.17	78	19	3.2
	6	5.4	2.6	2.4	172	<0.05	1.9	1.4	0.21	0.31	78	18	3.7
	8	5.4	4.1	2.1	203	<0.05	1.8	1.2	0.19	0.29	14	74	12
	10	4.9	2.8	1.9	201	<0.05	1.6	1.2	0.19	0.26	50	43	7.7
	12	5.9	2.2	1.9	72	<0.05	1.9	0.76	0.19	0.84	78	19	3.2
Caveira	**3**	5.3	3.8	3.8	402	0.42	6.6	0.8	0.082	1.1	50	40	10
	5	4	1.6	1.6	1790	<0.05	4.9	1.2	0.078	1.3	55	33	12
	7	4.2	2.5	2.2	425	0.08	2.2	0.7	0.057	0.46	57	35	8
	9	4.6	2	1.8	2439	0.07	6.7	1.2	0.066	1.2	54	36	10
	11	3.6	4.1	3.4	559	0.36	5.5	0.85	0.16	1.0	27	58	16
	13	4.2	5.1	4.3	459	0.24	5.2	0.74	0.15	1.1	21	62	17
	14	4.6	3.2	2.8	225	<0.05	4.2	0.93	0.086	0.068	25	61	14

***Sand** 0.050 < % < 2mm
***Silt** 0.002 < % < 0.050mm
***Clay** % < 0.002mm

4.3.2 Fractionation of mercury

The fractionation (Figure 11) revealed that in all samples mercury was mainly present in the semi-mobile phase (between 63 and 97%). The mobile fraction represented a much lower contribution to the total mercury content in both Caveira samples (between 0.29 and 2%), and Estarreja samples (median 1.3%). Two exceptions were observed, with samples 9 and 12 presenting an anomalous high percentage of mobile mercury (6.2% and 4.8%, respectively). The higher percentage of mobile mercury in these samples may be explained by the fact that these soils are used for agricultural purposes and are consequently subjected to human influence, including oxidation and the application of fertilizers. Also, characteristics of the soils may partially explain this occurrence, as will be clarified later.

Non-mobile mercury species were the second most abundant fraction present in Caveira soils, with percentages ranging between 1.3% and 35%. For Estarreja soils, however, mercury seems to be present in low contents both in mobile and non-mobile phase (less than 2% for both cases). These data could not be compared to others since there is no existing data about speciation of mercury in these areas.

Results from the fractionation of mercury in the soil samples can be seen in Table 5.

Recovery, defined as the sum of extracted mercury fractions divided by the independently determined total mercury concentration, ranged between 78 and 101 % and was considered satisfactory (Table 5). Recoveries higher than 100% can be explained by the heterogeneity associated with soils. Because mercury is not homogenously present in soil, it is likely than the aliquot taken for total mercury analysis does not have exactly the same mercury content as the one taken for mercury fractionation, despite the fact that each sample was thoroughly homogenised prior to analysis. Recoveries lower than 100% can result from losses of volatile mercury during the process. The same problem was observed by Kocman *et al.* (2004). Better recoveries were obtained for industrial soil samples, probably because of soil characteristics. Estarreja's soils are richer in sand particles and poorer in clay particles than Caveira's soil, which means that the extraction solutions can more easily access mercury in the first case.

As total mercury concentration of the fourteen samples ranged between 1.0 and 98 mg kg^{-1}, this method of fractionation proved to give good results both for high and low total mercury concentrations.

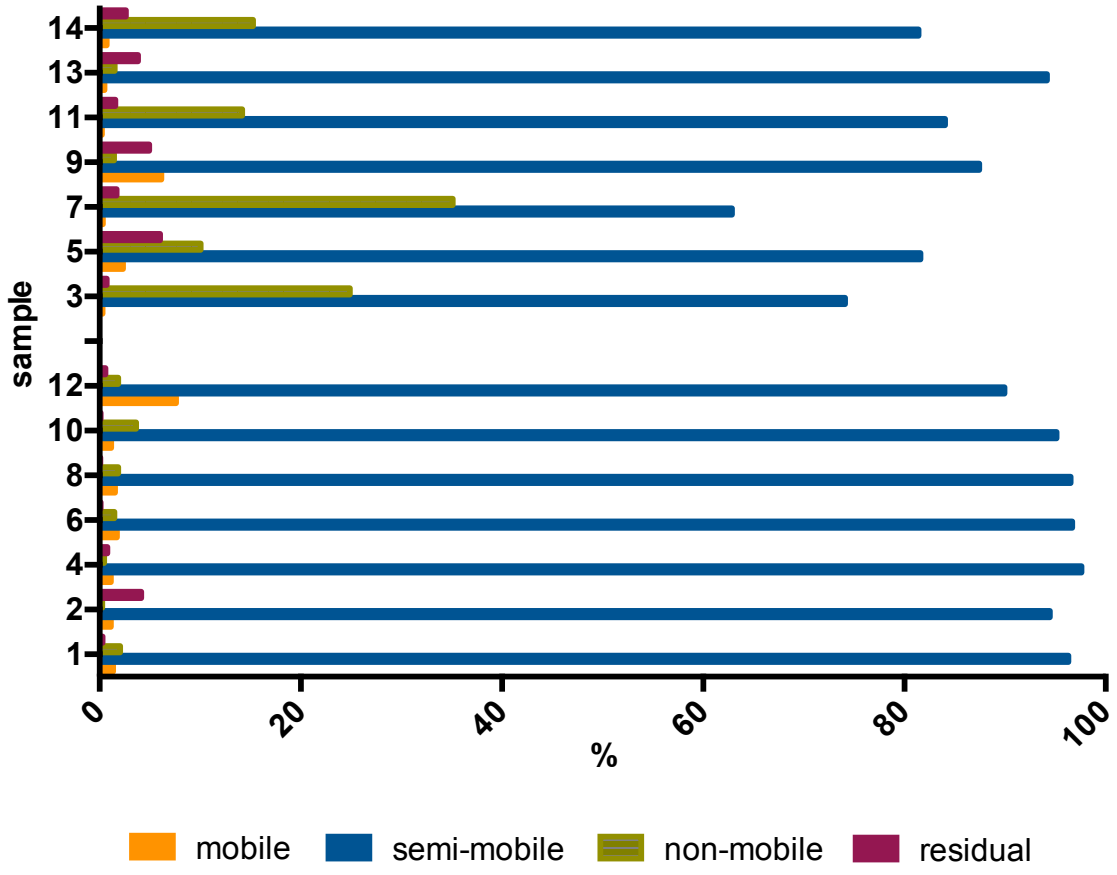

Figure 11. Distribution of mercury (percentage of mercury extracted) in Estarreja and Caveira soils, for mobile, semi-mobile, non-mobile and residual fractions.

4.3.3 Factor analysis

According to the criteria explained in the statistical analysis section, factor analysis was performed for each mercury fraction. Table 7 presents the loadings for all factors extracted, the respective communalities, and the variance explained by each factor as well as the cumulative variance. All communalities are elevated, demonstrating that the factors retained are fit to describe the correlational structure of the variables. The distribution of the samples according to the factor plots was examined for each fraction (Figure 12 to Figure 14).

Table 7. Rotated component matrix for soil data (n = 14). Total and cumulative percentage of variance explained and communalities are also presented.

Variable	Mobile fraction			Semi-mobile fraction				Non-mobile fraction			
	Factor 1	Factor 2	Communalities	Factor 1	Factor 2	Factor 3	Communalities	Factor 1	Factor 2	Factor 3	Communalities
Hg fraction*	0.068	0.84	0.71	-0.037	-0.42	0.82	0.85	0.068	0.21	0.88	0.82
pH	-0.72	0.15	0.53	-0.29	-0.68	0.27	0.61	-0.23	-0.71	-0.35	0.68
OrgC	0.43	-0,79	0.81	0.92	-0.08	-0.24	0.91	0.93	-0.08	0.23	0.92
Mn	0.71	0.67	0.96	-0.18	0.93	0.11	0.9	-0.15	0.92	-0.17	0.9
S	0.56	-0.66	0.75	0.8	0.11	-0.39	0.8	0.82	0.1	0.36	0.81
Fe	0.93	-0.029	0.87	0.53	0.72	-0.18	0.84	0.57	0.71	0.11	0.84
Al	-0.15	0.64	0.43	-0.34	0.19	0.82	0.82	-0.35	0.17	-0.78	0.75
clay	0.71	-0.34	0.62	0.73	0.46	0.115	0.75	0.69	0.47	-0.041	0.7
%variance explained	37	34 (71)		32	28 (60)	21 (81)		32	27 (59)	22 (81)	

For the mobile fraction, factor 1 explains 37% and factor 2 explains 34% of total variance. The mobile fraction has its highest loading on factor 2; the same factor also has high loadings for aluminium and manganese (positive) and organic carbon and sulfur content (negative). Factor 1 differentiates Caveira samples for their high content in manganese, iron and clay (Figure 12). Samples 5 and 9 are separated by factor 2, due to their high concentration of manganese, aluminium and particularly low concentration of organic carbon (Figure 12). As shown in this figure, factor 2, which includes the mobile fraction of mercury, did not separate samples by their different geographic origin. In contrast, factor 1 differentiates Caveira samples for their high content in manganese, iron and clay.

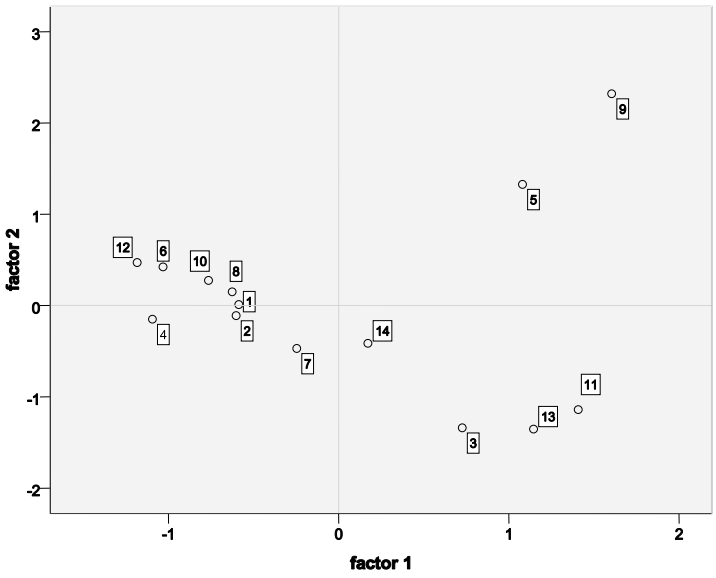

Figure 12. Factor scores for samples included in this study as obtained by factor analysis (rotated solution), considering mercury mobile fraction.

For the semi-mobile fraction, three factors were identified that, in total, explain 81% of variance (Table 7). The semi-mobile fraction has its highest loading on factor 3, as well as aluminium, indicating that the distribution of this variable is related with this particular fraction. As shown in Figure 13, samples 1, 6, 8, and 10 have the highest percentage of semi-mobile mercury and also of aluminium. This factor did not allow distinguishing Estarreja from Caveira samples (Figure 13). Both factor 1 (highest loadings of organic carbon, sulfur, and clay) and factor 2 (highest manganese and iron loadings) allowed to separate specific Caveira samples from

the dataset (factor 1: highest scores for samples 3, 11, 13; factor 2: highest scores for samples 5 and 9).

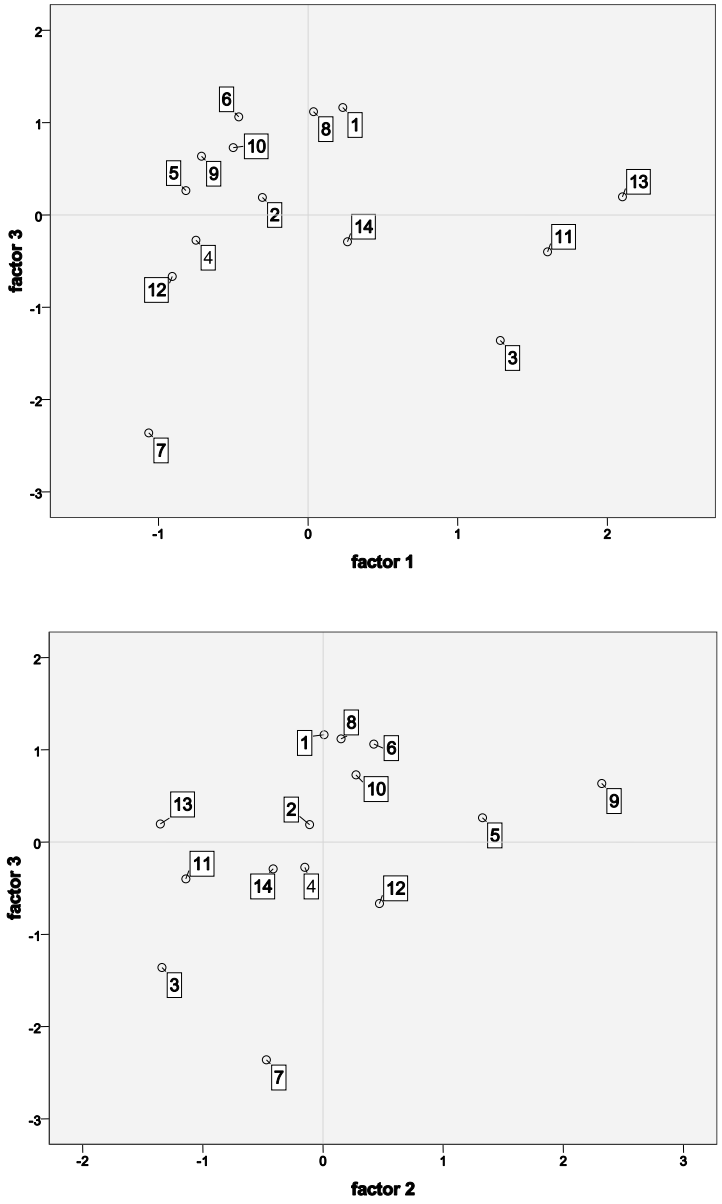

Figure 13. Factor scores for samples included in this study as obtained by factor analysis (rotated solution), considering mercury semi-mobile fraction.

Finally, factor analysis considering the non-mobile fraction allowed identifying three factors, with factor 3 exhibiting a 0.88 loading for the non-mobile fraction (Table 7). Aluminium has a strong, negative correlation with factor 3 (loading =.-0.78). pH also had a negative loading in factor 3 (Table 7). Although with low loadings values, a positive correlation between organic carbon and sulfur content and factor 3 was

observed (Table 7). Sample 7 has a high score in factor 3 and is clearly distinguishable from the rest (Figure 14), which relates to the presence of non-mobile species and a combination of relatively low pH and aluminium contents and medium organic carbon and sulfur levels.

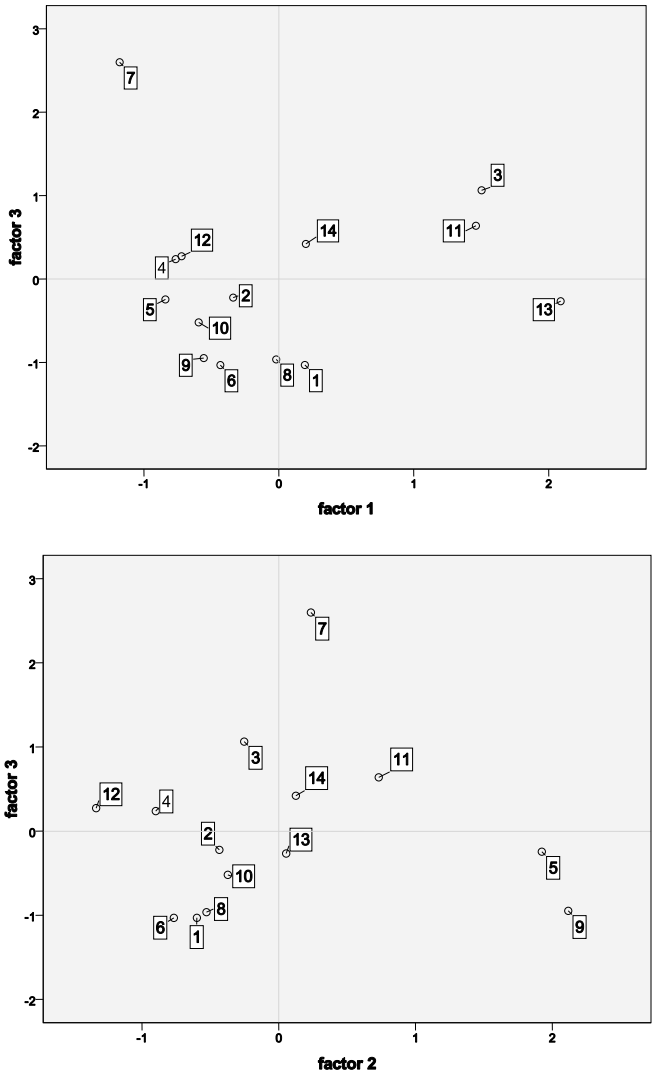

Figure 14. Factor scores for samples included in this study as obtained by factor analysis (rotated solution), considering mercury non-mobile fraction.

4.4 Discussion

Although the mercury fraction in the mobile phase generally did not exceed 2% of total mercury, given the high contamination of some samples this fraction may still represent significant amounts of bioavailable mercury. The importance of this fraction should not be underestimated, since it includes among others the alkyl species.

These mercury species are more mobile, more toxic and more readily bioaccumulated than any other mercury species (Han *et al.*, 2003). In the mobile fraction are also present soluble inorganic mercury species. These species, such as mercury chloride ($HgCl_2$) are more easily transported by natural processes than other inorganic mercury species and can also serve as substrates for mercury methylation (Bloom *et al.*, 1999; Han *et al.*, 2003). Combined, these extractable organomercury species and extractable soluble inorganic species contribute to the major portion of mercury potential toxicity in soils. Considering that the majority of these soils are predominantly used for agricultural and livestock purposes (Reis *et al.*, 2009), the presence of mobile and toxic mercury species, even in low concentrations, may be of concern.

Although the mobile mercury fraction (measured by HCl and ethanol extraction) is not entirely identical to *in-situ* soil pore water concentrations, it can be used as a first indicator for potential groundwater pollution or risk of metal leaching from soils. The Portuguese legislation defines a maximum admissible concentration of 0.0010 mg L^{-1} for mercury in groundwater to be used for drinking water supply (Decreto-Lei n. º 236, 1998). Thirteen of the fourteen samples analysed exhibited mobile mercury concentrations above this legal limit. The highest metal concentration observed in the liquid extracts reached 0.21 mg L^{-1} in Estarreja, and 0.087 mg L^{-1} in Caveira. The exceedance of the maximum admissible concentration in groundwater by mobile mercury contents may be an indication of environmental risk, confirming the need for a comprehensive assessment of the impacts of soil mercury contamination at these sites.

Despite the different characteristics of the soils from Estarreja and the soils from Caveira, when the mobile mercury fraction of both sets of samples was compared by means of the Mann-Whitney test, it proved that there was no difference between the two ($U=6.0$; $p=0.100$). This may be related to the fact that soil characteristics that were found to play most influence in the mobile fraction are similar for soils from both sampling sites.

The mercury species that fall into the **semi-mobile** category, such as elemental mercury, are less toxic than easily extractable mercury species (Han *et al.*, 2003). Such species include Hg^0 or amalgams of mercury with another metal, Hg^{2+} complexes, which can be also present in the mobile phase, and Hg_2Cl_2 to a small

extent (Table 4). Hg^0 is not the most toxic mercury species in soils, considering its low residence time in this compartment. Depending on the physico-chemical properties of the soil, vegetation and/or meteorological conditions (Gillis *et al.*, 2000; Zhang *et al.*, 2001), Hg^0 can be easily re-emitted to the atmosphere or oxidized to Hg^{2+}. In turn, inorganic mercury may be converted by microbial process to organic, methylated forms, such as methylmercury, raising the toxicity potential of the soil. Hg^{2+} can also complex with other ions present in soil, preferentially with OH^- and Cl^-, because of their abundance and stability. On the other hand, as mentioned previously, $HgCl_2$ and $Hg(OH)_2$ are more easily transported by natural processes than other inorganic mercury species and serve as the substrate for mercury methylation process. Therefore, although this fraction is not immediately available, its species can be easily converted into more readily available ones, as previously explained. The soils from Estarreja and Caveira presented different distribution of mercury in the semi-mobile phase (Mann-Whitney p=0.003), with soils of Estarreja showing higher concentration of semi-mobile mercury species. Considering that these soils are used for agricultural purposes, the presence of semi-mobile mercury species in significant concentrations can pose a risk of exposure.

The non-mobile fraction includes the less available and less toxic species of mercury, such as HgS, HgSe or Hg_2Cl_2 (Han *et al.*, 2003). The percentage of mercury in the **non-mobile** and **residual** fractions was different for mine and industrial soils, as confirmed by the Mann-Whitney test (p=0.018 for non-mobile fraction and p=0.018 for residual fraction), with mine soils exhibiting higher concentrations and higher variability in concentrations in both fractions.

In all samples, mercury was found within the residual fraction, despite the harsh extraction conditions applied to liberate previous fractions. This means that species present here are hardly available. Caveira soils have higher percentage of residual mercury species (median 2.6%) compared to industrial soils (median 0.29%). Considering that the percentage of non-mobile mercury is also higher in the first case, mine soils have elements that retain mercury tightly, so that it becomes less available, and, therefore, less dangerous.

4.4.1 Influence of soil properties on mercury fractions

Factor analysis suggested that specific soil properties play a relevant role in determining mercury mobility at both sampling areas. In general, aluminium and manganese contents have a positive influence on mercury mobility. The concentration of aluminium is particularly associated with the mercury semi-mobile fractions. In contrast, organic matter and sulfur contents contribute to mercury retention in the soil matrix and inhibit mercury mobility.

Several authors have regarded crystalline and amorphous aluminium as efficiently adsorbents for mercury in soils (He *et al.*, 2007; Kim *et al.*, 2004). As extractions were performed at low pH, the increasing acidity of the medium mobilized the aluminium ions and consequently mercury. This could explain the positive relation between mercury in mobile and semi-mobile fractions and the aluminium content.

The association of mercury mobility with the distribution of manganese can be explained by the fact that the presence of manganese oxides is known to significantly promote the solubility of HgS in an HCl solution (Fernández-Martínez *et al.*, 2005b). The influence of Mn on the mobility of mercury is evident, particularly in sample 9, which has one of the highest percentages of extracted mobile mercury and the highest content of manganese.

Organic carbon was one of the factors controlling mercury retention in soils. This was expected given the well know strong affinity of mercury to soil organic matter (Bloom *et al.*, 2003b).

Similarly, sulfur contributes to the retention of the metal in the non-mobile solid-phase. Cardoso Fonseca and Ferreira da Silva (2000) and Ferreira da Silva *et al.* (2005) reported the abundance of sulfides at the surface around the mine, explaining the occurrence of stable forms of mercury, such as cinnabar and other mercury sulfides, in the area of the Caveira mine. Therefore, the presence of mercury sulfides in soils from the area of Caveira possibly explains the inverse relationship between mobile mercury and sulfur percentages observed in this study, particularly given the low solubility of HgS in HCl (Fernández-Martínez *et al.*, 2005b).

Factor analysis did not clearly separate samples from Caveira and Estarreja, but did group some samples, according to their characteristics. Samples 5 and 9 (Caveira) are characterized by their high content in manganese and aluminium and

low organic carbon, which in turn favours mercury mobility. Samples 6, 10, 8, 1 (Estarreja) were characterised by higher semi-mobile mercury contents in association with higher aluminium levels. And finally, sample 7 (Caveira) was separated from the remaining samples due to conditions for higher retention of mercury in the solid-phase.

4.5 Conclusion

This study focused on the determination of the extractability of mercury in soils with different contamination sources and on the evaluation of the influence of specific soil properties on the behaviour of the contaminant. Results revealed that mercury was mainly present in the semi-mobile phase of soils from both locations. Analysis has also shown that the metal was more mobile in soils from the industrial sampling site than the mine area. The study conducted to evaluate the influence of soil properties in the distribution of mercury demonstrated that the presence of mercury in the mobile phase could be related to manganese and aluminium soil contents. A positive relation between mercury in the semi-mobile fraction and the aluminium content was also observed. In contrast, organic matter and sulfur contents contributed to mercury retention in the soil matrix reducing the mobility of the metal.

Despite known limitations of sequential extraction procedures, the methodology applied here for the fractionation of mercury in contaminated soil samples provided relevant information on mercury's relative mobility and it may be useful in the implementation of risk assessment methodologies in contaminated sites.

In relation to future assessments of risks to human health, crop quality and the environment it could be more useful to define a simple and robust approach that could give information on the distribution of mercury, considering not only its mobility, but also its reactivity and availability to plants and organisms.

Kinetic extractions

PART I: Extraction of mercury water-soluble fraction from soils: an optimisation study

PART II: Kinetic of desorption of mercury labile fractions from polluted soils

5 KINETIC EXTRACTIONS

I.5 Extraction of mercury water-soluble fraction from soils: an optimisation study

Highlights

- Procedure optimization for extraction of water-soluble mercury species from soils.
- Soil:water ratio did not influence results within the range of 1.5g:100mL to 20g:100mL.
- Kinetic study showed the extraction only reaches equilibrium at 24 hours.
- Laboratory procedure influences mercury quantification in the extracts.

Abstract

The procedure for extraction of water-soluble mercury species from soil was studied and optimised. Aspects studied included the soil:water ratio, time of extraction, separation technique (centrifugation vs. filtration) and analytical technique used to analyse the extract (pyrolysis-atomic absorption spectrometry vs. atomic fluorescence spectrometry). Results indicated that the process of extraction is not influenced by the soil:water ratio in the range studied (1.5:100 to 20:100). The kinetic study performed showed that it takes 24 hours for extraction to reach equilibrium, and that the mercury removal reaction takes place in two stages, a faster one ($0 < t < 6$ hours), followed by a slower stage ($t > 6$ hours). Hence, a two first-order reactions model was tested and proved to fit the experimental data. The particle size distribution seemed to have an influence on this process. Results also showed that filtration is preferable to centrifugation, as it avoids the presence of colloidal material in the leachate. Concerning the analytical technique used for quantification, atomic fluorescence spectrometry offers a lower limit of quantification; therefore it is more appropriate due to the low mercury concentrations often found in this fraction.

The conclusions of this study contribute to the refinement of an important step of sequential extraction procedures and soil toxicity assessment methods, and, ultimately, constitute a helpful tool for the prediction of long-term risks to the environment.

Keywords: water-soluble fraction; soil; mercury; extraction

I.5.1 Introduction

Measurement of the water-soluble fraction of mercury in soil is a particularly important tool for the assessment of the potential risk of groundwater contamination and the potential biological uptake and toxicity for aquatic organisms when leaching, runoff, and erosion occurs in polluted soils (Wahle *et al.*, 1997). In the literature there are several procedures reported for the extraction of mercury's water-soluble fraction, usually constituting the first step of a sequential extraction procedure. These procedures differ in soil:water ratio and/or time of extraction. Table 8 shows some examples of different water-soluble fraction extraction procedures used by different authors. Considering the environmental significance of this fraction, it is important that extractions are optimized to provide the most accurate estimation of the water-soluble mercury fraction and, hence, the most appropriate interpretation of the behaviour of water-soluble mercury species in soil. The optimization of the extraction procedure for estimation of water-soluble mercury species in soil may aid in providing an indication of the maximum potential metal extractability in water drainage and runoff, a helpful tool for the prediction of long-term risks to the environment. Therefore, in this work, experiments were conducted to establish optimal procedural conditions for extraction of the water-soluble fraction of mercury in soils. Parameters such as the soil:water ratio and the time of extraction were studied. The kinetic aspect is crucial to correctly predict the behaviour of the metal in soil, and although the study of the kinetic behaviour has been evaluated for other elements (Fangueiro *et al.*, 2002; 2005; Manouchehri *et al.*, 2006), it was only applied to mercury by Issaro *et al*. (2010), using sodium-thiosulfate as extractant. The influence of the separation technique (filtration vs. centrifugation) and the quantification methodology chosen to perform analysis (atomic fluorescence spectroscopy vs. direct mercury analyser) were also considered. This way, this study intends to contribute to the refinement of a crucial step of mercury sequential extraction procedures and soil toxicity assessment methods and, ultimately, improve the characterization of risk for terrestrial and aquatic systems, providing useful information to decision makers in terms of focusing site cleanup and remedial efforts.

Table 8. Soil:water ratio, time of extraction and percentage of mercury extracted in different extraction procedures for the water-soluble fraction found in literature. Procedures considered in this study (P1, P2, P3 and P4) are shown in bold.

Author(s)	Soil(g):water (mL) ratio	Time of extraction	Hg extracted (%)
(P1) Panyametheekul, 2004	**3:100**	**60 min**	**0%**
(P2) Renneberg and Dudas, 2001	**1.5:100**	**30 min**	**< 10%**
(P3) Biester and Scholz, 1997	**20:100**	**60 min**	**Chlor-alkali plant soil – 0.15%** **Mine soil (Idrija, Slovenia) – 0.12%**
(P4) Bloom et al., 2003	**1:100**	**18±4 hours**	**0.4 – 1.3%**
Bloom and Katon, 2000	1:100	18±3 hours	Gold mine tailings - 1.3% HgS mine soil – 0.01% Chlor-alkali plant soil – 0.18%
Neculita et al., 2005	10:100	2 hours	< 1.1%
Boszke et al, 2006	17:100	3 hours	1.00%

I.5.2 Materials and methods

I.5.2.1 Sampling and methodologies

Three soil samples from the industrial area of Estarreja (Industrial 1, Industrial 2, and Industrial 3) and three soil samples from the Caveira mine area (Mine 4, Mine 5, and Mine 6) were used in this study. More specifically, Mine 4 was collected from a tailing deposit, while samples Mine 5 and Mine 6 were collected at an agricultural field located approximately 1.7 km from the mine pit. A seventh sample collected at a non-contaminated area (Gandra 7) was used as reference site. The description of these locations is given in section 3.1.

Soil samples were analysed for the following parameters, according to the methodologies presented in sections 3.2and 3.3:

- Total mercury content;
- pH;
- Particle size distribution.

Quality control and quality assurance procedures applied in this work have already been described in section 3.5. Because certified reference materials are not available for mercury speciation, it was not possible to check the accuracy of the extraction. The relative standard deviation (RSD) among replicates varied between 0.28% and 5.6% (n = 4).

I.5.2.2 Extraction of water-soluble fraction from soils

Four water-soluble fraction extraction procedures were considered in this work: those of Panyametheekul *et al*. (2004) (procedure 1 – P1); Renneberg and Dudas (2001) (procedure 2 - P2); Biester and Scholz (1997b) (procedure 3 - P3); and Bloom *et al*. (2003a) (procedure 4 - P4). The operational conditions associated with each extraction procedure are presented in Table 8, in bold. These procedures were chosen based on their differences in soil:water ratio and time of extraction. Procedures P1 and P3 have the same time of extraction, albeit very different soil:water ratio, therefore allowing studying the effect of time of extraction. Procedure P4 has a longer extraction time. After shaking, the samples were centrifuged (3000 rpm) and the supernatant was acidified with concentrated HNO_3 and stored at 4°C until analysis. In all extractions distilled water (conductivity = 2 µS cm^{-1}) was used. Extractions were performed in triplicate for each sample.

I.5.2.3 Kinetic study

Two samples (Industrial 3 and Mine 6) were chosen to perform a kinetic study. The kinetic experiment was performed in duplicate for each sample, using a 1.5 g:100 mL soil:water ratio, as it is advisable to keep the soil:water ratio as low as possible (Issaro *et al.*, 2010) (see Results and Discussion). The mixtures (30 g of sample in 2000 mL of distilled water) were shaken, using an end-over-end shaker at a constant rate of 60 rpm. 50 mL of sample were removed for analysis at t = 0, 0.5, 1, 6, 18, 24, 48, and 72 hours, using a syringe. This step was performed as quickly as possible, before any settling of soil particles occurred, in order to ensure that a homogenous aliquot was removed and the soil:water ratio was maintained in the remaining suspension. Removed aliquots were filtered through a 0.45 µm filter with cellulose type membranes (Millipore®, USA), acidified with concentrated HNO_3 and stored at 4 °C until analysis (performed within 48 hours).

Mercury content in the extracts was measured by atomic fluorescence spectroscopy and also using the direct mercury analyser.

I.5.2.4 Data analysis

Statistical analysis was performed using SigmaPlot 11 and SPSS Statistics 17.0. Procedures and samples were compared by means of a two-way ANOVA. A multiple comparison procedure (Holm-Sidak method) was used to isolate the group or groups that differ from the others when statistically significant difference was identified in ANOVA analysis.

Kinetic data was modelled by nonlinear regression analysis, using GraphPad Prism 5 (trial version) that uses the least-squares fitting method and the method of Marquardt and Levenberg for adjusting the variables; this method blends the method of linear descent and the method of Gauss-Newton. Kinetic parameters, such as the quantity of mercury removed from soil, and the associated rate constants (k_1 and k_2) were determined for the two first-order reaction model. In order to assess the goodness of the fit to the experimental data, the coefficient of determination (R^2) and the standard deviation of residues ($S_{x/y}$) were analysed.

I.5.3 Results and discussion

I.5.3.1 Soil samples characteristics

All soils analysed had an acidic pH, with mean values of 5.1±0.4 for Estarreja soils and 4.6±0.7 for Caveira soils. The Caveira area is known to be affected by acid mine drainage (Cardoso Fonseca *et al.*, 2000) which may explain the lower pH. Percentages of total and organic carbon in Estarreja were 2.8±0.6 and 2.4±0.5%, respectively; in Caveira, the percentages were 2.5±1.5 and 2.4±1.2%.

Variable soil textures were obtained for these soils: loamy sand, sandy loam, loam and silt loam, with clay percentages between 8.3 and 13.6%. Soils from Caveira showed higher sand contents than soils from Estarreja. Mean sand contents obtained for Estarreja and Caveira were 11.8±1.5% and 52.9±2.6%, respectively.

Total mercury concentration in these soils was between 14 and 26 mg kg^{-1} in Estarreja and 16 and 97 mg kg^{-1} in Caveira.

I.5.3.2 Infuence of water:soil ratio and time on mercury extraction

Results for water-soluble mercury are expressed as absolute values and as percentages of total mercury contents in Figure 15, for each procedure. The water-soluble fraction was generally small - at most 0.49 mg kg^{-1}, for Mine 4, using Procedure 4 – and did not exceed 2% of the total mercury content, except in the control soil, Gandra 7, which has the lowest absolute mercury concentration. Mining soils showed slightly higher absolute concentrations of mercury than industrial soils; however when results were expressed as percentage of extracted mercury, this difference disappears.

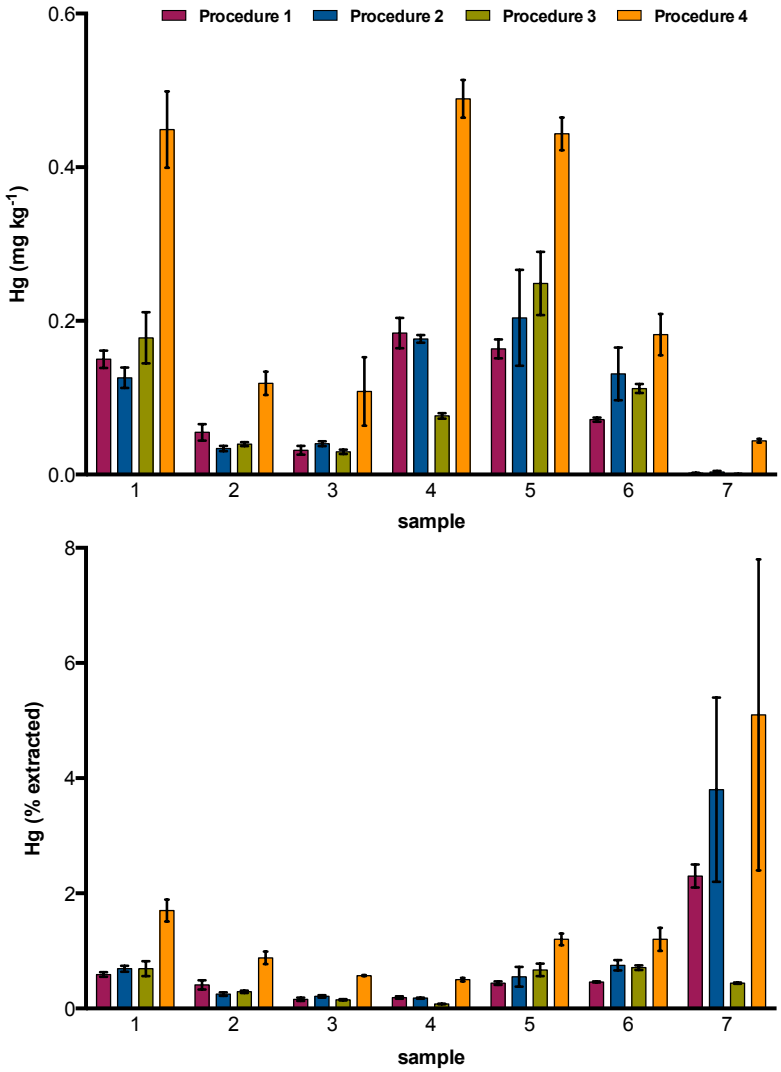

Figure 15. Absolute water-soluble mercury concentrations (mg kg^{-1}, above) and percentage of mercury extracted with water (below) when applying the four tested procedures.

Kruskal-Wallis One Way Analysis of Variance on Ranks revealed that Procedure 4 is statistically different from the other procedures for all soil samples ($p < 0.05$). This difference may be a consequence of the lengthier shaking period. Procedures 1 and 3 have the same extraction period (60 minutes), but different soil:water ratio (3g:100mL; 20g:100mL, respectively). No statistical differences ($p=0.866$) were found between these procedures. These results indicate that the period of extraction could have more influence in the extraction process than the soil:water ratio. Also, a low soil:water ratio ensures complete leaching of mercury species, as all the soil has contact with water.

The pH, organic matter and particle size distribution are among the most common factors that can affect metal extraction from the soil matrix (Gabriel *et al.*, 2004). For each procedure, the influence of these factors in mercury removal from the soil samples was considered but no significant correlations were observed.

I.5.3.3 Kinetic study

Kinetic extraction curves for samples Industrial 3 and Mine 6 are shown in Figure 16, where mercury extracted per unit weight of soil (mg kg^{-1}) is represented as a function of extraction time (hours). In both studied samples, a stationary state was reached at t=24 hours, which can be considered as an equilibrium state. The fact that the maximum mercury concentration in the water extract was only reached at 24 hours suggests that a longer shaking period than the one described in any procedure found in the literature may be need to fully evaluate the water-soluble fraction. Detailed observation of Figure 16 also reveals that the curves for the two samples are similar in shape and that two regions can be recognized in each curve: the first corresponds to short extraction times ($t < 6$ hours), with rapid release of metal, and the second to longer extraction times ($6 < t < 72$ hours), where the extraction kinetic is slower. This sort of extraction trend, with two well-defined extraction stages seems to be common (Fangueiro *et al.*, 2002; 2005; Manouchehri *et al.*, 2006), and models of multiple first-order reactions are frequently used to fit the experimental data.

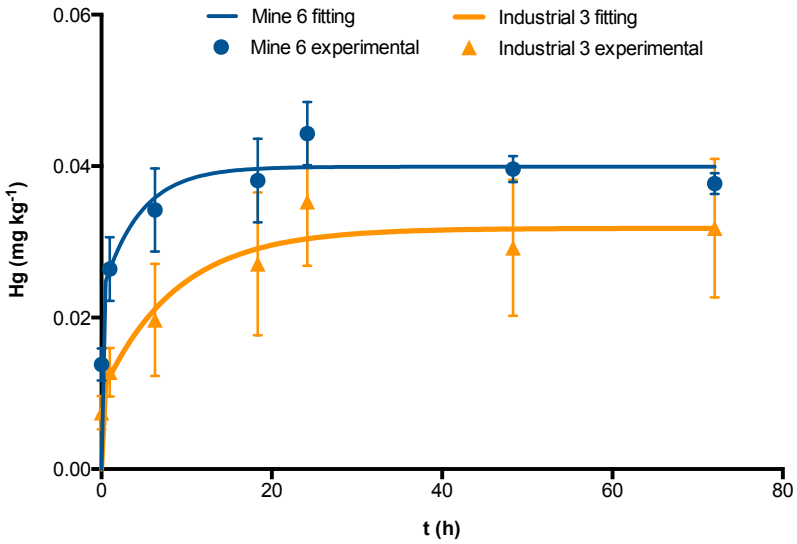

Figure 16. Kinetics of soil water-soluble fraction extraction and representative curves calculated from the two first-order reactions model for samples Industrial 3 and Mine 6 (exp: experimental; fit: fitted).

Usually the following two first-order reaction model is applied to describe this kinetic process.

Equation 4 $$\mathbf{C(t) = C_1\left(1 - \exp(-k_1 t)\right) + C_2\left(1 - \exp(-k_2 t)\right)},$$

where C_1 and C_2 (mg kg^{-1}) are mercury concentration extracted in the first and second stages, respectively, and k_1 and k_2 are the apparent rate constants. Fitting of the experimental data allowed determination of the kinetic parameters presented in Table 9 for the two samples. As the correlation coefficient was > 0.98 and the residual standard deviation was low, for both samples, the model was considered to fit the experimental data and satisfactory to explain mercury extraction from soil to water.

From a kinetic point of view, the two first-order reaction model reveals that some water-soluble mercury species are extracted more quickly than others, suggesting that they can be bound differently to the matrix. In both cases, k_1 was larger than k_2, confirming the two different kinetic stages and the fast removal rate during the first hours (though it should be mentioned that the standard error associated with the estimated rate constants is relatively high – Table 9). A possible explanation for the obtained results is that a diffusion process, such as intra-particle diffusion, may be controlling the release of the water-soluble mercury species from

soil and, if this is the case, soils with different porosity will exhibit different extraction rates. It is known that soil porosity typically decreases as particle size increases, since coarser surface soils are dominated by larger but fewer pores than finer textured surface soils which have an abundance of very small pores that give them higher total porosity and increased adhesion and resistance to compaction. According to this, water-soluble mercury species in clay soil will be extracted preferably in the second stage, since the extraction rate will be controlled by intra-particle diffusion, while in sandy soils, the water-soluble mercury species will be extracted mainly in the first stage. This statement is corroborated by the modelled results obtained with soils Industrial 3 and Mine 6 (Table 9). According to the results obtained with the two first-order reaction model, the relation between C_1 and C_2 differs in the two samples. For sample Industrial 3, a soil from Estarreja and with a lower sand content, the quantity of mercury removed in the first stage (C_1 represents ca. 31% of total mercury removed) is less than that extracted in the second stage (C_2). Sample Mine 6, a soil from Caveira with higher sand content, has an opposite behaviour with $C_1 > C_2$. For this sample, ca. 58% of the water-soluble mercury species are extracted in the first fraction (C_1). Therefore, the water-soluble mercury species in sample Mine 6 are more easily extracted than in sample Industrial 3 probably due to the texture of each soil. The results highlight the importance of increasing the extraction time to 24 hours; otherwise, an important mercury water-soluble fraction may not be extracted, depending on the soil type.

Table 9. Kinetic parameters of soils samples Industrial 3 and Mine 6 (mean±standard error).

Sample	Parameter	
Soil 3	C_1^0 (mg kg^{-1})	0.0099 ± 0.0038
	C_2^0 (mg kg^{-1})	0.022 ± 0.0039
	k_1	497 ± 596
	k_2	0.114 ± 0.053
	R^2	0.98
	$S_{x/y}$	0.003
Soil 6	C_1^0 (mg kg^{-1})	0.023 ± 0.003
	C_2^0 (mg kg^{-1})	0.017 ± 0.003
	k_1	326 ± 110
	k_2	0.226 ± 0.104
	R^2	0.99
	$S_{x/y}$	0.0024

C_1; C_2: mercury concentration extracted in the first and second stages, respectively
k_1; k_2 apparent rate constants of the first and second stages, respectively
R^2: coefficient of determination
$S_{x/y}$: standard deviation of residues

I.5.3.4 Laboratory methodological procedures that may influence the quantification of water-soluble mercury species in soils

The separation of the water-extract from the soil residue can be an important aspect in the laboratory methodology. Therefore, two methods of separation, centrifugation and filtration, were compared for soil samples Industrial 3 and Mine 6, at a soil:water ratio of 1.5 g:100 mL and extraction times of t=1 hour and t=18 hours. In sample Industrial 3, mercury concentrations of $3.2x10^{-2}$ and $1.1x10^{-1}$ mg kg^{-1}, after centrifugation, and $7.5x10^{-3}$ and $2.7x10^{-2}$ mg kg^{-1}, after filtration, were obtained at t=1 hour and t=18 hours, respectively. For Mine 6, at t=1 hour, mean mercury concentration was $7.2x10^{-2}$ and $2.6x10^{-2}$ mg kg^{-1}, while at t=18 hours, concentrations were $1.8x10^{-1}$ and $3.8x10^{-2}$ mg kg^{-1} for centrifugation and filtration, respectively. Therefore, mercury content in solution was higher when centrifugation was used, which may result from the inadequate removal of colloidal materials from soil suspension. As colloids in soils are known as potential carriers for trace metals (Zirkler *et al.*, 2012), their presence may enhance the measured mercury concentrations in the leachate analysis. Retaining the colloidal parts, hence avoiding their presence in the water-extract, through filtration may overcome this problem.

The influence of the quantification methodology used to perform mercury quantification was another studied aspect. Mercury in water-extracts of samples Industrial 3 and Mine 6 was quantified both by AFS and AAS (using a direct mercury analyser). Evidence of statistical significant differences was found between the two analytical methodologies (two-tailed t-test: t=-3.95; p=0.008), indicating an influence of the methodology of mercury quantification on the amount measured. As can be seen in Figure 17, where sample Industrial 3 is presented, the mercury content in water-extracts determined by CV-AFS is usually lower. A reasonable explanation is that while in pyrolysis-AAS all mercury forms present are quantified, including any soil colloids smaller than the filter pore (< 0.45 μm), when using CV-AFS only Hg^{2+} present in the solution (after irradiation) is quantified. Additionally, the higher limit of quantification of direct mercury analysers may constitute a problem when dealing with extracts low in mercury content, such as the ones coming from mercury water-soluble extractions. Hence, CV-AFS analysis may be the most adequate methodology for mercury quantification.

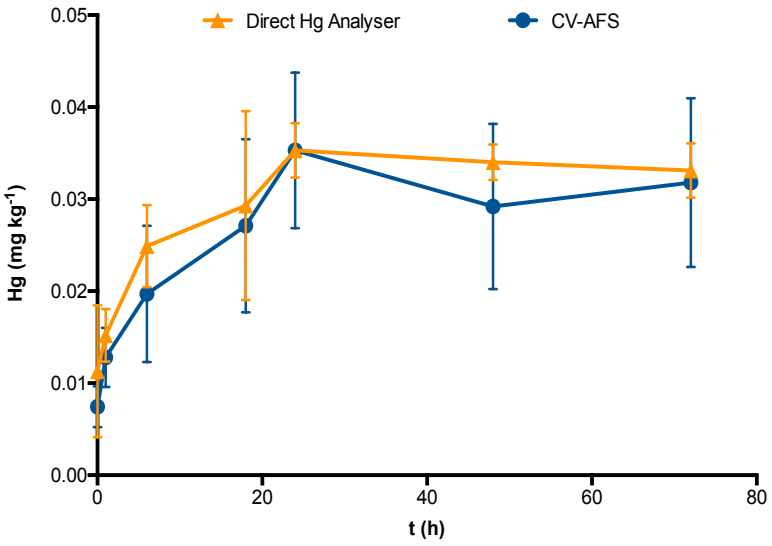

Figure 17. Mean mercury concentration±standard deviation (mg kg^{-1}) determined by atomic fluorescence spectroscopy and direct mercury analyser, in soil sample Industrial 3.

I.5.4 Conclusion

This study yielded a deeper knowledge of the extraction of mercury water-soluble fraction from soil. The results allow the following conclusions to be drawn: a) the soil:water ratio does not have a major influence on the extraction procedure, although it is advisable to keep the ratio as low as possible to guarantee that all soil sample has contact with water; b) the extraction time should be longer than the ones described by several researchers, as maximum mercury release was achieved at 24 hours; c) filtration is a superior separation technique to centrifugation, as it avoids the presence of colloidal material in the leachate; d) the analytical technique used to quantify mercury also influences the results, and atomic fluorescence spectroscopy may be the better choice.

A two first-order reactions model efficiently fitted the kinetic data obtained. Also the kinetic study indicated that there are two stages in the removal of water-soluble mercury from soils and the soil particle size distribution seemed to have an influence on this process.

II.5 Desorption kinetics of mercury labile fractions from contaminated soils

Highlights

- Effects of different reagents and soil:extractant ratios on the extraction of labile species were investigated and discussed.
- Kinetic models fitting the experimental data were proposed, which provide a good prediction for metal extraction from soil.
- The mercury extraction from soil is controlled by ion-exchange and diffusion processes.

Abstract

Kinetic studies are becoming more popular in fractionation of metals in soils. In this study the suitability of 1 mol L^{-1} ammonium acetate (NH_4Ac) pH 7, 0.1 mol L^{-1} and 0.5 mol L^{-1} hydrochloric acid (HCl) as reagents for extraction of labile mercury fractions from anthropogenic and geogenic contaminated soils was investigated. No statistical differences were found between 1 mol L^{-1} NH_4Ac and 0.1 mol L^{-1} HCl, but 0.5 mol L^{-1} HCl removed a higher percentage of mercury.

The soil:extractant ratio was also considered – 1.5g:100mL, 10g:100mL, and 20g:100mL. A higher percentage of mercury is extracted at lower ratios. In all cases, the rate of desorption was faster in the first 10 hours and declined after that period. Therefore, three fractions are obtained: labile, slowly labile, and un-extractable. The two first-order reactions and the diffusion models were used to fit the experimental data. Both fitted the dataset and allowed determining that diffusion of mercury is the rate limiting step. The Elovich equation fitted well the extraction data but does not present any physico-chemical meaning.

pH and particle size play an important role in the mercury desorption process from soil, as results suggested that acidic soil pH might reduce the ability of the soil to strongly retain metals. The particle size impacts the soil porosity and soils with higher porosity have lower rates of desorption.

Keywords: soil, mercury, kinetic fractionation, labile fraction, ammonium acetate, hydrochloric acid

II.5.1 Introduction

A common step in all extraction procedures for soil targets the so-called exchangeable fraction, which is the more mobile and bioavailable fraction of metal in soil. A large number of extractants have been used to assess plant available trace elements, including: i) chelating solutions, such as EDTA (Fangueiro *et al.*, 2005); ii) salt solutions such as NH_4Ac, $MgCl_2$, or $CaCl_2$, due to their capacity to release Mercury by ion-exchange (Gismera *et al.*, 2004; Han *et al.*, 2006); or iii) dilute solution of acid, for example HCl (Kashem *et al.*, 2007). Amongst these, the neutral (pH 7.00) 1.0 mol L^{-1} ammonium acetate (NH_4Ac) extraction is one of the most widely applied reagents for leaching the exchangeable fraction (Jing *et al.*, 2008). Additionally, due to its strong complexing power, acetate should prevent the re-adsorption or precipitation of the released metal ions (Filgueiras *et al.*, 2002). The bioavailable fraction of the metal in soil has also been estimated by the use of 0.1 mol L^{-1} HCl (Kashem *et al.*, 2007).

A simpler approach is to determine in one step the labile fraction of the metal in soil, by application of a single extraction procedure. This should include the more available species, such as water-soluble, exchangeable, and carbonate associated. Though this single extraction does not provided exactly the same geochemical information as sequential extraction does, it provides enough information about the more toxic and available species present in the soil, while it has the advantages of being faster, cost-effective, and require less technical skill and reagents (Sutherland *et al.*, 2008). Of the numerous reagents that can be used for extraction of the labile fraction, diluted HCl has been the most commonly applied (Andrews *et al.*, 2004; Snape *et al.*, 2004; Sutherland, 2002; Sutherland *et al.*, 2008).

In either case, these extractions should be optimized in order to better reflect the reactions taking place in the environment and recover the entire target fraction. One of the major problems of chemical extractions is the variety of procedures available in literature. Besides the already discussed variety of extractants, other operational conditions change as well, namely the time of extraction and the soil:extractant ratio. Studying the rate and extent of metal desorption from the matrix is important as on it depends the fate, transport, and bioavailability of metals in soils. Therefore, in this study we focused on establishing optimal procedural conditions for extraction of exchangeable and labile fractions of mercury in soils, considering

different soil:extractant ratios and the kinetics of desorption of mercury from contaminated soils. The kinetics involved in the mercury desorption from soil have occasionally been pondered (Issaro *et al.*, 2010; Reis *et al.*, 2014), but there is still shortage of information needed to help harmonize sequential extraction procedures. This way, this study intends to contribute for the understanding of mercury behaviour in soil, and optimize crucial steps of mercury extraction procedures and soil toxicity assessment methods.

Usually, metals of anthropogenic inputs tend to be in the first fractions of sequential extractions (exchangeable, carbonate bound, Fe and Mn oxide bound, organic matter bound) and therefore are more labile, while metals found in the residual fraction are of geogenic occurrence (Ratuzny *et al.*, 2009). For this work, soils from two contaminated areas were chosen - Estarreja (North-East Portugal) and Caveira (South-East Portugal). In both cases, soil contamination results from anthropogenic activities, but mercury in Estarreja soils results from the effluents of mercury-cells of a chlor-alkali plant (Reis *et al.*, 2009), while the latter is a mine area, situated in the Iberian Pyrite Belt (Barriga, 1990), and therefore of natural origin.

II.5.2 Sampling sites and methodology

One sample was collected in an agricultural field close to a former effluent stream of the Industrial Complex of Estarreja (Ullrich *et al.*, 2007). One Caveira mine sample was collected in at an agricultural field located near the mine pit. The description of these locations is given in section 3.1.

Soil samples were analysed for the following parameters, according to the methodologies presented in sections 3.2 and 3.3:

- Total mercury content;
- pH;
- Total carbon (TotC) and organic carbon (OrgC);
- Particle size distribution.

Quality control and quality assurance procedures applied in this work have already been described in section 3.5.

II.5.3 Extraction procedure

For the kinetic experiments, the effect of leaching time on extracted metal was evaluated The following reagents were studied as extractants: 1.0 mol L^{-1} NH_4Ac (pH 7.0), 0.1 mol L^{-1} HCl, and 0.5 mol L^{-1} HCl. NH_4Ac at pH 7.0 and 0.1 mol L^{-1} HCl were investigated to study the exchangeable and bioavailable fraction of mercury in soil. 0.5 mol L^{-1} HCl was employed to assess the labile fraction of mercury. For the three extractants, soil:extractant ratios considered were 1.5 g:100 mL, 10 g:100 mL and 20 g:100mL. As soils are very heterogeneous media, samples were thoroughly homogenized prior to weighting. The mixtures (12 g, 80 g, and 160 g of sample in 800 mL of extractant) were shaken at room temperature (23 ± 5 °C), using an end-over-end shaker at a constant rate of 60 rpm. 8 mL of sample were removed for analysis, using a syringe, at t = 30 seconds, 15 minutes, 30 minutes, 1, 2, 4, 6, 18, and 24 hours, and then every 24 hours until equilibrium. This step was performed as quickly as possible, before any settling of soil particles occurred, in order to ensure that a homogenous aliquot was removed and that the soil:extractant ratio was preserved in the remaining suspension. Removed aliquots were immediately filtered through a 0.45 µm filter with cellulose type membranes (Millipore®, USA) and stored at 4°C until analysis (performed within 24 hours).

Possible variations in the pH could affect the extraction process; therefore, the pH of the suspension was controlled during the experiment, after different extraction periods.

Note: Water-soluble fraction is negligible is these soils (Reis *et al.*, 2014) and its extraction was not performed in order to reduce to minimum other sources of error; extractants may alter the surface chemical characteristics of the soil, resulting in more exposed reactive surfaces that, in turn, may potentiate metal sorption and redistribution among the remaining fractions during the extraction process.

II.5.4 Kinetic data fitting

In order to perform kinetic fitting, the results were expressed as mercury extracted per unit of soil (mg kg^{-1}) between extraction initiation time (t_0) and t_i, and as a function of the volume of extractant solution (V) and sample mass (m).

Equation 5 $Hg_{t_0<t<t_i} = [Hg_{t_i} - Hg_{t_0}] \times \frac{V}{m}$

The removal rate per unit of time (mg kg^{-1} h^{-1}), between extraction initiation time (t_0) and t_i was determined as:

Equation 6 $Removed\ Hg_{t_0<t<t_i} = \frac{Hg_{t_i} - Hg_{t_0}}{t_i - t_0}$

The data obtained for mercury extracted per unit of soil was modeled by nonlinear regression analysis, using GraphPad Prism 5 (trial version) that uses the least-squares fitting method and the method of Marquardt and Levenberg for adjusting the variables; this method blends the method of linear descent and the method of Gauss–Newton.

The most common models and fitting equations were used to fit the extraction rate data: the two first-order reactions model, the diffusion model and the Elovich equation. Each of the kinetic models was tested for data fitting. In order to assess the goodness of the fit to the experimental data the coefficient of determination (R^2) and the standard deviation of residues ($S_{x/y}$) were determined. A relatively high R^2 and low value of $S_{x/y}$ were used as criteria for best fit. For each case, the fitting was tested using the mean of the whole set of extraction data.

II.5.4.1 Two first-order reactions model

This model has been regarded as the most appropriate model to explain the kinetics involved in metal fractionation in the solid fraction. It advocates that desorption of the metal from soil takes place in multiple steps (first-order reactions) and that reaction rates are independent from each other. This implies that metals are bound to distinct sites available in soil, resulting in a readily extractable (C_1) and a less extractable (C_2) metal fractions. In addition, the total non extractable metal fraction (C_3) can be estimated through the difference between total mercury and C_1+C_2. The two first-order reactions model is described as:

Equation 7 $C = C_1(1 - e^{k_1 t}) + C_2(1 - e^{k_2 t})$,

Where C_1 and C_2 (mg kg^{-1}) are mercury concentration extracted in the first and second stages, respectively, and k_1 and k_2 are the associated apparent rate constants.

II.5.4.2 Diffusion model

The diffusion model assumes that the desorption of metals from the solid matrix is initially fast but the rate is limited by the diffusion from the mineral lattice or the intra-particle diffusion from pores of inner soil surfaces (Gismera *et al.*, 2004). According to Gismera *et al*. (Gismera *et al.*, 2004), the metal desorption rate of a solid fraction due to diffusion-controlled kinetics may be described as:

Equation 8 $\frac{\partial C}{\partial t} = \frac{kDS\,(C_{eq} - C)}{V\delta}$,

where C is the removed metal concentration; C_{eq} is the metal concentration at the equilibrium; t is the time; D is the diffusion coefficient; S is the surface area of the solid particle, V is the solution volume; δ is the thickness of the diffusion layer around the particle; and k is a constant of proportionality. Including the parameters D, S, δ and V in the constant k and rearranging and solving Equation 8, we obtain a first-order equation:

Equation 9 $C = C_{eq} \times (1 - e^{-kt})$

II.5.4.3 Elovich equation

The Elovich equation is generally used to describe adsorption and desorption mechanism in nature, and is particularly valid for heterogeneous systems. The following integrated form of the Elovich equation was used:

Equation 10 $C = \frac{1}{b}\ln(1 + abt)$,

where C is the amount of mercury desorbed per kg of soil at time t, and a and b are constants during the experiment, frequently used to estimate the reaction rates (a decrease in b and/or an increase in a would increase the reaction rate).

II.5.5 Results and discussion

II.5.5.4 Soil samples characteristics

Estarreja sample has a total mercury content of 70.0 mg kg^{-1}, and is characterised by being loamy sand soil (sand 78.1 %; silt 18.8 %; clay 3.15 %), with a pH of 6.0 and a percentage of organic carbon of 1.9 %.

Caveira sample has lower total mercury content and pH of 6.3 mg kg^{-1} and 3.3, respectively. Organic carbon constitutes 3.5%, and the soil is classified as silt loam (sand 27.0 %; silt 57.5 %; clay 15.5 %).

II.5.5.5 Mercury desorption from soil

The results of mercury removal per unit of time are depicted in Figure 18, while Table 10 presents the mercury removed per kg of soil, and percentage of desorbed mercury (percentage of mercury released in comparison with total mercury).

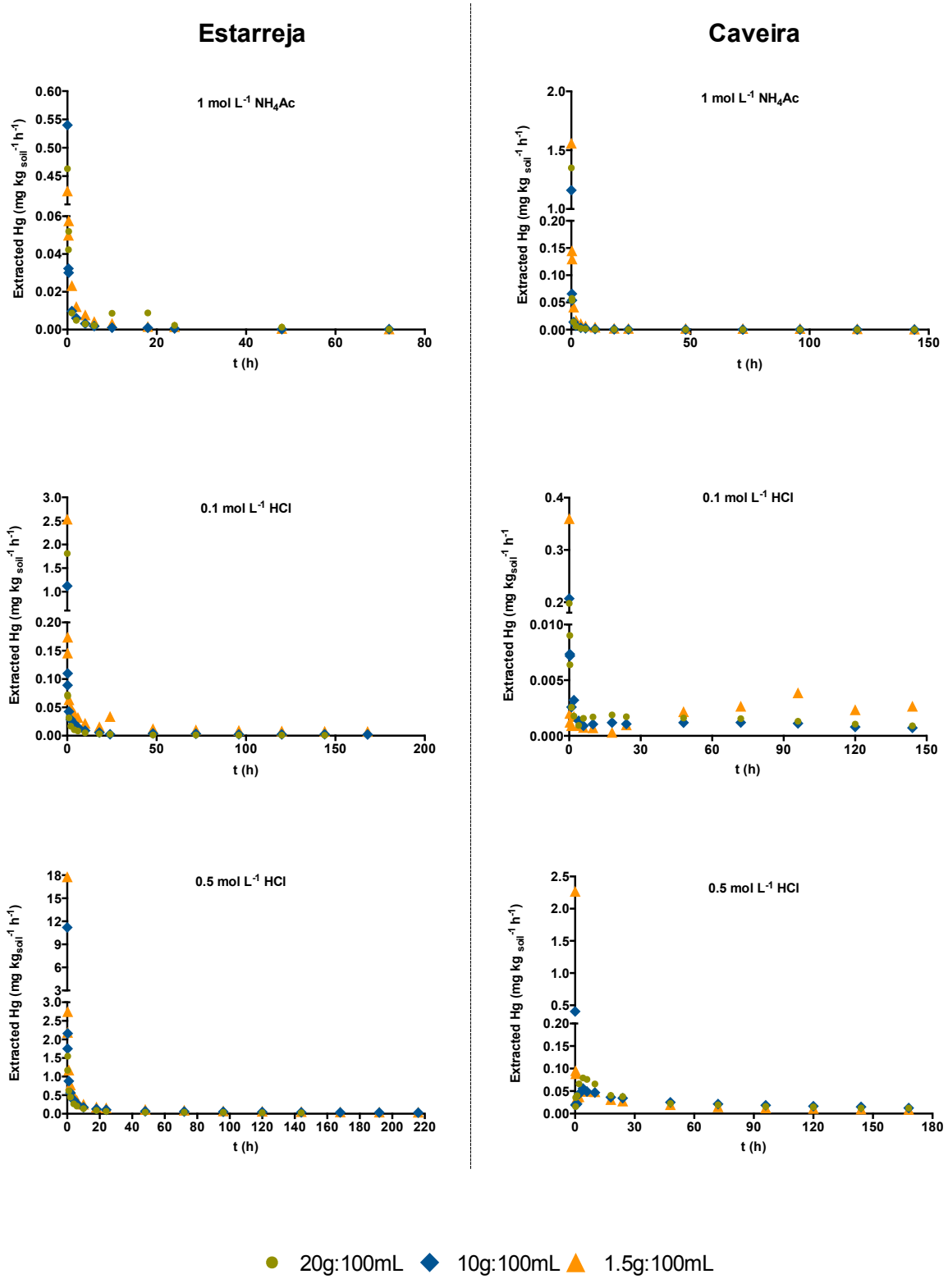

Figure 18. Extracted mercury for the three soil:extractant ratios (mg kg^{-1} of soil) per hour from Estarreja (left) and Caveira (right) samples. Extractants are, from top to bottom, 1 mol L^{-1} NH_4Ac pH 7, 0.1 mol L^{-1} HCl, and 0.5 mol L^{-1} HCl.

Table 10. Amounts of mercury extracted by NH_4Ac, 0.1 mol L^{-1} HCl, and 0.5 mol L^{-1} HCl solutions at three soil:extractant ratios. Amounts are expressed as mg of mercury extracted by kg of soil in t ≤ 10 hours, t > 10 hours, and total extracted (in equilibrium). Percentage of total mercury extracted was determined in comparison to total mercury in soil.

			Hg removed (mg kg^{-1}) t<10h	C_1	Relative error	Hg removed (mg kg^{-1}) t>10h	C_2	Relative error	total Hg removed (mg kg^{-1})	total Hg removed (%)	C_{eq}	Relative error
Estarreja (total Hg 70 mg kg^{-1})	1.0 mol L^{-1} NH_4Ac	20:100	0.086	0.06	43	-0.023	n.e.	-	0.063	**0.09**	0.069	-8.4
		10:100	0.01	0.0089	13	0.0059	0.0049	20.4	0.016	**0.023**	0.013	23
		1.5:100	0.032	0.029	12	0.0044	n.e.	-	0.037	**0.053**	0.029	27
	0.1 mol L^{-1} HCl	20:100	0.051	0.035	47	0.048	0.062	-22.4	0.1	**0.14**	0.083	20
		10:100	0.089	0.064	39	0.28	2.4	-88.3	0.37	**0.53**	0.41	-9.8
		1.5:100	0.22	0.46	-52	1	n.e.	-	1.3	**1.8**	1.1	15
	0.5 mol L^{-1} HCl	20:100	1.4	0.93	51	2.4	3.9	-39.5	3.8	**5.4**	3.5	7.4
		10:100	1.7	1.1	57	4.4	5.4	-19.4	6.1	**8.7**	5.9	3.1
		1.5:100	2.6	1.4	84	6.8	8.1	-15.6	9.4	**13**	8.9	5.8
Caveira (total Hg 6.3 mg kg^{-1})	1.0 mol L^{-1} NH_4Ac	20:100	0.0074	0.016	-54	0.0022	0.009	-76	0.0096	**0.15**	0.0091	5.3
		10:100	0.012	0.016	-24	-0.0027	0.0083	-132	0.0095	**0.15**	0.011	-13
		1.5:100	0.047	0.028	67	0.16	0.19	-18	0.2	**3.2**	0.21	-3.3
	0.1 mol L^{-1} HCl	20:100	0.018	0.15	-88	0.1	n.e.	-	0.12	**1.9**	0.14	-14
		10:100	0.01	0.13	-92	0.09	n.e.	-	0.1	**1.6**	0.13	-23
		1.5:100	0.0072	0.48	-98	0.25	0.000026	972054	0.26	**4.1**	0.49	-47
	0.5 mol L^{-1} HCl	20:100	0.66	0.62	6.3	1.4	2.5	-44	2.1	**33**	1.9	7.9
		10:100	0.47	0.24	95	1.6	2.2	-26	2.1	**33**	2.2	-5
		1.5:100	0.49	0.51	-4.5	1.1	1.7	-35	1.6	**25**	1.5	6.7

C_1; C_2: mercury concentration extracted in the first and second stages, respectively

C_{eq}: metal concentration at equilibrium

n.e. non-estimated

In general, all the curves are similar in shape: a fast desorption rate in the first hours (t < 10 hours) that becomes slower after that period. This type of extraction rate data, with two distinct desorption stages has been observed for extraction of the water-soluble fraction (I.5) and in other studies concerning metal desorption from soil (Bermond *et al.*, 2005; Issaro *et al.*, 2010; Reis *et al.*, 2014). The first stage corresponds to desorption of mercury ions that are weakly adsorbed to the matrix; the second stage corresponds to desorption of mercury complexes more intricately associated with the matrix and that need more time to dissociate. In terms of the environment, the more labile portion has more impact because it's easily mobilized to the soil solution, becoming readily available for plant uptake and contaminating crops or the aquatic compartment. As shown in Figure 18, the metal displacement from soil by all extractant solutions was almost instantaneous. Comparing to the total mercury concentration that was desorbed, at the end of the first 10 hours a higher percentage of mercury had been released when NH_4Ac was used and this percentage was superior in Estarreja soil. In the particular cases of Estarreja 20g:100mL, and Caveira 10g:100mL, mercury concentration when equilibrium is reached is lower than concentration at t=10 hours. This means that, during the experiment, re-adsorption of mercury occurs. Re-adsorption problems are one of the disadvantages recognized to chemical extraction procedures (Bacon *et al.*, 2008). In 0.1 mol L^{-1} HCl extraction, 17-51% of total mercury desorption happens in the first 10 hours, while in Caveira soil the percentages are lower (3-15%). Thus, mercury in Caveira is present in less labile species that need more time to dissociate from the matrix. Extraction with 0.5 mol L^{-1} HCl in the first hours was equivalent for both samples, although in total, more mercury was extracted in the Caveira sample.

Metal availability can be dependent on source - anthropogenic or geogenic – and it is generally recognized that metals are easily extractable in anthropogenic-contaminated soils. The results of our investigation, however, differ as a total higher percentage of mercury was extracted in the Caveira sample (although at an apparent slower rate), a mine soil where mercury is of geogenic origin, when compared to the percentage extracted in the Estarreja sample, where contamination results from a chlor-alkali plant. Caveira soil also has the physico-chemical characteristics to retain metals more efficiently: higher content of organic matter, sulfur and clay. This behaviour may be due to the influence of soil pH, since this parameter has a strong influence on mercury desorption from soil. The pH was adjusted to 7 in the initial

NH_4Ac solution and that pH was controlled during the reaction time, but changes were not significant. For Estarreja soil, pH varied between 6.6 and 6.9, and for Caveira between 5.2 and 5.8 (pH was slightly higher in the 1.5g:100mL ratio). In the experiments using HCl it was impossible to correctly measure pH, due to the strong acidity of the solution. However, Caveira soil is considerable more acid that Estarreja soil (3.3 versus 6.0), therefore, and due to soil's buffering capacity, it is expected that the final suspension also has lower pH. The increased tendency for a soil to release metals with decreasing pH has been well documented, due to H^+ removing and replacing the metal cations (Gabriel *et al.*, 2004). Also, Sutherland and Tack (Sutherland *et al.*, 2008) showed that metal extraction with diluted HCl was greater in soil richer in finer particles, as is the case of Caveira.

For environmental relevance, it is more interesting to ponder the actual mercury concentration that is, in fact, released. Total concentration in both samples is very different; hence a small fraction of a large amount represents considerably more than a large fraction of a small amount. Indeed, when considering absolute concentrations, mercury found in extracts from Caveira is in lower concentration.

As can be seen, for each sample-extractant-soil:extractant ratio combination there is a maximum quantity of mercury that can be extracted, which differs from the total metal concentration in the original sample. In terms of extraction efficiency, the percentage was higher when 0.5 mol L^{-1} HCl was applied, followed by 0.1 mol L^{-1} HCl and NH_4Ac. Therefore, desorption increases with decreasing pH. Both HCl and NH_4Ac promote mercury release by cation exchange (H^+ and NH_4^+, respectively), but exchange sites at soil's clay minerals and organic matter have more affinity to H^+ than NH_4^+. To test the statistical difference among the three procedures, Friedman's test, followed by post-hoc test for pairwise comparison, was performed for each sample and each soil:extractant ratio. It is particularly interesting to compare 0.1 mol L^{-1} HCl and 1 mol L^{-1} NH_4Ac, as both are used to estimate the bioavailable fraction. The results presented in Table 10 show that more mercury is extracted when using 0.1 mol L^{-1} HCl, and that the difference is larger in Caveira soil. The Friedman's test showed that there is a significant difference between the 0.1 mol L^{-1} HCl and NH_4Ac procedures in the 10g:100mL and 1.5g:100mL of the Estarreja sample. In all other cases, the test did not show statistical differences (Table 11). This means that, although these solutions are often used for the same purpose, our experiment shows

that under certain circumstances, the results obtained by the two extractions are not equivalent.

Frequently used in single extractions of the labile fraction of a metal in solid media (Sutherland *et al.*, 2008), 0.5 mol L^{-1} HCl provides information on the most environmental significant fraction. As more mercury was extracted using this reagent when compared to the other extractants considered in this study (percentage extracted in each procedure is presented in Table 10 – "total Hg removed"), this signifies that the bioavailable fraction is only a small part of the labile fraction of mercury in these soils. Friedman't test also revealed that extraction procedure with 0.5 mol L^{-1} HCl is statistically different from the other two procedures (Table 11).

Table 11. Friedman's test (p-value; α = 0.05) for extraction procedure comparison.

		20g:100mL	10g:100mL	1.5g:100mL
Estarreja	1 mol L^{-1} NH_4Ac - 0.1 mol L^{-1} HCl	p=0.102	p=0.014	p=0.015
	1 mol L^{-1} NH_4Ac - 0.5 mol L^{-1} HCl	p=0.0001	p=0.0005	p=0.0003
	0.1 mol L^{-1} HCl - 0.5 mol L^{-1} HCl	p=0.004	p=0.014	p=0.014
Caveira	1 mol L^{-1} NH_4Ac - 0.1 mol L^{-1} HCl	p=0.855	p=0.465	p=0.068
	1 mol L^{-1} NH_4Ac - 0.5 mol L^{-1} HCl	p=0.002	p=0.0001	p=0.0003
	0.1 mol L^{-1} HCl - 0.5 mol L^{-1} HCl	p=0.003	p=0.003	p=0.018

Determining the effect of soil:extractant ratio is important but rarely considered in desorption studies. Data showed that the higher the ratio, the higher is the concentration of metal in solution, which may assist to overcome any problems with detection limits. Mercury desorption was, in fact, favoured by lower soil:extractant ratios, and according to Table 10, the highest percentage is removed when using 1.5 g of sample per 100 mL of extractant when compared to the other ratios (with the exception of extraction with 0.5 mol L^{-1} HCl in Caveira soil). A high ratio also can lead to extractant saturation and implies a lengthier filtration process, due to filter clogging, meaning that the soil is in contact with the solution for longer time. Often, in the 20g:100mL ratio, one filter was not enough to filter the aliquot removed (c.a. 8 mL), representing an increase in the cost of the extraction. Therefore, it is better to use the lowest soil:extractant ratio possible to improve leaching of the mercury species.

II.5.6 Kinetic fitting

To test for the fitting of the kinetic equations the results of mercury removed per kg of soil were plotted against time (hours), in Figure 19. A close inspection of the results presented in Figure 18 indicated that desorption behaviour of mercury could be resolved into two different phases: a fast desorption phase and a relatively slower one, as previously discussed. Based on this observation, the experimental data were fitted into the two first-order reactions model (Equation 7), as this model considers a biphasic desorption behaviour and, therefore, seemed appropriate for our dataset. The kinetic parameters are presented in Table 12 and Table 13.

The kinetic constant k_1 is always larger than k_2, confirming the two different kinetic stages and the fast removal rate during the first hours. For extraction with HCl (both concentrations) k_1 and k_2 from Estarreja are superior to k_1 and k_2 from Caveira. Several phenomenons can explain this desorption behaviour. The analysed samples have different textures: Caveira soils are richer in clay particles, which results in a soil with higher porosity. In turn, the high porosity of this soil suggests that mercury released may be controlled by intra-particle diffusion. This desorption mechanism had already been observed in the study of the water-soluble fraction and a thorough explanation can be found in section I.5.3.3. Additionally, the smaller particle size of Caveira soil increases its metal retention capacity. In soil chemistry, metal desorption is also dependent on solid-extractant equilibrium (extractant solution may become saturated) and on the strength of the bound between the metal and the solid particles.

In general, the C_2 fraction estimated by the two first-order reactions model was larger than the C_1 fraction and both increased with decreasing soil:extractant ratio (exception for Caveira soil, 0.5 mol L^{-1} HCl). However, the model is not able to fit accurately the experimental data from all extraction conditions (signalled in red), and in some situations, the model shows limitations in predicting some parameters (particularly C_2 and k_2). This means that a good fitting should not be used as the only evidence for the suitability of a kinetic model and all parameters should be analysed with care.

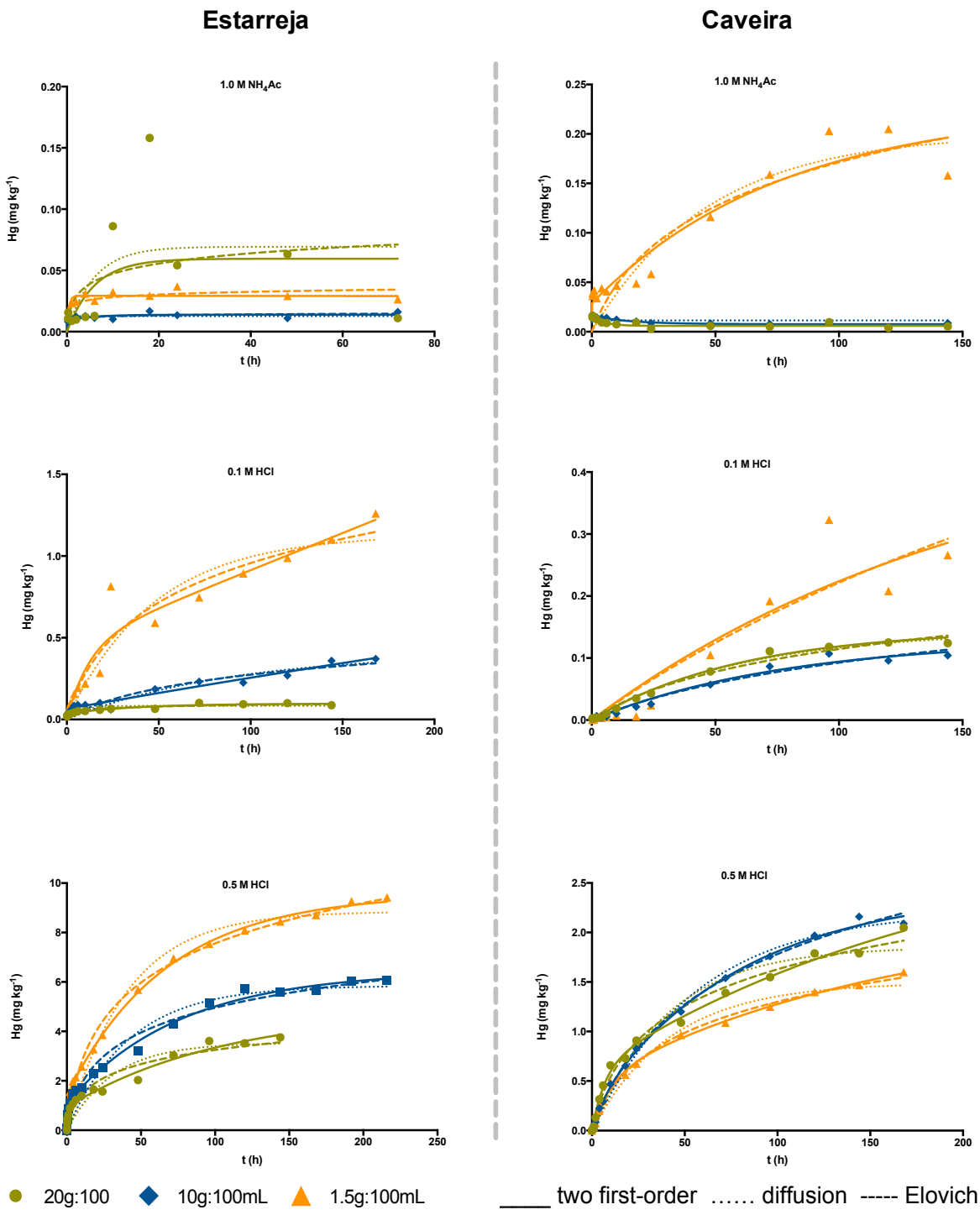

Figure 19. Extraction kinetics of soil-Hg, using 1 mol L^{-1} NH_4Ac pH 7, 0.1 mol L^{-1} HCl, and 0.5 mol L^{-1} HCl (top to bottom), and representative curves calculated from the two first-order reactions and diffusion models, and Elovich equation for samples Estarreja (left) and Caveira (right).

Table 12. Parameters of the kinetic models for the Estarreja sample (mean±standard deviation).

Sample	Kinetic model	Parameters	1 mol L^{-1} NH_4Ac			0.1 mol L^{-1} HCl			0.5 mol L^{-1} HCl		
			20:100	10:100	1.5:100	20:100	10:100	1.5:100	20:100	10:100	1.5:100
Estarreja	two-first order	C_1	$6.0x10^{-2}$±$5.7x10^{-2}$	$8.9x10^{-3}$±$1.0x10^{-3}$	$2.9x10^{-2}$±$3.0x10^{-3}$	$3.5x10^{-2}$±$6.0x10^{-3}$	$6.4x10^{-2}$±$1.2x10^{-2}$	0.46±0.33	0.93±0.12	1.1±0.1	1.4±0.1
		k_1	0.17±0.32	83±58	2.3±0.7	2.8±1.5	1.4±1.1	$8.3x10^{-2}$±$7.6x10^{-2}$	1.3±0.1	2.0±0.7	1.9±0.4
		C_2	*~ -3.3x10^{-8}*	$4.9x10^{-3}$±$1.6x10^{-3}$	*~ -6.9x10^{-2}*	$6.2x10^{-2}$±$7.0x10^{-3}$	2.4±7.8	*~ 221*	3.9±0.7	5.4±0.2	8.1±0.1
		k_2	*~ -7.9x10^{-12}*	0.18±0.17	*~ 6.1x10^{-5}*	$2.7x10^{-2}$±$1.0x10^{-2}$	$8.4x10^{-4}$±$3.0x10^{-3}$	*~ 2.1x10^{-5}*	$9.5x10^{-3}$±$3.4x10^{-3}$	$1.3x10^{-2}$±$1.0x10^{-3}$	$1.5x10^{-2}$±$1.0x10^{-3}$
		95% Confidence Intervals									
		C_1	-0.071 - 0.19	0.0058 - 0.012	$2.3x10^{-2}$ - $3.5x10^{-2}$	2.310^{-2} - $4.7x10^{-2}$	$3.7x10^{-2}$ - $9.2x10^{-2}$	-0.25 - 1.2	0.67 - 1.2	0.86 - 1.3	1.3 - 1.6
		k_1	-0.56 - 0.89	-48.2 - 215.0	0.69 - 3.9	-0.39 - 5.9	-0.89 - 3.7	-0.081 - 0.25	$9.4x10^{-2}$ - 2.6	0.44 - 3.6	1.0 - 2.8
		C_2	(Very wide)	0.0011 - 0.0086	(Very wide)	$4.7x10^{-2}$ - $7.7x10^{-2}$	-15 - 20	(Very wide)	2.4 - 5.5	4.9 - 5.7	7.9 - 8.4
		k_2	(Very wide)	-0.21 - 0.56	(Very wide)	$4.3x10^{-3}$ - $4.8x10^{-2}$	$5.7x10^{-3}$ - $7.4x10^{-3}$	(Very wide)	$2.0x10^{-3}$ - $1.7x10^{-2}$	$1.0x10^{-2}$ - $1.6x10^{-2}$	$1.3x10^{-2}$ - $1.6x10^{-2}$
		Goodness of Fit									
		R^2	**0.388**	**0.8424**	**0.9051**	**0.9483**	**0.9718**	**0.9539**	**0.9853**	**0.9947**	**0.9987**
		Sy.x	**$4.1x10^{-2}$**	**$2.1x10^{-3}$**	**$4.0x10^{-3}$**	**$8.0x10^{-3}$**	**$2.2x10^{-2}$**	**0.1**	**0.17**	**0.18**	**0.13**
	diffusion	Ceq	$6.9x10^{-2}$±$2.0x10^{-2}$	$1.3x10^{-2}$±$1.0x10^{-3}$	$2.9x10^{-2}$±$1.0x10^{-3}$	$8.3x10^{-2}$±$6.0x10^{-3}$	0.41±0.08	1.1±0.1	3.5±0.3	5.9±0.3	8.9±0.0
		k	0.17±0.17	4.1±1.6	2.3±0.6	0.14±0.05	$1.1x10^{-2}$±$4.0x10^{-3}$	$2.1x10^{-2}$±$5.0x10^{-3}$	$3.2x10^{-2}$±$8.0x10^{-3}$	$2.4x10^{-2}$±$4.0x10^{-3}$	$2.5x10^{-2}$±$3.0x10^{-3}$
		95% Confidence Intervals									
		Ceq	0.025 - 0.11	$1.1x10^{-2}$ - $1.4x10^{-2}$	$2.6x10^{-2}$ - $3.2x10^{-2}$	$7.1x10^{-2}$ - $9.7x10^{-2}$	0.24 - 0.57	0.92 - 1.3	2.9 - 4.2	5.3 - 6.4	8.2 - 9.6
		k	-0.22 - 0.54	0.57 - 7.6	1.1 - 3.6	0.049 - 0.23	$2.3x10^{-3}$ - 0.021	$9.8-10^{-3}$ - $3.2x10^{-2}$	$1.5x10^{-2}$ - $5.0x10^{-2}$	$1.5x10^{-2}$ - $3.2x10^{-2}$	$1.8x10^{-2}$ - $3.2x10^{-2}$
		Goodness of Fit									
		R^2	**0.4089**	**0.7349**	**0.9050**	**0.7900**	**0.9017**	**0.9250**	**0.8866**	**0.9474**	**0.9673**
		Sy.x	**0.0366**	**$2.4x10^{-3}$**	**$3.6x10^{-3}$**	**$1.5x10^{-2}$**	**$3.9x10^{-2}$**	**0.12**	**0.44**	**0.53**	**0.64**
	Elovich	a	0.057±0.154	10.9±20.3	2.3±2.9	$9.2x10^{-2}$±$3.0x10^{-3}$	$7.5x10^{-3}$±$2.5x10^{-3}$	$4.0x10^{-2}$±$1.1x10^{-2}$	0.37±0.12	0.37±0.08	0.51±0.07
		b	82±69	918±177	317±54	74±8	6.4±1.8	2.5±0.5	1.2±0.2	0.65±0.06	0.41±0.03
		95% Confidence Intervals									
		a	−0.2814 - 0.3954	−34 - 56	−4.2 - 8.9	$3.4x10^{-3}$ - 0.18	$2.5x10^{-3}$ - $1.2x10^{-2}$	$1.5x10^{-2}$ - $6.5x10^{-2}$	0.12 - 0.62	0.21 - 0.53	0.36 - 0.67
		b	−70.69 - 234.5	529 - 1308	197 - 439	56 - 91	2.6 - 10	1.5 - 3.5	0.82 - 1.5	0.53 - 0.78	0.35 - 0.47
		Goodness of Fit									
		R^2	**0.2857**	**0.8524**	**0.8658**	**0.9321**	**0.9157**	**0.9447**	**0.9443**	**0.9742**	**0.9876**
		Sy.x	**0.0402**	**$1.7x10^{-3}$**	**$4.3x10^{-3}$**	**$8.5x10^{-3}$**	**$3.6x10^{-2}$**	**0.11**	**0.31**	**0.37**	**0.4**

C_1; C_2: mercury concentration extracted in the first and second stages, respectively

k_1; k_2 apparent rate constants of the first and second stages, respectively

C_{eq}: metal concentration at equilibrium

k; a; b: constants

R^2: coefficient of determination

$S_{x/y}$: standard deviation of residues

Table 13. Parameters of the kinetic models for the Caveira sample (mean±standard deviation).

Sample	Kinetic model	Parameters	1 mol L^{-1} NH_4Ac			0.1 mol L^{-1} HCl			0.5 mol L^{-1} HCl		
			20:100	10:100	1.5:100	20:100	10:100	1.5:100	20:100	10:100	1.5:100
Caveira	two-first order	C_1	$1.6x10^{-2}$±$1.0x10^{-3}$	$1.6x10^{-2}$±$1.0x10^{-3}$	$2.8x10^{-2}$±$8.0x10^{-3}$	0.15±0.15	0.13±0.23	0.48±0.63	0.62±0.09	0.24±0.11	0.51±0.09
		k_1	149±54	113±26	72±149	$1.7x10^{-2}$±$1.0x10^{-2}$	$1.3x10^{-2}$±$2.0x10^{-2}$	$6.3x10^{-3}$±$9.9x10^{-3}$	0.15±0.03	0.14±0.08	0.10±0.02
		C_2	$9.9x10^{-3}$±$1.4x10^{-3}$	$8.3x10^{-3}$±$8.6x10^{-4}$	0.19±0.1	~ $-8.2x10^{-8}$	~ $1.1x10^{-3}$	$2.6x10^{-5}$±0.8	2.5±0.7	2.2±0.1	1.7±0.3
		k_2	0.21±0.1	$6.2x10^{-2}$±$2.0x10^{-2}$	$1.3x10^{-2}$±$6.0x10^{-3}$	~ $-7.1x10^{-11}$	~ $6.8x10^{-10}$	$2.2x10^{-10}$±53	$4.9x10^{-3}$±$2.5x10^{-3}$	$1.3x10^{-2}$±$2.0x10^{-2}$	$6.1x10^{-3}$±$2.5x10^{-3}$
		95% Confidence Intervals									
		C_1	$1.3x10^{-2}$ - $1.9x10^{-2}$	$1.4x10^{-2}$ - $1.7x10^{-2}$	$1.1x10^{-2}$ - $4.7x10^{-2}$	-0.19 - 0.48	-0.37 - 0.63	-0.90 - 1.9	0.41 - 0.83	$4.8-10^{-3}$ - 0.47	0.30 - 0.71
		k_1	31 - 267	56 - 170	-253 - 397	-0.011 - 0.044	-0.024 - 0.049	-0.015 - 0.028	0.068 - 0.23	-0.039 - 0.32	$5.1x10^{-2}$ - 0.15
		C_2	$-1.3x10^{-2}$ - $-6.8x10^{-3}$	$-1.0x10^{-2}$ - $-6.4x10^{-3}$	0.11 - 0.29	(Very wide)	(Very wide)	$-1.9x10^{7}$ - $1.9x10^{7}$	0.91 - 4.1	2.0 - 2.4	1.0 - 2.3
		k_2	$3.0x10^{-2}$ - 0.38	$1.9x10^{-2}$ - 0.10	$-2.4x10^{-4}$ - $2.6x10^{-2}$	(Very wide)	(Very wide)	-114 - 114	$-5.4x10^{-4}$ - $1.0x10^{-2}$	$8.6x10^{-3}$ - $1.7x10^{-2}$	$6.6x10^{-4}$ - $1.2x10^{-2}$
		Goodness of Fit									
		R^2	**0.8673**	**0.9322**	**0.9351**	**0.9927**	**0.9791**	**0.8967**	**0.9962**	**0.9982**	**0.998**
		Sy.x	**$1.8x10^{-3}$**	**$1.3x10^{-3}$**	**$1.9x10^{-2}$**	**4.8x10-3**	**$6.7x10^{-3}$**	**$3.9x10^{-2}$**	**$4.9x10^{-2}$**	**$3.9x10^{-2}$**	**$2.9x10^{-2}$**
	diffusion	Ceq	$9.1x10^{-3}$±$1.1x10^{-3}$	$1.1x10^{-2}$±$1.0x10^{-3}$	0.21±0.03	0.14±0.01	0.13±0.01	0.49±0.32	1.9±0.1	2.2±0.1	1.5±0.1
		k	~ $2.7x10^{13}$	227±250	$2.1x10^{-2}$±$7.0x10^{-2}$	$1.7x10^{-2}$±$2.0x10^{-2}$	$1.3x10^{-2}$±$3.0x10^{-3}$	$5.9x10^{-3}$±$5.4x10^{-3}$	$2.5x10^{-2}$±$3.0x10^{-2}$	$1.8x10^{-2}$±$1.0x10^{-3}$	$2.4x10^{-2}$±$3.0x10^{-3}$
		95% Confidence Intervals									
		Ceq	$6.8x10^{-3}$ - $1.1x10^{-2}$	$9.3x10^{-3}$ - $1.3x10^{-2}$	0.14 - 0.26	0.13 - 0.16	0.10 - 0.16	-0.19 - 1.2	1.7 - 2.1	2.1 - 2.4	1.4 - 1.6
		k	(Very wide)	-309 - 764	$6.0x10^{-3}$ - $3.7x10^{-2}$	$1.3x10^{-2}$ - $2.0x10^{-2}$	$6.9x10^{-3}$ - $1.8x10^{-2}$	$-5.7x10^{-3}$ - $1.8x10^{-2}$	$1.7x10^{-2}$ - $3.3x10^{-2}$	$1.5x10^{-2}$ - $2.0x10^{-2}$	$1.8x10^{-2}$ - $2.9x10^{-2}$
		Goodness of Fit									
		R^2	**0.2492**	**0.4163**	**0.8649**	**0.9932**	**0.9791**	**0.8967**	**0.9684**	**0.9952**	**0.9826**
		Sy.x	**$4.0x10^{-3}$**	**$3.5x10^{-3}$**	**$2.6x10^{-2}$**	**$4.3x10^{-3}$**	**$6.2x10^{-3}$**	**$3.7x10^{-2}$**	**0.13**	**$5.9x10^{-2}$**	**$7.9x10^{-2}$**
	Elovich	a			$6.6x10^{-3}$±$2.7x10^{-3}$	$2.8x10^{-3}$±$3.7x10^{-4}$	$1.8x10^{-3}$±$1.1x10^{-3}$	$2.9x10^{-3}$±$9.7x10^{-4}$	$8.8x10^{-2}$±$1.1x10^{-2}$	$5.6x10^{-2}$±$3.0x10^{-3}$	$6.1x10^{-2}$±$4.0x10^{-2}$
		b			13±4	14±2	13±3	2.3±2.6	1.7±0.1	1.1±0.1	1.9±0.1
		95% Confidence Intervals	n.e	n.e							
		a			$7.6x10^{-4}$ - $1.2x10^{-2}$	$1.9x10^{-3}$ - $3.6x10^{-3}$	$1.1x10^{-3}$ - $2.4x10^{-3}$	$7.9x10^{-4}$ - $4.9x10^{-3}$	$6.5x10^{-2}$ - 0.11	$4.9x10^{-2}$ - $6.3x10^{-2}$	$5.2x10^{-2}$ - $7.0x10^{-2}$
		b			4.6 - 22	9.5 - 18	5.6 - 20	−3.3 - 7.8	1.5 - 1.9	1.0 - 1.2	1.8 - 2.2
		Goodness of Fit									
		R^2	**0.2306**	**0.3895**	**0.8602**	**0.9858**	**0.9729**	**0.8941**	**0.9908**	**0.9975**	**0.9967**
		Sy.x	**$4.1x10^{-3}$**	**$3.5x10^{-3}$**	**$2.6x10^{-2}$**	**$6.2x10^{-3}$**	**$7.1x10^{-3}$**	**$3.7x10^{-2}$**	**$7.2x10^{-2}$**	**$4.3x10^{-2}$**	**$3.5x10^{-2}$**

C_1; C_2: mercury concentration extracted in the first and second stages, respectively

k_1; k_2 apparent rate constants of the first and second stages, respectively

C_{eq}: metal concentration at equilibrium

k; a; b: constants

R^2: coefficient of determination

$S_{x/y}$: standard deviation of residues

n.e. non-estimated.

Two other kinetic models were tested – the diffusion model (Equation 9) and the Elovich equation (Equation 10). As can be seen in Table 12 and Table 13, the R^2 values obtained with the diffusion model and the Elovich equation are generally slightly lower than the ones obtained with the two first-order reactions model. Nevertheless, in many cases the three models fitted the experimental data. This good agreement between the experimental and fitted curves is also visible in Figure 19. On the other hand, the standard deviation of residues obtained was, in most cases, higher. Data referring to extraction with NH_4Ac rarely fitted to any of the adopted models. The phenomenon of re-adsorption observed during the extraction process, and particularly noted for this extractant solution caused a more "irregular" dataset, hampering its fit. The C_{eq} values estimated by the diffusion model increase in the order NH_4Ac < 0.1 mol L^{-1} HCl < 0.5 mol L^{-1} HCl, and decrease as soil:extractant ratio increases. Also, C_{eq} in Estarreja is higher than C_{eq} in Caveira soil sample. The kinetic constant, k, is larger in 0.5 mol L^{-1} HCl than in 0.1 mol L^{-1} HCl, meaning that desorption reaction occurs faster in the presence of more concentrated acid. However, between the two samples, there is no meaningful difference in the constant k, although Caveira's is slightly lower. The explanation for the slower reaction in Caveira is due to the sample texture and was already discussed.

Although the meaning of constants *a* and *b* in the Elovich equation is very unclear (Fangueiro *et al.*, 2005), some investigators have used them as estimators for the reaction rate, even though this is questionable (Sparks, 1999). The *b* constant was generally similar in both samples but the *a* constant was larger in Estarreja soil, confirming that desorption is faster in this sample. Nevertheless, the utility of the Elovich equation is debatable, as it has no clear physical meaning, it should only be applied to predict the quantity of metal extracted at times not studied experimentally (Fangueiro *et al.*, 2005; Sparks, 1999).

The relative error between the experimental and the estimated values of C_1, and C_2, both from the two first-order reactions model and C_{eq} from the diffusion model were calculated. The experimental value of C_1 was defined as the amount of mercury extracted per unit of soil, respectively, at t=10 hours; the experimental value of C_2 was calculated by the difference between the amount of mercury extracted at equilibrium and C_1. C_{eq}, in the diffusion model, was defined as the amount of mercury extracted per unit of soil at t=equilibrium. The relative error associated with C_1 and C_2 is not satisfactory as it ranges from 6% to approximately 60% and, in a

very few cases, is as high as 95%. Both under and overestimation of the experimental value occurred. The error associated with C_{eq} is considerable lower, meaning that this constant better estimates the real concentration reached at equilibrium.

In summary, both the two first-order reactions and the diffusion models fit the experimental data, meaning that mercury desorption from the studied soils occurs in two concurrently stages and that desorption is limited by diffusion of less labile mercury complexes. Still, the error associated with mercury concentration in the first and second stages (constants C_1 and C_2 of two first-order reactions) cannot be disregarded when estimating mercury release at a given time, using this equation.

II.5.7 Conclusion

In the desorption of mercury as a function of time two stages were distinguishable: one, for short extraction times (t ≤ 10 hours), corresponding to faster metal extraction rate and a second where the slower desorption of the metal indicates its release from sites of relatively higher bonding energy. Therefore, two mechanisms seem to be involved in mercury desorption from soil: “chemical” desorption of cations that are in more exposed, reactive sites, and diffusion from the intricate mineral lattice or from pores of inner soil surfaces that need more time to dissociate. This is a common phenomenon associated to metal desorption in solid matrices, as demonstrated by other studies, even though different metals and/or extractant solutions were studied (Bermond *et al.*, 2005; Issaro *et al.*, 2010; Varrault *et al.*, 2011).

The two first-order reactions and diffusion models, and the Elovich equation have been tested to fit the experimental data obtained for mercury extraction with NH_4Ac and HCl. On one hand, the extraction with NH_4Ac was not fitted by any of the equations but, on the other hand, the three equations allow a good fitting for experimental data obtained with HCl extractions. The two first-order reactions model was adequate for the two-stage desorption behaviour of mercury, associated to two kinetically distinct rates of desorption, while the diffusion model allowed determining that the diffusion of mercury complexes is the limiting rate of the extraction.

It was demonstrated that more mercury was released when the soil to extractant ratio was lower, and that ammonium acetate and 0.1 mol L^{-1} HCl, both

used in estimation of metal bioavailability in soil, do not yield statistically different results in the majority of the operational conditions, although more problems of re-adsorption were observed with the first reagent. 0.5 mol L^{-1} HCl, used to extract all labile fractions of metal in soil, removes a higher percentage of mercury, but extraction is strongly affected by the sample characteristics.

The results obtained in this study can be considered fairly good, taking into account the heterogeneous nature of soil samples. However, the models are still not capable of accurately estimate all constants. Re-adsorption problems may be one of the reasons behind this problem. Performing extraction in continuous flow mode, for example, may help overcome this problem.

Studying desorption processes in heterogeneous systems such as soils has clear difficulties. This is largely due to the complexity of the soil and the numerous components that it is constituted of. These components interact with each other resulting in a multitude of sites for metal adsorption with different reactivity. Additionally, the presence of different sized particles results in a variety of textures and porosity in soil, which strongly influenced the mercury desorption rate. The comparison of results of both samples analysed allowed concluding that pH and particle size play an important role in mercury desorption from soil. The results concerning the Caveira soil sample, in particular, show the importance of performing fractionation studies even in samples where mercury would be expected to exist as more stable species.

The results also demonstrate that kinetic extraction of mercury from soil appears to be an efficient and adequate alternative to study the metal fractionation. The change from thermodynamic (i.e. equilibrium) to kinetic control of the leaching process has been claimed to represent more accurately environmental processes such as the percolation of rainwater through a soil profile (Bacon *et al.*, 2008). However, the utilization of kinetic chemical extractions for providing detailed insight on metal fractionation, and most importantly, on metal bioavailability, is still in need of more supplementary information, particularly in the case of mercury, where there is a serious lack of studies. In order to achieve the most accurate information possible through kinetic extraction, studies connecting results on metal mobility from laboratory kinetic speciation with “real-world” investigations, i.e. *in-situ* or similar to field conditions, must be conducted.

An international proficiency-test as a tool to evaluate the current mercury determination status in organic and inorganic matrices

6 AN INTERNATIONAL PROFICIENCY TEST AS A TOOL TO EVALUATE THE CURRENT MERCURY DETERMINATION STATUS IN ORGANIC AND INORGANIC MATRICES

Highlights

•Describes the design and organisation of an inter-laboratory proficiency test for mercury determination in environmental matrices.

•Compares methods for total mercury determination.

•Assesses the reproducibility of extraction procedures aiming the organometallic and exchangeable fractions.

•Evaluates laboratory bias and analytical performance against consensus values.

•Calls for a collaborative trial to define future strategies in mercury speciation.

Abstract

A proficiency-testing scheme (denominated ILAE-Hg-02) targeting total mercury determination in soil, sediment, fish and human hair was organised in order to evaluate the consistency of results obtained by different laboratories, applying their routine methods to the same test samples. Additionally, single extractions by 1 mol L^{-1} ammonium acetate solution, 0.1 mol L^{-1} HCl and 0.1 mol L^{-1} $CaCl_2$, as well as extraction of the organometallic fraction were proposed for soil; the last was also suggested for sediment and fish. Objectives included allowing participants to test the reliability of their analytical and quality control procedures, and assessing the variability of the obtained results. Participants' performance was evaluated by z-scores; the assigned value was obtained from consensus of participants. Results for the four matrices indicated that, out of the 29 participants, 74% had a satisfactory performance ($|z\text{-score}| \leq 2$), 8% had questionable performance and 18% require action ($|z\text{-score}| > 3$). Best results were obtained for soil, while fish yielded the highest-biased results, which can reflect the analytical problems of quantifying mercury at low concentrations. The influence of sample pretreatment and analytical procedures used for quantification was studied, but no direct relationship between these variables and bias in the determination of total mercury was observed. The four participants that returned results for mercury extractions reported different

mercury concentrations in soil and sediment; extraction of organic mercury in fish yielded more reproducible results. This study was important to update the knowledge on analytical techniques that are being used for mercury quantification and confirmed the need to develop analytical techniques for mercury determination at very low concentrations, to improve and standardize mercury extraction techniques, and to implement effective strategies for quality control in mercury determination.

Keywords: Interlaboratory study; Proficiency testing; Mercury; Mercury extraction; Homogeneity test; Assigned value; z-score; Soil; Sediment; Fish; Human hair

6.1 Introduction

The problems associated with the presence of mercury in the environment have been previously discussed. Therefore, analytical techniques capable of detecting both trace and high amounts of this element are fundamentally important. Several techniques exist and are currently used in mercury quantification in various matrices, in different areas (environmental, food products, clinical, etc.). Cold-vapour atomic absorption spectroscopy (CV-AAS) and cold-vapour atomic fluorescence spectroscopy (CV-AFS) are among the most widely used methods in mercury determination (Clevenger *et al.*, 1997); they allow direct determination of the metal without the need of an atomizer, due to the high vapour pressure of mercury. Other techniques still in use include atomic absorption spectroscopy, which offers good detection limits despite suffering from matrix interference (Brown *et al.*, 1995; Clevenger *et al.*, 1997), and inductively coupled plasma optical emission or mass spectrometry. Radiochemical methods, like neutron activation analysis (Delft *et al.*, 1988), although rapid and sensitive for trace concentrations, are less commonly applied, as are electrochemical methods. Thermo-desorption atomic absorption spectroscopy (TD-AAS), has been gaining more popularity due to its wide applicability in solid and liquid samples of organic or inorganic composition, without requiring time-consuming sample preparation or digestion methods (Costley *et al.*, 2000).

But do these techniques yield the same results? An approach that has been working very well to both improve the analytical quality and to quantify the

uncertainty in analytical data is to promote inter-laboratory comparison studies (Frazzoli *et al.*, 2005; Pereira *et al.*, 2008b). Participation in these exercises is extremely important and one of the most accurate forms of external quality control (Thompson *et al.*, 2006; Vander Heyden *et al.*, 2007). Due to the large variety of analytical methods and techniques currently used for mercury determination, it is of upmost importance to test the consistency of the results obtained by the different laboratories, applying their routine methods to the same controlled, "blind" samples. The analysis of blind samples provides more objective information on the technical competence of a laboratory than the analysis of certified reference materials (CRM). Comparison of the obtained results enables detection of errors as a result of a specific procedure or malpractices of a given laboratory, which will help participants to comply with quality assurance (QA) and quality control (QC) requirements. Furthermore, proficiency-testing is an essential part of the accreditation of analytical laboratories (Vander Heyden *et al.*, 2007)

Following the successful results of ILAE-Hg-01 (Pereira *et al.*, 2008b) in 2008, a second inter-laboratory exercise, targeting mercury determination in solid samples (ILAE-Hg-02) was organised. This time, test materials considered for total mercury determination were soil, sediment, fish and human hair. Additionally, chemical extraction procedures were included.

Chemical extraction procedures have been regularly applied to determine the availability and mobility of mercury (Boszke *et al.*, 2008; Reis *et al.*, 2012). However, along with proliferation in application have grown questions about the operational nature of the extraction procedures and the comparability of the data produced. If data from one study are to be compared with those from another study then consistency of methodologies and extraction conditions becomes important (Bacon *et al.*, 2008). Information regarding the reproducibility of chemical extraction procedures is necessary but scarce. For this inter-laboratory study, simple and common extraction procedures were chosen to be applied to the soil test material: 0.1 mol L^{-1} $CaCl_2$, frequently used as indicative of soil-to-plant transfer (Sahuquillo *et al.*, 2002); 1 mol L^{-1} NH_4Ac and 0.1 mol L^{-1} HCl, used to estimate the exchangeable fraction, i.e. the species that can be easily released into the environment (Filgueiras *et al.*, 2002). Extraction of the organometallic fraction, which contains the more toxic species, was also purposed for soil, sediment, and fish. In general, most analytical methods for organic mercury fractions determination combine acid or alkaline

extraction with solvent extraction (Válega *et al.*, 2006). While in fish organometallic mercury species can account for over 85% of total mercury (Mieiro *et al.*, 2011; Tavares *et al.*, 2011), their concentration in soils and sediments is low (usually below 3%) (Canário *et al.*, 2007; Rimondi *et al.*, 2012); still the risk incurred by their presence must not be neglected.

Essentially, this work intends to provide more information about the analytical techniques that are currently used in mercury quantification, the results they provide, and to identify underlying problems, which is crucial for the effectiveness of mercury strategies. Additionally, it is expected that divulging the results of this study will emphasise the importance of performing selective extractions in mercury-contaminated samples and, consequently, of developing adequate extraction and respective quality control procedures.

6.2 Experimental section

6.2.1 Participation in ILAE-Hg-02

The ILAE-Hg-02 was announced via e-mail, with the collaboration of LECO®, together with a subscription form and the outline of the study. The subscription occurred in two stages: first, during May 2011; second, during December 2011-January 2012. Thirty-eight laboratories expressed their interest in participating in this study, including public and private laboratories, universities and public research facilities, from six countries - Denmark (1), Finland (1), Nicaragua (1), Poland (1), Portugal (10) and Spain (24). Each participant was randomly assigned a laboratory code. For confidentiality purposes, the institutions' names are omitted. Of the 38 participants, 29 submitted results.

6.2.2 Test materials

The ILAE-Hg-02 was organized for the determination of mercury concentrations in four matrices: soil, sediment, fish and human hair. The participants were offered the opportunity to perform the range of analyses described in Table 14. The chosen extractions are widely used to evaluate environmental risk, as they provide valuable

information on mercury mobility and availability to plant, animal, and ultimately man (Filgueiras *et al.*, 2002; Jing *et al.*, 2008; Mieiro *et al.*, 2011; Sahuquillo *et al.*, 2002).

Table 14. Materials used in ILAE-Hg-02 and respective range of analysis available.

Soil	• Total Hg concentration; • Organometallic Hg concentration; • Hg concentration after single extraction by 1 mol L^{-1} NH_4Ac (pH adjusted to 7.0 with NH_4OH); • Hg concentration after single extraction by 0.1 mol L^{-1} HCl; • Hg concentration after single extraction by 0.1 mol L^{-1} $CaCl_2$.
Sediment	• Total Hg concentration; • Organometallic Hg concentration;
Fish	• Total Hg concentration; • Organometallic Hg concentration;
Human hair	• Total Hg concentration;

6.2.3 Preparation of test materials

Soil was collected in a contaminated area of Estarreja (Portugal). Sampling was performed using a plastic spatula and samples were placed in plastic bags during transport to the laboratory, where they were pre-treated within one hour. The soil sampling depth was 0–15 cm. Once in the laboratory, soil samples were air dried at room temperature to constant weight. Stones were removed and soil aggregates were crushed and homogenized, during the drying stage. The dried samples were sieved to <250 µm using a nylon sieve.

A contaminated sediment was collected in the Laranjo basin of Ria de Aveiro (Portugal; 40°43'45.77"N; 8°36'59.61"W) and a non-contaminated sediment was collected at Vagos (Portugal; 40°33'33.47"N; 8°40'36.26"W). Both were sampled using a plastic spatula and placed in plastic bags during transport to the laboratory, where they were pre-treated within one hour. Once in the laboratory, sediments were air dried at room temperature to constant weight. Stones and shells were removed and aggregates were crushed and homogenized, during the drying stage. The dried samples were sieved to <150 µm using a nylon sieve. The two sediments were then combined in order to achieve the desired mercury concentration.

Catfish (*Pangasius hypophthalmus*) from a Vietnamese aquaculture was purchased in a local supermarket. The muscle was freeze-dried, homogenized and sieved (<150 µm).

Human hair was collected in local barbershops and hairdressing salons. The hair was washed, minced, sieved (<150 µm) and blended.

6.2.4 Homogeneity testing

Homogeneity was tested according to the procedure described in "The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories" (Thompson *et al.*, 2006), by selecting 12 bottles of each material from the lot and analysing them for their total mercury content. Each bottle was analysed in duplicate, by the organizers, using a direct mercury analyser (LECO® AMA-254).

Cochran's C test was used to study and identify the homogeneity of variances, using the critical value at 95% level of confidence. The remaining data was tested using analysis of variance (ANOVA) to estimate the between-sample ($\mathbf{s_{sam}^2}$) and analytical ($\mathbf{s_{an}^2}$) variances, according to Equation 11.

Equation 11 $\mathbf{s_{sam}^2 = \frac{(MS_{between} - MS_{within})}{2}}$, with $\mathbf{s_{an}^2 = MS_{within}}$.

The material was considered homogeneous if it passed the final test for sufficient homogeneity ($\mathbf{s_{sam}^2} < c$), where:

Equation 12 $\mathbf{c = F_1 \sigma_{all}^2 + F_2 s_{an}^2}$, with $\mathbf{F_1}$=1.79 and $\mathbf{F_2}$=0.86 (n=12; α=0.05)

Equation 13 $\mathbf{\sigma_{all}^2 = (0.3\sigma)^2}$, and

Equation 14 $\mathbf{\sigma = \chi \times CV}$

χ is the mean of results and **CV** is the coefficient of variation, established as 10% for this study

6.2.5 Sample dispatch

On the 17th April 2012, samples were sent to the participants, according to their requirements. Each participant received only the matrices for which they had subscribed. Samples were sent to participants (100 g of soil, 5 g of sediment, 4 g of fish and 2 g of hair), packed in amber glass bottles with polyethylene caps.

Information sheets containing a description of the samples, requirements, deadlines and instructions on how to report the results, a data reporting form and a questionnaire regarding general information on the participating laboratory and specific analysis details (use of pretreatment, analytical method used, etc.) were sent together with samples. Additionally, suggested extractions procedures were sent to participants who desired to perform them. The purposed procedures are described in Annex I.

6.2.6 Evaluation of participants performance

Participants' performance was evaluated by determination of the z-score, which is calculated by comparing the difference between the participants' results (x) and the assigned value (χ) for the test material.

6.2.7 Assigned value

The assigned value (χ) was determined by consensus of participants, and equals the robust mean, after winsorisation of the data by the Huber method. Winsorisation replaces the outliers with cutting-point values, rather than discarding them, making more use of the information available. It was done as follows:

(a) the dataset was analysed for invalid results (expressed in wrong or dubious units), which were removed;

(b) exploratory statistical analysis of the remaining data was performed, together with a boxplot and Grubbs' test for identification of outliers;

(c) outliers, if present, were removed from the dataset and a "new" median and MAD (median absolute deviation) were determined,

(d) data was then allocated according to:

if a value > median + 1.5MAD, x_i it's changed to "median + 1.5MAD";

if a value < median - 1.5MAD, x_i it's changed to "median - 1.5MAD";

an improved robust mean and standard deviation were then calculated and used as reference value and standard deviation, respectively, in z-score

Uncertainty associated to the estimated value (u_x) was determined according to Equation 15, using the procedure described in “The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories” (Thompson *et al.*, 2006).

Equation 15 $\mathbf{u_x = \sigma_{rob} \times \sqrt{n}}$

$\boldsymbol{\sigma_{rob}}$ is the robust standard deviation
n is the number of participants

6.2.8 z-score

z-scores were determined according to Equation 16.

Equation 16 $\mathbf{z - score = \frac{(x - \chi)}{\sigma_{rob}}}$

x is the participant result
χ is the assigned value

z-scores are interpreted as:
$|z| \leq 2$ satisfactory performance
$2 < |z| < 3$ questionable performance
$|z| \geq 3$ requiring action

6.3 Results and discussion

6.3.1 Test materials homogeneity

All test materials passed the homogeneity test and were considered appropriate for the interlaboratory study (Table 15). Moreover, Cochran's test results for within-sample variation indicated good analyst precision at a 95% level of confidence (critical value is 0.54 for n = 12) in the conduct of analyses in four matrices.

Table 15. Homogeneity test results for the four matrices used in ILAE-Hg-02.

	soil	sediment	fish	hair
mean, X	27	44.6	2.66×10^{-2}	1.46
Chocran's C	0.387	0.464	0.347	0.409
outliers	no	no	no	no
s^2_{sam}	0.242	2.16	1.21×10^{-6}	1.86×10^{-3}
c	1.2	6.06	1.39×10^{-6}	3.67×10^{-3}
$s^2_{sam} < c$	passed	passed	passed	passed

6.3.2 Evaluation of participants performance

6.3.2.1 Total mercury determinations

Participants' results for total mercury in the four test materials are presented in Table 16, together with the respective z-scores.

Out of the twenty participants that returned results for total mercury determination in soil, 80% had a satisfactory performance, as they obtained a $|z| \leq 2$; among them, 69% obtained a $|z| \leq 1$, indicating a very good performance (Table 17). Participants with questionable performance comprised 5%, while 15% had $|z| > 3$, and should undertake immediate action to improve the quality of their results. In soil, results ranged from 20.1 to 35.0 mg kg^{-1}, which resulted in a mean of 27.4 mg kg^{-1} and a median of 27.1 mg kg^{-1} (Table 17). Grubbs test did not confirm the presence of any outlier and winsorisation of the dataset generated a robust mean of 27.1 mg kg^{-1}; therefore, an assigned value of 27.1±0.3 mg kg^{-1} was attributed to soil.

Table 16. Participants' results for total mercury in the four test materials and respective z-scores.

		001	002	003	005	006	008	009	010	011	012	013	015	017	018	019
	Pre-treatment	**WM* No****	**No**	**No**	**No**	**No**	**WM**	**No**	**No**	**No**	**WM**	**No**	**WM**	**No**	**No**	**No**
	Analytical technique	**ICP-OES* TD-AAS****	**TD-AAS**	**TD-AAS**	**TD-AAS**	**TD-AAS**	**AAS**	**CV-AAS**	**TD-AAS**	**AAS**	**CV-AAS**	**TD-AAS**	**AFS**	**TD-AAS**	**TD-AAS**	**TD-AAS**
Soil	**n**	5	2		5			5		5		3	2	5	5	
	THg $_{mean}$ (mg kg^{-1})	20.1	32.1		24.8			30.3		27.3		25.4	35.9	26.8	27.0	
	SD	0.3	-		0.5			0.6		0.3		1.2	-	16.4	0.2	
	% RSD	1.5	-		2.14			2.04		0.94		4.73	-	61.3	0.59	
	z-score	**-4.89**	**3.47**		**-1.45**			**2.25**		**0.14**		**-1.16**	**6.09**	**-0.20**	**-0.09**	
Sediment	**n**	5	2					5		5		2	2	5	5	
	THg $_{mean}$ (mg kg^{-1})	27.0	43.1					46.5		45.0		43	62.0	56.4	42.2	
	SD	1.7	4.7					1.4		0.6		-	-	17.3	0.2	
	% RSD	6.45	10.8					2.95		1.42		-	-	30.6	0.51	
	z-score	**-6.10**	**-0.13**					**1.12**		**0.57**		**-0.170**	**6.86**	**4.80**	**-0.48**	
Fish	**n**	5				5	2	5	5	5	0	2	2		5	5
	THg $_{mean}$ (mg kg^{-1})	2.70×10^{-2}				1.86×10^{-2}	<0.1	4.28×10^{-2}	2.22×10^{-2}	2.01×10^{-2}	<0,025	1.35×10^{-1}	1.19×10^{-2}		2.15×10^{-2}	1.72×10^{-2}
	SD	1.58×10^{-3}				5.48×10^{-4}	-	3.56×10^{-3}	1.79×10^{-3}	2.70×10^{-4}	-	-	-		2.30×10^{-4}	8.87×10^{-4}
	% RSD	5.9				2.94	-	8.33	8.06	1.34	-	-	-		1.07	5.16
	z-score	**2.01**				**-0.71**	**-**	**7.47**	**0.31**	**-0.37**	**-**	**40.5**	**-3.09**		**0.170**	**-1.39**
Hair	**n**	5		5				5		5		2			5	
	THg $_{mean}$ (mg kg^{-1})	1.39		1.26				1.48		1.41		1.49			1.48	
	SD	0.04		0.03				0.04		0.03		-			0.01	
	% RSD	2.7		2.04				2.39		2.23		-			0.42	
	z-score	**-0.08**		**-1.22**				**0.71**		**0.10**		**0.80**			**0.71**	

THg: total mercury; SD: standard deviation; RSD: relative standard deviation

WM: Wet mineralization; PyrAAS: pyrolysis-atomic absorption spectroscopy; AAS: atomic absorption spectroscopy; CVAAS: could-vapour atomic absorption spectroscopy; AFS: atomic fluorescence spectroscopy

Table 16. Continuation.

		020	021	022	023	025	029	030	031	032	033	034	036	037	038
	Pre-treatment	**No**	**No**	**No**	**No**	**No**	**No**	**WM**	**WM**	**No**	**No**	**No**	**No**	**WM**	**No**
	Analytical technique	**TD-AAS**	**TD-AAS**	**TD-AAS**	**TD-AAS**	**TD-AAS**	**TD-AAS**	**TD-AAS**	**CV-AAS**	**TD-AAS**	**TD-AAS**	**TD-AAS**	**TD-AAS**	**CV-AAS**	**TD-AAS**
Soil	**n**	3	5		5	5		5	4		5	5	3	3	5
	THg $_{mean}$ (mg kg^{-1})	27.1	25.4		25.7	25.7		29.6	26.5		27.6	28.0	29.6	26.6	27.4
	SD	0.7	1.4		0.7	1.9		1.3	0.4		1.1	0.9	1.0	2.4	0.3
	% RSD	2.69	5.70		2.71	7.28		4.47	1.35		4.14	3.20	3.39	9.02	1.00
	z-score	**0.03**	**-1.17**		**-0.97**	**-0.97**		**1.74**	**-0.41**		**0.35**	**0.56**	**1.74**	**-0.34**	**0.21**
Sediment	**n**	4	5	5	5	5	3	5	4	5	5	5	3	3	5
	THg $_{mean}$ (mg kg^{-1})	40.3	37.1	41.1	43.2	40.4	16.1	48.4	44.8	40.3	46.2	45.6	44.2	44.7	44.0
	SD	1.8	1.4	0.4	1.8	1.6	2.8	1.4	0.4	1.1	1.0	0.5	0.7	3.7	0.4
	% RSD	4.58	3.75	0.96	4.21	3.91	17.3	2.95	0.87	2.77	2.16	1.15	1.54	8.19	0.90
	z-score	**-1.17**	**-2.34**	**-0.87**	**-0.10**	**-1.14**	**-9.99**	**1.83**	**0.50**	**-1.17**	**1.02**	**0.79**	**0.27**	**0.46**	**0.20**
Fish	**n**	5	5		5	5	2		4		5	5		2	5
	THg $_{mean}$ (mg kg^{-1})	1.95×10^{-2}	2.32×10^{-2}		1.92×10^{-2}	3.06×10^{-2}	1.74×10^{-2}		2.85×10^{-2}		1.68×10^{-2}	2.12×10^{-2}		8.40×10^{-2}	1.95×10^{-2}
	SD	1.80×10^{-3}	2.86×10^{-3}		6.38×10^{-4}	3.65×10^{-3}	-		2.65×10^{-3}		1.28×10^{-3}	9.05×10^{-4}		-	1.37×10^{-3}
	% RSD	9.24	12.3		3.33	11.9	-		9.28		7.59	4.27		-	7.0
	z-score	**-0.37**	**0.65**		**-0.71**	**3.24**	**-0.37**		**2.69**		**-1.39**	**-0.37**		**21.4**	**-0.53**
Hair	**n**	3	5		5	5			3		5	5	3	3	5
	THg $_{mean}$ (mg kg^{-1})	1.15	1.23		1.47	1.36			1.29		1.65	2.26	1.36	2.48	1.25
	SD	0.00	0.03		0.01	0.05			0.02		0.04	0.05	0.02	0.28	0.02
	% RSD	0.119	2.50		0.78	4.03			1.44		2.52	2.40	1.53	11.2	1.8
	z-score	**-2.18**	**-1.48**		**0.54**	**-0.34**			**-0.95**		**2.21**	**7.56**	**-0.34**	**9.49**	**-1.31**

THg: total mercury; SD: standard deviation; RSD: relative standard deviation

WM: Wet mineralization; TD-AAS: thermo-desorption atomic absorption spectroscopy; AAS: atomic absorption spectroscopy; CVAAS: could-vapour atomic absorption spectroscopy; AFS: atomic fluorescence spectroscopy

Table 17. Descriptive statistics regarding the analysis of total mercury in the four test materials and overall z-score percentage for each matrix.

		soil	sediment	fish	hair
raw data	**n**	20	22	21	16
	median (mg kg^{-1})	27.1	43.6	2.10×10^{-2}	1.41
	MAD (mg kg^{-1})	1.3	2.6	3.00×10^{-3}	0.08
	mean (mg kg^{-1})	27.4	42.8	3.18×10^{-2}	1.52
	SD (mg kg^{-1})	3.1	8.8	3.05×10^{-2}	0.36
	RSD	11.5 %	20.60%	96.0%	24.2%
	Grubbs test	no outliers	1 outlier	3 outliers	2 outliers
	minimum (mg kg^{-1})	20.1	16.5	1.19×10^{-2}	1.15
	maximum (mg kg^{-1})	35.9	61.9	1.35×10^{-1}	2.48
without outliers	**n**		21	16	14
	median (mg kg^{-1})		44.0	2.00×10^{-2}	1.38
	mean (mg kg^{-1})		44.1	2.11×10^{-2}	1.38
	SD (mg kg^{-1})	not applicable	6.7	4.81×10^{-3}	0.13
	RSD		15.1%	22.8%	9.6%
	minimum (mg kg^{-1})		27.0	1.20×10^{-2}	1.15
	maximum (mg kg^{-1})		61.9	3.06×10^{-2}	1.65
winsorised	**n**	20	22	19	16
	median (mg kg^{-1})	27.1	43.60	2.08×10^{-2}	1.39
	robust mean (mg kg^{-1}	27.1	43.3	2.11×10^{-2}	1.39
	robust SD (mg kg^{-1})	1.4	2.7	2.94×10^{-3}	0.11
	RSD	5.3 %	6.20%	13.9%	7.9%
	reallocated data	7	6	7	6
z-score	**$\|z\| \leq 2$**	16 (80%)	17 (77%)	12 (63%)	12 (75%)
	$2 > \|z\| \leq 3$	1 (5%)	1 (5%)	2 (11%)	2 (12.5%)
	$\|z\| > 3$	3 (15%)	4 (18%)	5 (26%)	2 (12.5%)

n: number of participants; SD: standard deviation; RSD: relative standard deviation; MAD: median absolute deviation

Twenty-two participants returned results for total mercury determination in sediment. Results ranged from 16.5 to 61.9 mg kg^{-1}, with a mean of 42.8 mg kg^{-1} and a median of 43.6 mg kg^{-1} (Table 17), and the presence of one outlier was confirmed. An assigned value of 43.3±0.6 mg kg^{-1} was attributed to sediment; therefore, 77% of the participants had a satisfactory performance; among them, 65% obtained a $|z| \leq 1$, indicating a very good performance. Additionally, most of these participants also returned results with good repeatability (Table 16). On the other

hand, 18% of participants attained a |z|>3 and should take immediate action in order to understand the reasons for this extreme bias.

Reported mercury concentrations for fish had the highest range of variation, from $1.2x10^{-2}$ to $1.4x10^{-1}$ mg kg^{-1}, which was reflected on the percentage of participants that had a |z|>3 (26%) (Table 17). 63% of the participants had a satisfactory performance, with a $|z| \leq 2$, while 11% had questionable performance.

Sixteen participants returned results for total mercury quantification in hair, which ranged from 1.2 to 2.5 mg kg^{-1}, and an assigned value of 1.39±0.03 mg kg^{-1} was attributed to this test material. 75% of the participants had a satisfactory performance; 13% of participants had a questionable performance, the same percentage that had a z-score above 3 (Table 17).

A general overview of the z-scores obtained for all participants in the four matrices is depicted in Figure 20. Results indicate that, in the total of the four matrices, 74% of participants had a satisfactory performance, with 8% showing questionable performance and 18% requiring action. The highest percentage of satisfactory performance was obtained for soil, while the lowest was observed for fish, which may reflect the analytical problems of quantifying mercury at low concentrations. Moreover, there are additional analytical challenges when analysing fish, as mercury in this matrix is almost entirely in organometallic forms (Ullrich *et al.*, 2001) that are more volatile and easily lost during analysis; hence extra careful manipulation of the samples is needed.

An overview of the results reveals that out of the 21 participants who returned results for more than one matrix, only half had a full set of acceptable z-scores and, out of the 8 participants that chose to analyse only one matrix, 5 had a good performance. However, to properly assess the quality of the results, the repeatability of the independent replicate measurements performed by the participants was also appraised and is represented in Figure 21. It must be noted that a participant with an accurate mean value can still have a large scatter of results. For example, while the majority of participants reported values with good repeatability for soil, participant 017 despite being close to the assigned value, had a poor repeatability (Figure 21). Similar cases were not observed for the other matrices, where participants combined both the proximity to the assigned value with good repeatability. Despite a good overall precision of the participants, it should be highlighted that some provided

inconsistent results, while others only performed two replicate measurements. General reasons for achieving unsatisfactory results might be the lack of experience with this type of samples, random errors in the sample preparation procedure, calculation errors, and/or the incorrect application of internal quality control procedures. Low-biased results, in particular, may result from mercury losses during analysis or deficient calibration of the measurement equipment. Especial attention should be given to potential losses of mercury during pretreatment of samples to be analysed, particularly when involving digestion steps, like wet mineralization for example. High-biased results could originate from contamination during either sample preparation or analysis.

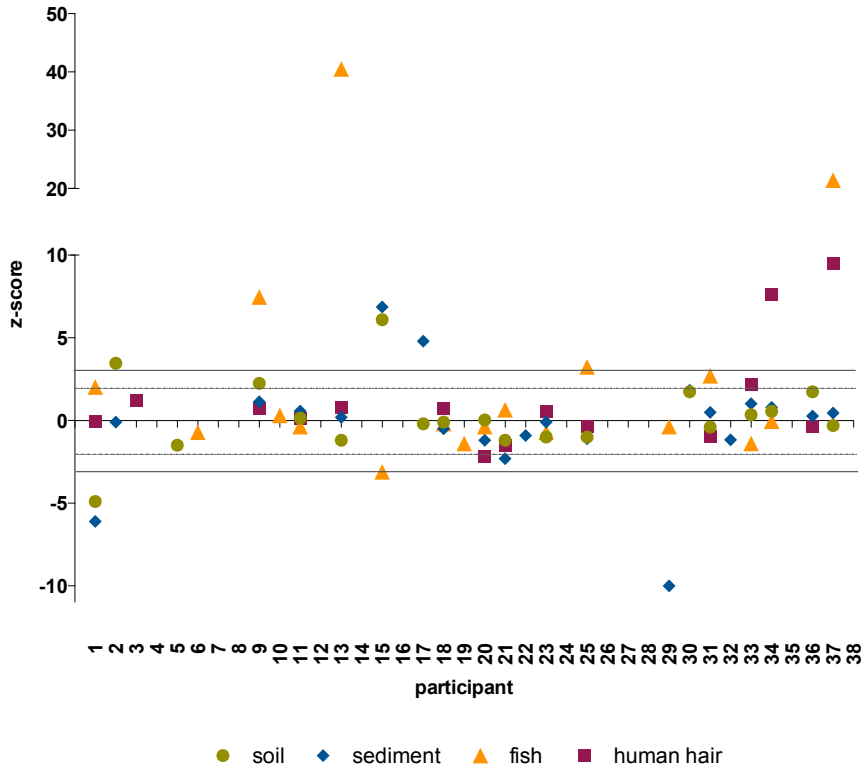

Figure 20. Overview of z-scores of all participants, for the determination of total mercury in the four matrices. Solid line corresponds to z=-3 and z=3; dashed line indicates z=-2 and z=2.

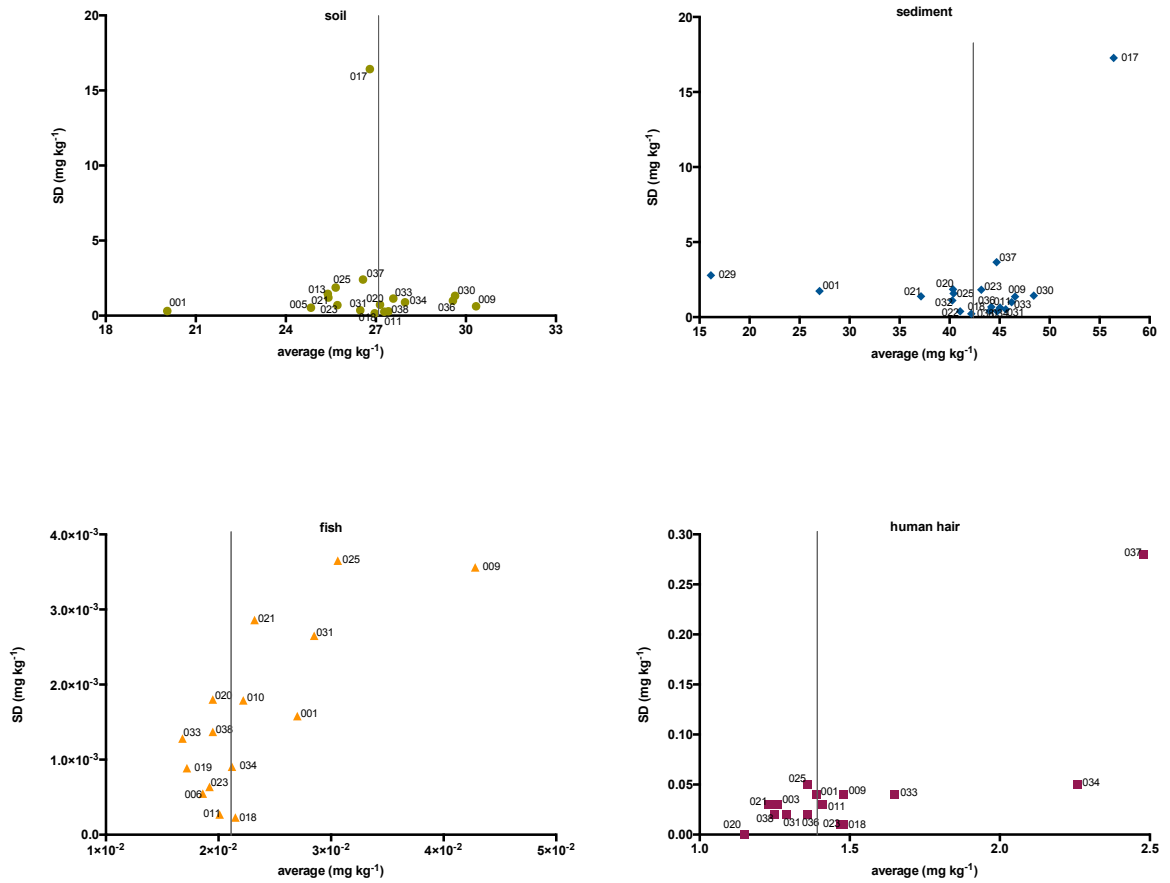

Figure 21. Distribution of mean and standard deviation of replicate measurements: for soil, sediment, fish, and human hair.

The relationship between total mercury concentrations and the analytical procedures used was studied, as well as the influence of pretreatment (wet-mineralization) of the sample (Table 18). Regarding the latter, concentrations were comparable to when wet-mineralization was not performed; fish was an exception, as mean total mercury was almost three times higher when wet mineralization was performed. However, a more careful analysis of the results revealed that these larger median and mean values are a consequence of the results reported by participant 037 and mainly by participant 013, whose errors may have another source. Therefore, excluding these participants and considering the results obtained for the other three matrices, it can be concluded that wet mineralization did not influenced the results.

Table 18. Influence of pretreatment and analytical procedures on total mercury concentration achieved for the four matrices.

	Soil		Sediment		Fish		Hair	
pre-treatment	**yes**	**no**	**yes**	**no**	**yes**	**no**	**yes**	**no**
n	5	15	5	17	3	16	3	13
THg (mg kg^{-1}) mean	27.3	27.348	45.4	42.0	8.25E-02	2.18E-02	1.75	1.44
THg (mg kg^{-1}) median	26.6	27.1	44.8	43.1	8.40E-02	1.98E-02	1.49	1.39
AAS								
n	1		1		1		-	
THg (mg kg^{-1})	27.3		45		2.20E-02		-	
AFS								
n	1		1		1		1	
THg (mg kg^{-1})	35.9		62		1.35E-01		1.49	
CVAAS								
n	3		3		3		3	
THg (mg kg^{-1}) mean	27.8		45.3		5.18E-02		1.75	
THg (mg kg^{-1}) median	26.6		44.755		4.28E-02		1.48	
ICP-OES								
n	1		1		-		-	
THg (mg kg-1)	20.1		27		-		-	
TD-AAS								
n	14		16		13		11	
THg (mg kg^{-1}) mean	27.1		42.0		2.03E-02		1.44	
THg (mg kg^{-1}) median	27.1		43.1		1.95E-02		1.36	

n: number of participants; THg: total mercury
AAS: atomic absorption spectroscopy; AFS: atomic fluorescence spectroscopy; CVAAS: could-vapour atomic absorption spectroscopy; ICP-OES: inductively coupled plasma optical emission spectrometry; TD-AAS: thermo-desorption atomic absorption spectroscopy;

Concerning the analytical techniques chosen to quantify total mercury, TD-AAS and AAS yielded good results. With exception of hair, AFS yielded larger concentrations than the other analytical techniques. Participant 015 was the only to use AFS; the high-biased results may result from other analytical problems rather than from the use of AFS. Another participant used ICP-OES for soil and sediment. As only one participant used this technique, it is impossible to infer general patterns.

A comparison of the results obtained for soil, sediment and fish in ILAE-Hg-02 with the results of the previous study, ILAE-Hg-01 (Pereira *et al.*, 2008a), reveals that the quality of laboratories' performance decreased for all comparable matrices, as a higher percentage of participants had a $|z|>3$, especially in the case of fish. This can be a consequence of the lower mercury concentration of the fish test material used in the current study when compared to the one used in ILAE-Hg-01, a result that demonstrates the challenges of mercury determination at low levels. However, it

should be mentioned that other authors also reported low percentages of satisfactory results ($|z| \leq 2$) for fish analysis with higher mercury contents. For example, Coquery *et al.* (1997) reported 15% and 45% for fish with a mercury contents of 0.1 and 2.2 mg kg^{-1}, respectively, while Frazzoli *et al.* (2005), using blue-fin tuna (*Thunnus thynnus*) with a mercury content of 3.3 mg kg^{-1} obtained 54% of satisfactory results.

From an overall point of view, the results of this study indicate that, while the performance in total mercury determination is satisfactory, further efforts are needed to improve it and minimize differences between laboratories, particularly at the low range of mercury concentrations.

6.3.2.2 Selective extractions of mercury

Only four participants submitted results for the purposed selective extractions. Therefore, due to the low number of results and the differences in the reported values, it was decided not to perform further statistical treatment, and no assigned values were determined.

Three participants reported results for extraction of organometallic mercury fraction and extraction with $CaCl_2$ and four participants reported results for extractions with HCl, and NH_4Ac in soil (Table 19). In all cases, the concentrations obtained were very low. Organometallic mercury had the lowest concentration, while extraction with HCl yielded the largest concentrations. The results concerning the organometallic fraction were distinct among participants (Table 19). However, it is impossible to indicate which participant had the best performance. Out of the three participants that performed the extraction of organometallic mercury in sediment, two (034 and 038) reported very similar concentrations; participant 009 obtained an organometallic mercury concentration about two times lower. Low mercury concentrations were expected in these extracts, as these fractions generally account for very small percentages of total mercury in soil and sediment (Filgueiras *et al.*, 2002; Issaro *et al.*, 2009). This constitutes one problem because most analytical techniques are not sensitive enough to detect such low concentrations.

Table 19. Results of selective extraction for soil, sediment, and fish.

		Organic			0.1 M HCl			0.1 M $CaCl_2$			1 M NH_4Ac		
	lab code	mean	SD	%RSD	mean	SD	%RSD	mean	SD	%RSD	mean	SD	%RSD
soil	009	$1.00x10^{-3}$	0	0	$1.10x10^{-2}$	0	0	$3.20x10^{-3}$	$4.47x10^{-4}$	13.9	$5.00x10^{-3}$	0	0
	011	-	-	-	$1.78x10^{-2}$	$1.81x10^{-3}$	10.2	$5.82x10^{-3}$	$5.51x10^{-4}$	9.46	$1.31x10^{-2}$	$1.81x10^{-3}$	8.96
	034	$<1.60x10^{-2}$	-	-	$9.74x10^{-3}$	$3.97x10^{-4}$	4.08	$<1.60x10^{-2}$	-	-	$3.48x10^{-3}$	$6.76x10^{-4}$	19.4
	038	0.011018	0.001181	10.7166	0.001845	7.94E-05	4.301687	-	-	-	0.000408	2.14E-05	5.243442
sediment	009	$2.00x10^{-4}$	0	0									
	034	$3.68x10^{-2}$	$9.74x10^{-3}$	26.46145									
	038	0.041406	0.001813	4.378483									
fish	006	$1.60x10^{-2}$	$7.07x10^{-4}$	4.419417									
	009	$4.00x10^{-4}$	0	0									
	034	$2.43x10^{-2}$	$1.07x10^{-2}$	44.06756									
	038	0.016942	0.000341	2.014193									

Regarding the extraction of organometallic mercury in fish, participant 009 presented a very distinct concentration from the other three participants (Table 19). It should be noted that participant 034 reported a concentration of organometallic mercury greater than the concentration of total mercury, which cannot be correct. Still, organic mercury extraction in fish yielded more reproducible results than for soil and sediment, and accounted for circa 92% of total mercury. The procedure suggested for this extraction is well established (Válega *et al.*, 2006), is commonly used (Coelho *et al.*, 2008b; Mieiro *et al.*, 2011) and seems to be adequate for this matrix.

6.4 Conclusion

The outcome of ILAE-Hg-02 provided valuable information on the quality of total mercury determinations in organic and inorganic matrices; furthermore, as the participants were representative of six countries, and different operational conditions were used, a representative study was accomplished. The main conclusion was that the majority of participants performed satisfactorily ($|z|<2$) in the determination of total mercury in the four different matrices. Still, significant bias was identified for 18% of laboratories. Therefore, it is expected that the results of this exercise serve as internal quality control for all participants and that they help to detect underlying problems in the analytical work, such as calibration errors, reagent contamination, miscalculations, the cleanliness of the working environment and material and inadequate quality control and quality assurance procedures.

Difficulties were mainly expected in the proposed extractions of specific mercury fractions. The assessment of the accuracy of these particular analyses was an important goal of ILAE-Hg-02. Unfortunately, the low number of results returned did not allow undertaking any definite conclusions, but the low number of participants returning results is, itself, indicative of the reluctance of laboratories to perform mercury extractions. An ongoing limitation to the use of extraction procedures has been the lack of validation and quality control (Quevauviller, 1998). In this context, proficiency testing schemes can be a helpful tool in producing valuable data and information towards the validation of extraction and respective quality control procedures; therefore, in future proficiency test schemes the importance of performing selective mercury extractions must be highlighted to participants.

A thermo-desorption technique for mercury speciation

PART I: Development and validation

PART II: Improvements

7 DEVELOPMENT AND VALIDATION OF A SIMPLE THERMO-DESORPTION TECHNIQUE FOR MERCURY SPECIATION IN SOILS AND SEDIMENTS

I.7 Development and validation of the analytical technique

Highlights

- Simple technique for fast and easy mercury speciation within soils and sediments.
- Thermo-desorption of mercury species, using direct mercury analyser equipments.
- Minimum sample manipulation and no reagents or waste.
- No mercury losses or cross contamination.

Abstract

An innovative technique for rapid identification and quantification of mercury species in soils and sediments was developed, using a direct mercury analyser. Speciation was performed by the continuous thermal-desorption of mercury species (temperature range 76-770 °C), in combination with atomic absorption spectrophotometry detection. Standard materials $HgCl_2$, Hg bound to humic acids and HgS were characterized; thermo-desorption curves of each material showed one well-resolved peak at specific temperature intervals: 125-225 °C, 100–250 °C and 225–325 °C, respectively. Certified reference materials (CRM) BCR® 142R, RTC® CRM 021, NRC® MESS-3 and PACS-2 were tested. Although the CRM were not certified for mercury species, the sum of mercury species obtained was compared to the certified value for total mercury; recoveries were 92%, 100%, 97%, and 95%, respectively. One sediment and three soil samples from mercury contaminated areas (total mercury concentrations 0.067–126 mg kg^{-1}) were analysed as well. It was possible to compare peaks of thermo-desorption curves from the samples with those from standard materials and thereby distinguish different mercury species in solid samples. Generally, mercury was present as bound to chloride or humic substances. The precision was satisfactory, as reflected by the relative standard deviations determined for standards and certified reference materials (<11%; n=10).

Keywords: mercury; speciation; soils; sediments; thermo-desorption

I.7.1 Introduction

In the previous chapters, mercury fractionation was obtained by the application of "chemical" procedures. Due to the above-mentioned disadvantages of conventional sequential extractions, it was critical to develop an approach to address the issue of mercury speciation in a more efficient and less expensive manner. Methods based on species release from the matrix according to their desorption temperature have been previously tested (Biester *et al.*, 1999; Biester *et al.*, 1997a; Biester *et al.*, 1997b; Bollen *et al.*, 2008). So far, such measurements have been carried out with self-constructed apparatus consisting of a sample vessel located within an electric furnace that is directly connected to a heated quartz cell. The pyrolysis unit with the measuring cell was placed inside the detection unit of an atomic absorption spectrometer (Biester *et al.*, 1997a; Biester *et al.*, 1997b; Bollen *et al.*, 2008). Since measurements were carried out under varied operational conditions (for example different heating rates and gas flow) and little is reported about accuracy and reproducibility of the results (Biester *et al.*, 1997a), it is difficult to compare data from literature. Recently, Shuvaeva *et al.* (2008) used a mercury analyser (RA-915+ of Lumex Ltd) for mercury speciation with some "in-house" modifications, in order to perform speciation using this equipment.

In this study the aim was to develop and test a simple procedure for mercury speciation by thermo-desorption, using a direct mercury analyser without modification of the equipment. Even though thermo-desorption techniques are not new, the use of a direct mercury analyser to do so is a significant improvement, as operational conditions can be easily standardised, allowing the intercomparison of results. For this particular work, the Advanced Mercury Analyser (AMA-254), from LECO® was used. To date, this kind of equipments have been used only in determination of total mercury contents or in the quantification of previously chemical extracted mercury species (Pereira *et al.*, 2008a). In-house prepared standard materials were tested in order to characterize mercury compounds. Certified reference materials as well as sediment and soil samples were subsequently analysed. The results obtained by the thermo-desorption method for soil samples

were later compared with those obtained by a sequential extraction method (Reis *et al.*, 2010).

I.7.2 Material and methods

I.7.2.1 Sampling sites and methodology

To test the applicability of the procedures, one soil sample was collected in an agricultural field in Estarreja (sample Industrial 1) and two soil samples were chosen from Caveira mine (samples Mine 2 and Mine 3) collected near the mine pit. One sediment sample from Laranjo Bay was also considered (sample Sediment 4). The description of these locations is given in section 3.1

Soil samples were analysed for the following parameters, according to the methodologies presented in sections 3.2 and 3.3:

- Total mercury content;
- pH;
- Total carbon (TotC) and organic carbon (OrgC);
- Particle size distribution.

Quality control and quality assurance procedures applied in this work have already been described in section 3.5

I.7.2.2 Mercury speciation by thermo-desorption: development and validation of the technique

This technique of thermo-desorption speciation was developed using a LECO® model AMA-254, an equipment commonly used for mercury analysis. The main change introduced was the variation of the temperature at the quartz combustion tube and thereby controlling the release of the different mercury species from the solid matrix. While the temperature cannot be directly controlled, it can be increased by successively increasing the number of active furnaces. LECO® provided a set of 10 points, where temperature is given according to the number of active furnaces. After plotting the number of active furnaces (F) as a function of temperature (T), the

equation that best described the dataset was determined as $T(°C) = -0.096F^2 + 5.2F + 71$; $R^2 = 0.9993$ (Figure 22). Using this equation, temperature was determined according to the number of furnaces that were active at each time.

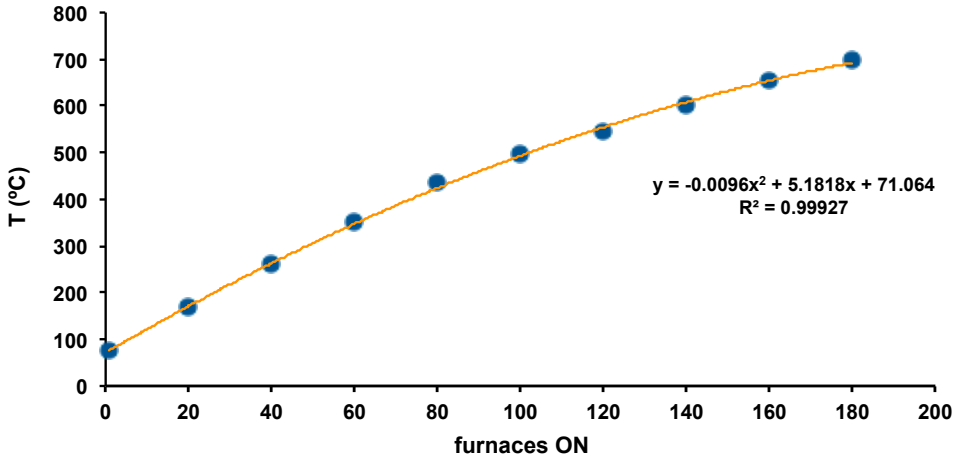

Figure 22. Temperature as a function of number of furnaces ON in LECO® AMA-254.

I.7.3 Standard materials

Four standard materials were used in this work. Synthetic red cinnabar was purchased from Riedel-de-Haën and $HgCl_2$ from Panreac (both pure analytical quality grade). Natural cinnabar was scraped off from a natural mineral specimen, while humic acid-mercury complex was obtained by extraction from a soil sample, according to a procedure adapted from the International Humic Substance Society (International Humic Substance Society, 2008). Since mercury concentrations in these materials were too high to be measured directly, they were diluted by thoroughly mixing with aluminium oxide in an end-over-end shaker, for a period of 10-12 hours. Each material was analysed at least 10 times. Mercury species were characterised by the temperature range they were released at, which consists of the temperature at which thermal-release starts, reaches the maximum and returns to baseline.

I.7.4 Repeatability and accuracy

The thermal-desorption method was applied to four CRM: light sandy soil BCR® 142R and sandy loam RTC® CRM 021 for soil, and marine sediments NRC® MESS-3 and PACS-2 (total mercury concentrations are indicated in Table 20). Although these CRM are not certified for mercury fractions, to determine the accuracy of the procedure the sum of mercury fractions obtained by thermal-desorption was compared to the certified value for total mercury using a t test. The experimental t (t_{exp}) was calculated using Equation 17. No significant difference between t_{exp} and critical t for n-1 was considered the null hypothesis. The repeatability was determined through the relative standard deviation (RSD).

Equation 17 $$\mathbf{t_{exp} = \frac{(x_{exp} - x_{certified}) \times \sqrt{n}}{\sigma_{exp}}}$$

where:

x_{exp} - mean sum of mercury concentration after the thermal-release analysis;

σ_{exp} – standard deviation associated to x_{exp};

n – number of replicates analysed.

The four CRMs were also tested daily to check the equipment's accuracy. At least, three replicates of each material were analysed. Total mercury concentration was found to be within the confidence interval for certified values with recoveries in the range 81–113% and the relative standard deviation (RSD) among replicates was <10%.

Table 20. Sum of mercury fractions obtained at each desorption temperature and recovery compared to the certified value and to total mercury, as determined daily.

CRM	Sum of Hg fractions[a] (mg kg^{-1})	Certified value (mg kg^{-1})	Recovery[b] (%)	Total Hg[c] (mg kg^{-1})	Recovery[d] (%)
BCR-142R	0.058 ± 0.002	0.067 ± 0.011	87	0.063 ± 0.003	92
CRM021	4.9 ± 0.3	4.7 ± 0.4	104	4.9 ± 0.2	100
MESS-3	0.095 ± 0.005	0.091 ± 0.009	104	0.098 ± 0.002	97
PACS-2	2.76 ± 0.21	3.04 ± 0.20	91	2.90 ± 0.12	95

[a] mean ± standard deviation (n=10)
[b] Recovery = (mean sum Hg fractions/certified value)x100
[c] as determined daily (mean ± standard deviation)
[d] Recovery = (mean sum Hg fractions/total Hg daily determination)x100

I.7.5 Results and discussion

I.7.5.1 Analytical performance and validation

Standard materials

The thermo-desorption curves (TDC) obtained for standard materials are shown in Figure 23 (mean and standard deviation). Temperatures of release of $HgCl_2$ and Hg bound to humic acids are similar. Results of solid-phase thermal-desorption indicate that $HgCl_2$ is released in the range of 125-225 °C (Figure 23a), while Hg bound to humic acids is released between 100 °C and 240 °C (Figure 23b); therefore, it was not possible to differentiate the two species. Synthetic HgS is released in the range of 225–325 °C (Figure 23c) and shows a well-resolved peak. In contrast, natural cinnabar shows an “irregular” thermo-desorption curve (Figure 23d). Because it was prepared from a scraping of a natural mineral specimen, it is not guaranteed that a pure substance was achieved. However, the more reasonable explanation is that natural cinnabar decomposes through several steps due to the breakdown of the mineral lattice. The $HgCl_2$, Hg bound to humic acids and synthetic HgS standards were mixed (1:10:1) and analysed. The results (Figure 24) confirm this observation, as only two peaks can be identified: $HgCl_2$ and Hg bound to humic acids overlap. However, the differentiation of these species from cinnabar is attainable, giving a good indication of how reactive mercury present in a sample can be.

The RSD of $HgCl_2$, Hg bound to humic acids, and HgS was 10.8%, 5.9%, and 10.9%, respectively; which were considered acceptable precision values.

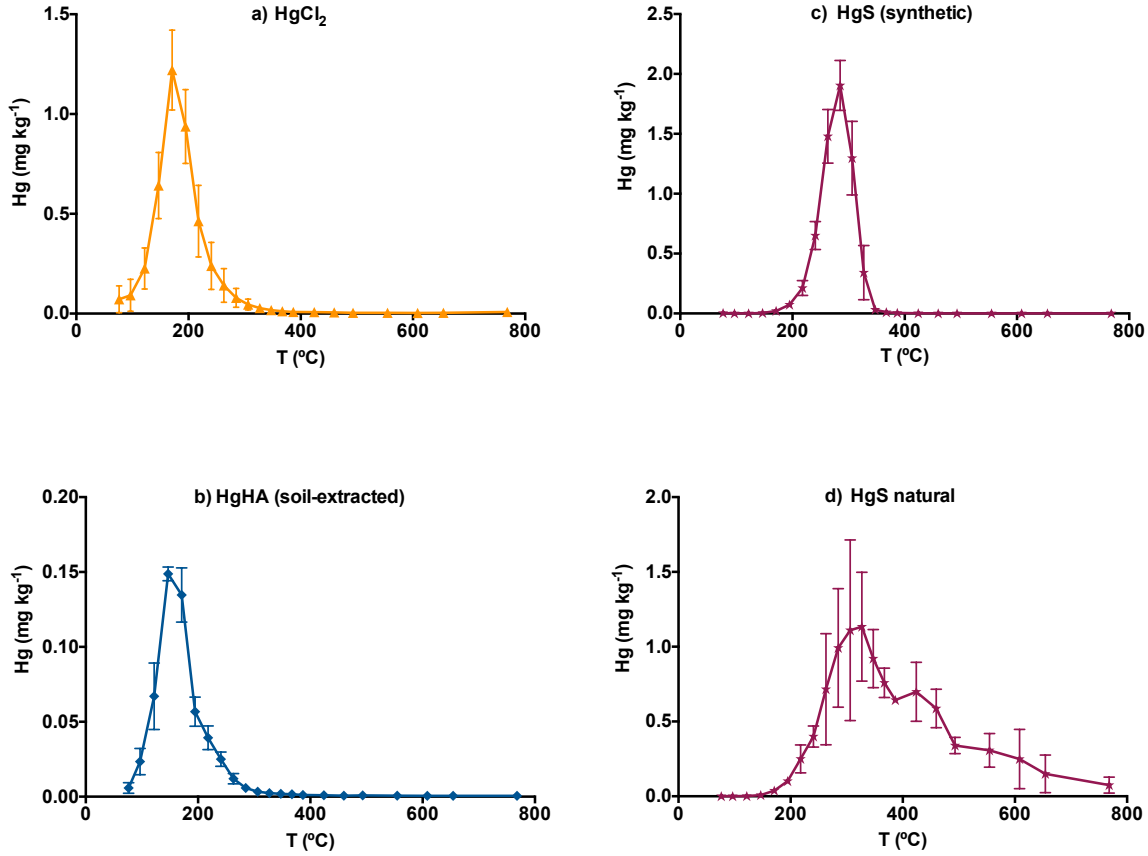

Figure 23. Thermo-desorption curves (mean±standard deviation; n=10) of standard materials. (a) HgCl2; (b) Hg-humic acids; (c) synthetic red cinnabar; (d) natural cinnabar.

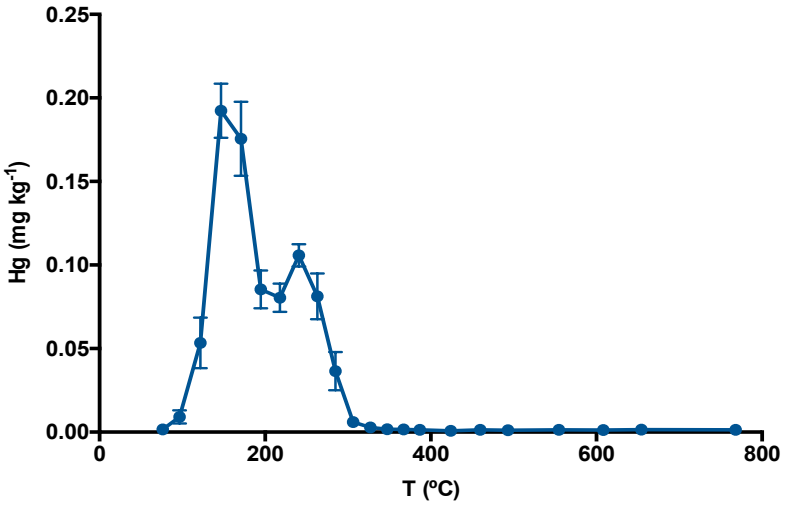

Figure 24. Thermo-desorption curve (mean±standard deviation; n=10) of the $HgCl_2$, Hg-HA and synthetic HgS mixture.

Certified reference materials

The thermo-desorption curves obtained for the CRM are displayed in Figure 25 (mean curve and standard deviation). For BCR® 142R (Figure 25a), one major peak was identified in the temperature range 220–260 °C, which is consistent with $HgCl_2$ and/or Hg bound to humic acids. A second smaller peak was identified at 600–650 °C, which could not be assigned to any mercury compound analysed in this study. In CRM 021 (Figure 25b), the majority of mercury is released at 150-170 °C, which suggests that, again, chloride and/or humic acids species are present in this soil. A second peak can be seen at temperatures above 500 °C, which, according to Biester *et al*. (1999) may correspond to HgO. For MESS-3 (Figure 25c), one single and well-resolved peak was identified at 220–240 °C, which partially overlaps the $HgCl_2$/Hg bound to humic acids region. As can be seen in Figure 25d, PACS-2 only has one peak, in the range of 140-220 °C, which is equivalent to $HgCl_2$ and/or Hg bound to humic acids.

Recovery was within the range of 87–104% (Table 20) and mercury concentration was within the certified confidence interval. In Table 20 is also presented the recovery comparing the thermo-desorption of the different CRM against the mean of total mercury determined daily. This approach is important considering that the response of the equipment is dependent on the condition of the catalytic tube. It is a fact that the equipment's accuracy decreases with time due to deterioration of this component. However, the frequent replacement of the catalytic tube would be extremely expensive and time consuming. Therefore, it continues to be used while the value determined for a CRM is within the certified confidence interval. When recovery was re-calculated considering the concentration obtained daily for each CRM, it improved to 92-100%. The values of t_{exp} for the four CRM analysed were lower than the respective critical value ($p = 0.01$), which indicates that there are no significant differences between the certified and measured values; therefore, the accuracy of the method is considered satisfactory.

The low %RSD (3.4%, 6.1%, 5.3%, 7.6% for BCR 142R, CRM021, MESS-3 and PACS-2, respectively) denotes a good repeatability of the method.

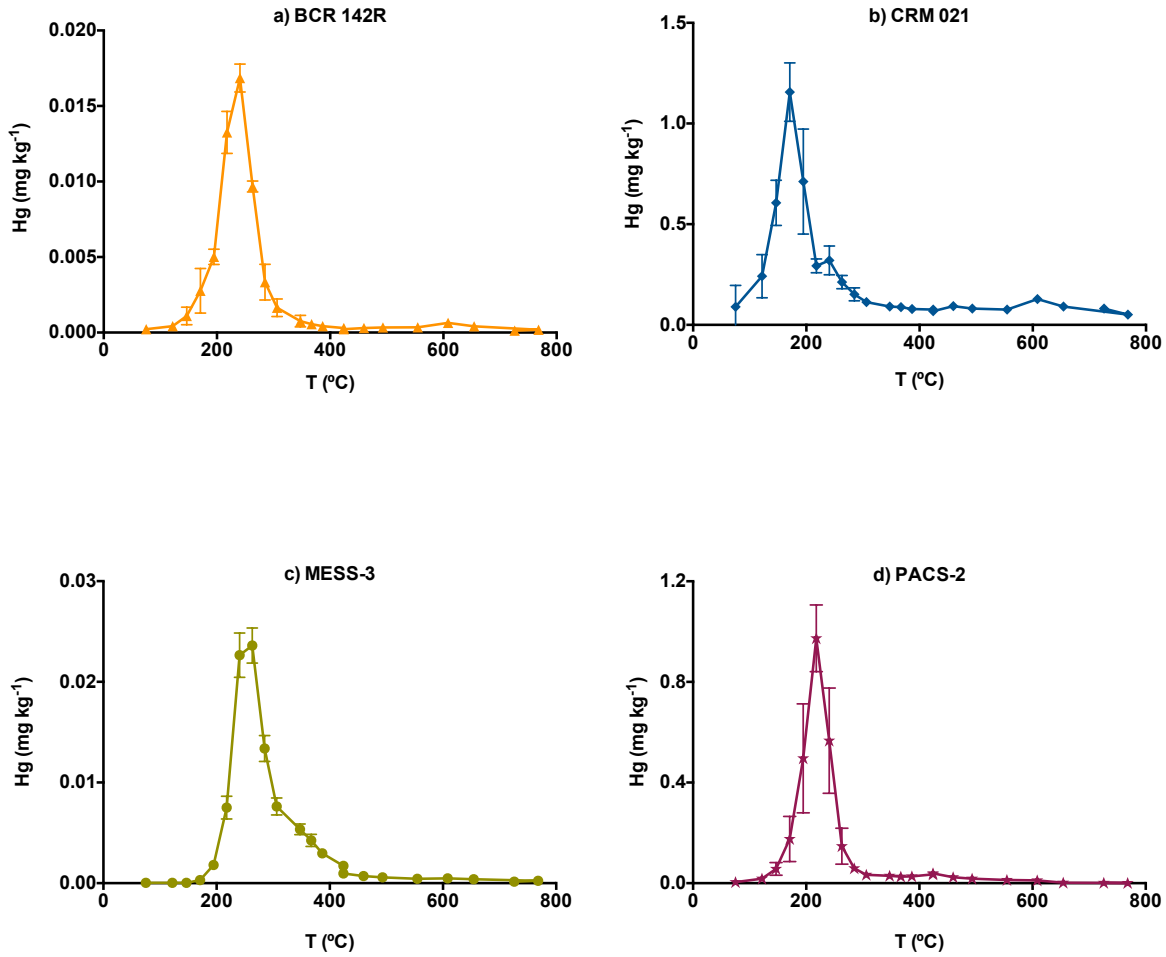

Figure 25. Thermo-desorption curves (mean±standard deviation; n=10) for standard reference materials. (a) BCR 142R; (b) CRM 021; (c) MESS-3; (d) PACS-2.

Soil and sediment samples

The TDC of the four samples analysed are shown in Figure 26 (mean curve and standard deviation). Sample Industrial 1 (Figure 26a) shows one peak, consistent with $HgCl_2$ and/or Hg bound to humic acids and it represents 10.7±0.4 mg kg^{-1} (91% of total mercury). The species have a homogenous distribution, indicated by the low RSD (3.4%, n=7). Hg^0, which is known to be the main species emitted from chlor-alkali plants, was not detected in sample Industrial 1. According to Biester *et al.* (Biester *et al.*, 1997b) this species should be release at temperatures between 70-120 °C, which was not verified in any sample. Lack of Hg^0 may result from re-emission to atmosphere or oxidation to Hg^{2+}. Caveira soils (Mine 2 and Mine 3 – Figure 26 b and c) appeared to contain the same species, because one peak was identified between 125 and 275 °C in the two samples. In both cases, these species

represent a significant percentage of total mercury concentration (84% and 85%, respectively), corresponding to concentrations of 106±6 mg kg^{-1} and 56.2±4.2 mg kg^{-1}. As found for sample Industrial 1, $HgCl_2$ and/or Hg bound to humic acids in Caveira soils also shows a homogeneous distribution, as indicated by the low RSD (< 8%). Mine2 also exhibits a second smaller peak at 450–650 °C, consistent with HgO. This species represents 1.8% of total mercury and has a RSD of 20.3%, indicating that the distribution of this compound in the sample is comparatively more heterogeneous.

A comparison of results of mercury speciation with those from sequential extraction is shown in Figure 26. A previously performed sequential extraction procedure (Reis *et al.*, 2010) revealed that mercury was mainly present (74-98%) as semi-mobile species in the soil samples (mostly Hg^0 and Hg^{2+} complexes - (Han *et al.*, 2003)), with a significant amount of non-mobile mercury (HgS, HgSe - (Han *et al.*, 2003)) being detected in Mine 2 as well (25%). The results of both procedures (thermo-desorption and sequential extraction) are in agreement, considering that $HgCl_2$ or Hg bound to humic matter were the main species identified in all samples, and a stable species (released only at higher temperature) was also identified in Mine 2. Sediment 4 showed two peaks: a major peak is visible at 150–300 °C; that represents a concentration of 0.096±0.005 mg kg^{-1} (78% of total mercury); it also has low RSD (5.0%, n=7). The identification of this compound is not clear, as it partially overlaps the $HgCl_2$ and humic matter peaks; however, the release of mercury at a slighter higher temperature suggests that mercury may be chemically bound to the matrix instead of physically adsorbed (Biester *et al.*, 2002a). Sediments from this area have higher content in organic matter (about 10%) (Válega *et al.*, 2008) than the studied soils (2-3%) (Reis *et al.*, 2009), which may justify the stronger bond to the matrix. A second smaller peak was released in the temperature range of 375-500 °C and it does not correspond to any of the standards analysed in this study. However, Biester *et al.* (1999) found that $HgSO_4$ and HgO were the only compounds to be released above 400 °C. As $HgSO_4$ is not stable under environmental conditions (Biester *et al.*, 1999), it is unlikely that it is present in Sediment4; therefore, HgO is the most reasonable justification for the second peak observed. This species is responsible for 8.7% of total mercury in the sample and

exhibits a higher RSD (19.5%), which can indicate that HgO is heterogeneously distributed. The same was observed in sample Mine 2.

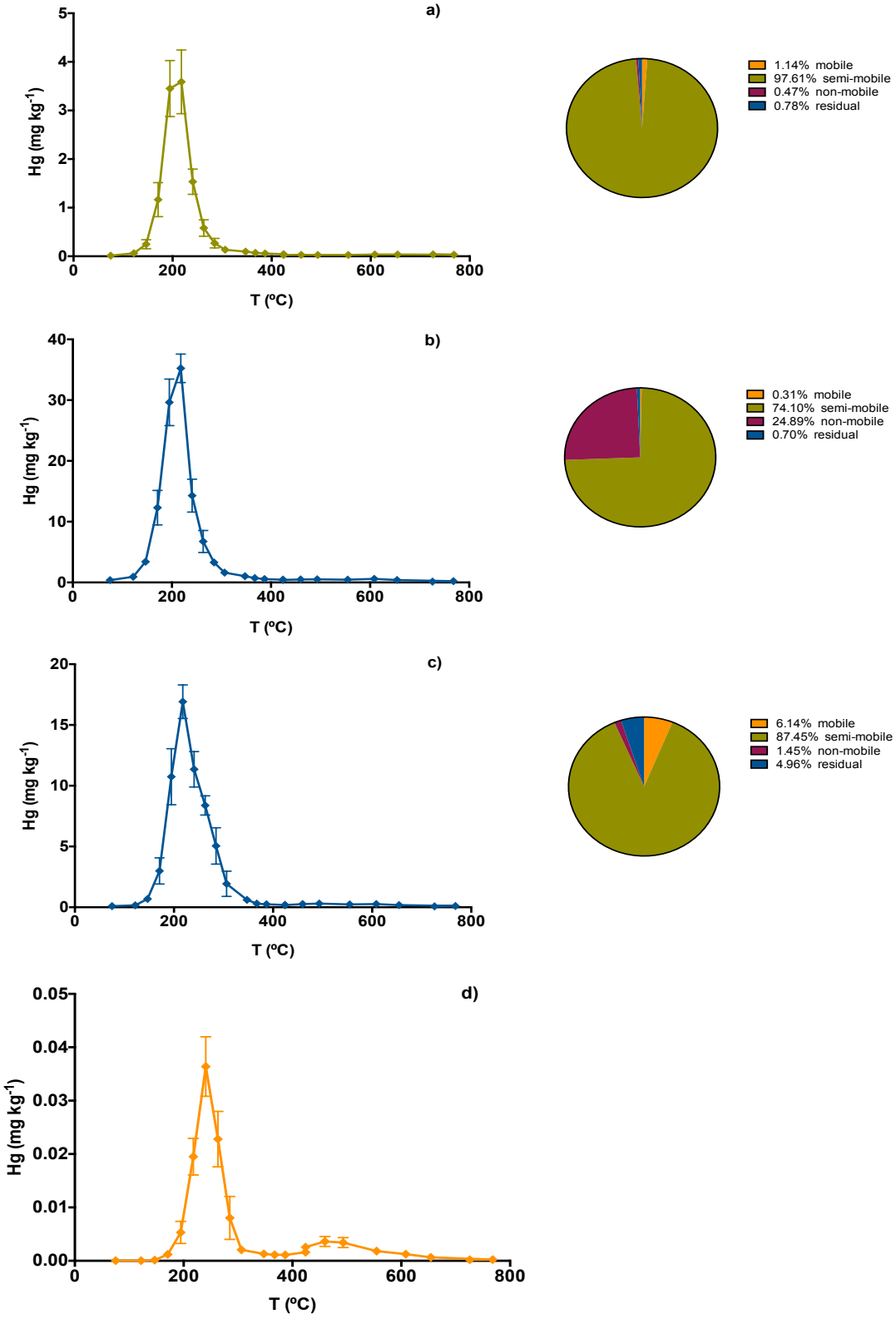

Figure 26. Left: Thermo-desorption curves (mean±standard deviation; n=7) for samples a) Industrial 1; b) Industrial 2; c) Industrial 3; and d) Sediment 4. Right: Hg distribution according to its extractability using Kingston sequential extraction procedure (Chapter 1).

I.7.6 Conclusion

The thermo-desorption technique provides an attractive alternative in mercury speciation, as it allows a fast and relatively easy identification and quantification of mercury species within soil and sediment samples. In this study it was possible to obtain thermo-desorption curves for standard materials such as $HgCl_2$, Hg-humic acids and HgS using an automatic mercury analyser, since each material showed one well-resolved peak at specific temperature intervals: 125-225 °C for $HgCl_2$, 100–250 °C for Hg-humic acids and 225–325 °C for HgS.

The results obtained by the two methods (thermo-desorption and sequential extraction) are consistent, but the thermo-desorption technique offers many advantages over conventional methods for mercury speciation: it is selective, sensitive, allows the prompt identification of several mercury species, is free of cross-contamination, can be applied to a vast range of total mercury concentrations, requires no or little sample preparation which also prevents the loss of volatile mercury-compounds, since the analysis is performed directly on the solid sample. No residues are produced because no reagents are used, and a small quantity (<1 g) of sample is required. It was found that the RSD depends on the occurring mercury compound, but overall, the repeatability of the method is good. Since the equipment used is commercially available, operational conditions can be standardized and results obtained by different laboratories can be easily compared.

The developed technique can be an important contribution for the preliminary screening of the potential risk associated with mercury contamination at a given locale. Even though the complete separation, identification and quantification of all mercury species is still not possible, indication on how they interact with the matrix is attainable, providing relevant information on the potential mobility and availability of the samples' mercury species. In the future, several aspects will be studied, mainly targeting the separation of $HgCl_2$ from Hg bound to humic acids and identification of the additional mercury species.

II.7 Improvements of a simple thermo-desorption technique for mercury speciation in soils and sediments

Highlights

- Standards of iron oxide and humic acids were characterised.
- A separation of mercury bound to humic matter and the mineral fraction in soil and sediment was achieved.
- With increasing temperature, mercury species are released in the following order: $HgCl_2$=Hg associated with Fe_2O_3 > Hg bound to humic acids > HgS > HgO.
- Influence of sample pretreatment and storage on mercury speciation was studied.

Abstract

Mercury speciation by thermo-desorption is a promising alternative to laborious sequential chemical procedures; therefore its popularity has increased in the last years. In this work, with the goal of improving the information obtained by mercury speciation through thermo-desorption, the optimization of a previously developed technique is presented. The thermo-desorption behaviour of mercury bound to iron oxides was characterized, as well as a new Hg-humic acids synthetic standard. Contrary to previous studies, the peak corresponding to the mercury fraction bound to humic acids was clearly separated from the mineral fraction, and identified in some samples. With increasing temperature, mercury species are released in the following order: $HgCl_2$=Hg associated with Fe_2O_3 > Hg bound to humic acids > HgS > HgO. Hence, there is an overlap of $HgCl_2$ and Hg associated with iron oxides.

We also evaluated the effects of sample pretreatment and storage on mercury speciation. It was found that sieving to the < 2 mm fraction improved the sample homogeneity, and the importance of fast sample analysis was highlighted, given that after 10 days of storage at room temperature, volatile Hg^0 could no longer be identified in the sample.

Keywords: Mercury; speciation; thermo-desorption; soil; sediment

II.7.1 Introduction

In the first part of this work (section I.7), the use of a direct mercury analyser for mercury speciation analysis was described, but only a limited range of standard materials was available. However, as mercury behaviour in soil and sediment is complex, and additional materials may act as mercury sorbents, further method development has been undertaken to expand the information that can be obtained. Two new standard materials were considered: Hg associated with iron (III) oxide (Fe_2O_3) and Hg bound to humic acids. The study of Hg-iron oxides complexes in soil and sediment is important due to the role of iron oxides in controling mercury mobility in these matrices. Humic acids had been considered previously. However, while in the previous workthe humic acid-Hg complex was obtained by extraction from a soil sample, in this work, a synthetic humic acid sodium salt was used. Thus all standards were of synthetic origin, and their composition known and well-characterised.

The applicability of the method was tested by analysing soil and sediment samples with different characteristics and mercury origins (natural vs. anthropogenic), and distinct total mercury content. Additionally, as part of the overall method optimization, the influence of sample pretreatment and time passed between sampling and analysis was also assessed. It has been reported that common pretreatment procedures such as air-drying, homogenation, sieving, or storage in plastic bags can be a source of error, particularly in the case of volatile Hg^0 that can easily be lost (Rasemann *et al.*, 1995).

II.7.2 Materials and methods

II.7.2.1 Sampling sites and methodologies

Surface (0-15 cm) samples of soils were collected in the industrialized area of Estarreja (North-East Portugal) and mining areas of Caveira (South-East Portugal), Almadén (Central Spain) and Asturias (Northern Spain). Estuarine sediment samples were collected at the Laranjo basin, Ria de Aveiro (Portugal). The sediment core was then sliced into 1 cm layers for vertical profile characterization.

The description of these locations is given in section 3.1.

Soil samples were analysed for the following parameters, according to the methodologies presented in sections 3.2 and 3.3:

- Total mercury (Tot Hg);
- pH;
- Total carbon (Tot C) and organic carbon (Org C);
- Total iron (Fe) and iron oxide (Fe_ox);
- Sulfur (S);
- Particle size distribution.

Quality control and quality assurance procedures applied in this work have already been described in section 3.5.

II.7.3 Mercury speciation by thermo-desorption

Mercury speciation analysis was carried out using the solid phase thermo-desorption technique presented in section I.7.2.2 Basically, this method involves the thermal release of mercury compounds from the matrix, according to their desorption temperatures. Temperature was increased from 76 ºC to 768 ºC and results are depicted as mercury thermo-desorption curves (TDC), which show mercury release (mg kg^{-1}) plotted against temperature (ºC). The mercury species were characterized by the temperature range at which they were released, from the temperature at which thermal-release starts, through the peak maximum, to the point where the curve returns to baseline. Standard materials were used to identify mercury species.

In the first part of this study, the thermal release behaviour of $HgCl_2$, Hg-humic acids (extracted from a soil) and (red-)HgS was studied. Two new standard materials were now considered: iron (III) oxide (Fe_2O_3) from Panreac and humic acid sodium salt, from Sigma Aldrich. Both were purchased in technical grade. They were found to have a total mercury content of 0.045 and 0.26 mg kg^{-1}, respectively.

Each standard or sample was analysed at least three times, and depending on its total mercury content, 0.5 - 40 mg were weighted for each analysis.

II.7.4 Effect of sample pretreatment and storage

To test the effects that pretreatment and storage may have on mercury speciation, one sample from Estarreja (the sampling location nearest to the laboratory, in order to reduce to minimum the effects of transport) was collected, using the sampling procedure described in section 3.2, and brought to the laboratory where it was immediately analysed (original sample – day 1). The same sample was then analysed after air-drying for 24 hours and sieved to <2 mm (day 2). This fraction was re-analysed after 5 and 10 days. During this time, the sample was stored in a double plastic bag, at room temperature. This storage procedure was chosen to mimic those typically used in soil sampling campaigns.

II.7.5 Results and discussion

The thermo-desorption curves (TDC) obtained for standard materials are shown in Figure 28. A full characterization of the thermo-release behaviour of $HgCl_2$ and synthetic red-HgS can be found in section I.7.5. In the same work, a standard of Hg bound to humic acids was considered, with a release peak between 100 and 240 ºC that overlapped $HgCl_2$. While that humic acid-Hg complex was obtained by extraction from a soil sample, in the current work synthetic humic acid sodium salt was used. In this case, mercury thermo-release behaviour occurs between 194 and 424 ºC and is characterised by a main peak immediately follwed by two smaller peaks. To explain this three-step release behaviour it is important to consider Hg^{2+}-humic acids interaction. Humic acids offer more than one reactive site to which Hg^{2+} can bind. Mercury will preferetially form covalent bonds with reduced sulfur atoms in reactive sites, hence the overlapping of the second peak with that of HgS. However, as only about 2% of these sites actively take part in the binding of mercury, they easily become saturated and additional mercury ions have to bind to oxygen- and nitrogen-containing groups, such as phenolic, carboxylic and amine groups (Gismera *et al.*, 2007). The different bound forms have different stability constants, which lead to the three-step thermo-release of mercury from humic acids. The comparison of the thermo-desorption curves of soil-extracted and synthetic HgHA (Figure 27a) shows the difference between the temperature releases. Humic acids were extracted

from soil using 1 mol L^{-1} HCl, which most likely extracted all labile species; therefore, soil-extracted HgHA is released at lower temperature than synthetic HgHA.

Mercury associated with iron oxides is mainly released between 100 and 285 ºC, while a second, much smaller peak can be observed at 500-610 ºC (Figure 27b). This overlaps with $HgCl_2$. Therefore, it is not possible to distinguish the two compounds if present in a sample. However, characterisation of the sample and consideration of its origin may help to infer the species that is most likely to be present, as will be exemplified below.

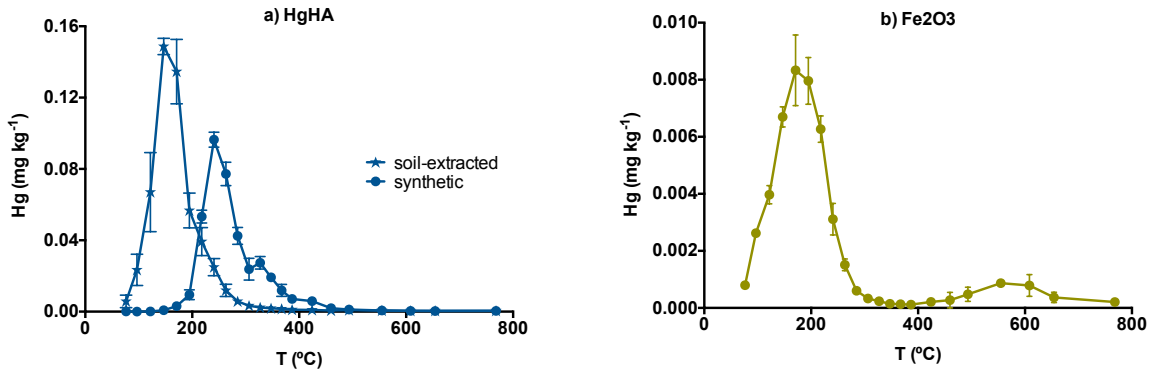

Figure 27. a) Comparison of Hg bound to humic acids standards: HgHA extracted from a soil (★) and synthetic (•); b) Hg associated with iron oxide standard (mean±standard deviation).

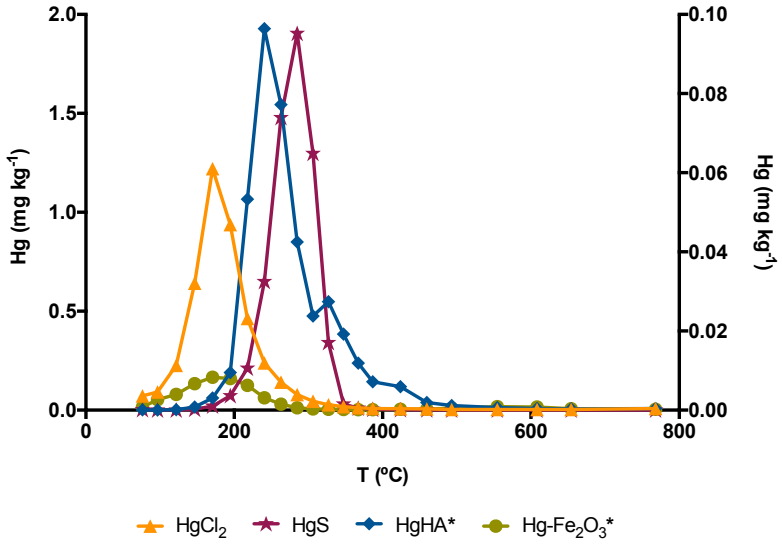

Figure 28. Thermo-desorption curves for Hg species standards: mercury (II) chloride ($HgCl_2$), mercury associated with iron oxides (HgFe) and humic matter (HgHA); cinnabar (HgS).

II.7.5.1 Samples characterisation

Samples characterisation is given in Table 21. In Asturias sample and sediment samples particle size distribution was not determined. Sediment samples were however characterised for their fine fraction (< 0.63 µm) percentage. Organic matter in these samples was estimated by their LOI content.

II.7.5.2 Soil samples speciation

In Estarreja soil sample, thermal-desorption ocurred between 146 and 424 ºC (Figure 29a), although mercury was mainly released in the 146 – 320 ºC temperature interval, which is consistent with Hg bound to humic acids. This is in accordance with the physical-chemical characterisation of the sample, as the acidic pH favours the adsorption to organic matter, and this is in higher abundance than the other soil components likely to bind mercury.

Soil sample Caveira 1 shows one major peak at 125-250 ºC (Figure 29b). This peak does not directly match any of the studied standards, but can be considered as what Biester *et al.* called "matrix-bound mercury " (Biester *et al.*, 1997b). This means that mercury can either be chemically bound to functional groups of organic matter or physically adsorbed to mineral surfaces, but it is difficult to distinguish the species. In fact, both processes can happen, as Hg(II)-organic complexes may be specifically adsorbed onto the mineral surfaces of the matrix, forming organo-mineral mercury complexes. There is also evidence of the presence of cinnabar in the 400-600 ºC interval.

Sample Caveira 2 shows three clearly distinguishable peaks (Figure 29c). The first, released at 120-210 ºC is consistent with $HgCl_2$ or Hg bound to iron oxides. The second peak suggests the presence of complexes of Hg^{2+} with organic matter. The last species that can be identified is possibly cinnabar. Although the TDC does not completely match that of the HgS standard, it has been reported that mercury in natural cinnabar is released at higher temperatures when compared to synthetic HgS (Biester *et al.*, 2000). This is mainly due to the breakdown of the cinnabar lattice, in a process that causes the sudden release of ‘pulses’ of mercury; hence, decomposition occurs in several steps, which explains the presence of more than one "peak" at this stage.

Table 21. Soil and sediment samples characterisation.

PORTUGUESE SOIL SAMPLES										
Sample	**Hg (mg kg^{-1})**	**pH ($CaCl_2$)**	**Org C (%)**	**Fe (%)**	**Fe_ox (%)**	**S (%)**	**sand (%)**	**silt (%)**	**clay (%)**	**USDA texture class**
Estarreja	1.2	4.8	1.66	1.14	0.65	0.11	18.62	71.00	10.38	silt loam
Caveira 1	6.9	3.3	0.51	4.2	10.42	<0.05	25.26	61.28	13.46	silt loam
Caveira 2	34.2	2.9	0.93	6.56	11.23	0.42	49.87	39.77	10.36	loam

SPANISH SOIL SAMPLES									
Sample	**Hg (mg kg^{-1})**	**pH ($CaCl_2$)**	**Org matter (%)**	**Fe (%)**	**S (%)**	**sand (%)**	**silt (%)**	**clay (%)**	**USDA texture class**
Asturias	153.6	6.5	9.9	4.26	0.23	-	-	-	
Almadén	64.8	5.4	1.3	2.7	0.12	43.16	30.6	26.24	loam

SEDIMENT SAMPLES						
Sample depth	**Hg (mg kg^{-1})**	**pH ($CaCl_2$)**	**LOI (%)**	**Fe (%)**	**Fe_ox (%)**	**Fraction <0.63 µm (%)**
Laranjo$_{0\text{-}1cm}$	8.8	6.5	41.2	3.19	1.89	33
Laranjo$_{2\text{-}3cm}$	4.3	6.1	30.2	4.03	2.54	34
Laranjo$_{5\text{-}6cm}$	6.3	6.1	22.5	3.56	2.56	21
Laranjo$_{10\text{-}11cm}$	5.8	6.2	28.4	3.28	2.17	26
Laranjo$_{14\text{-}15cm}$	11.5	6.1	31.2	3.39	0.57	30
Laranjo$_{20\text{-}21cm}$	6.9	6.2	31.3	3.47	0.56	20
Laranjo$_{25\text{-}26cm}$	50.9	6.4	32.8	4.04	0.58	32
Laranjo$_{30\text{-}31cm}$	26.1	6.8	30.7	3.94	0.52	26
Laranjo$_{38\text{-}39cm}$	0.7	6.9	26.4	3.15	0.50	28

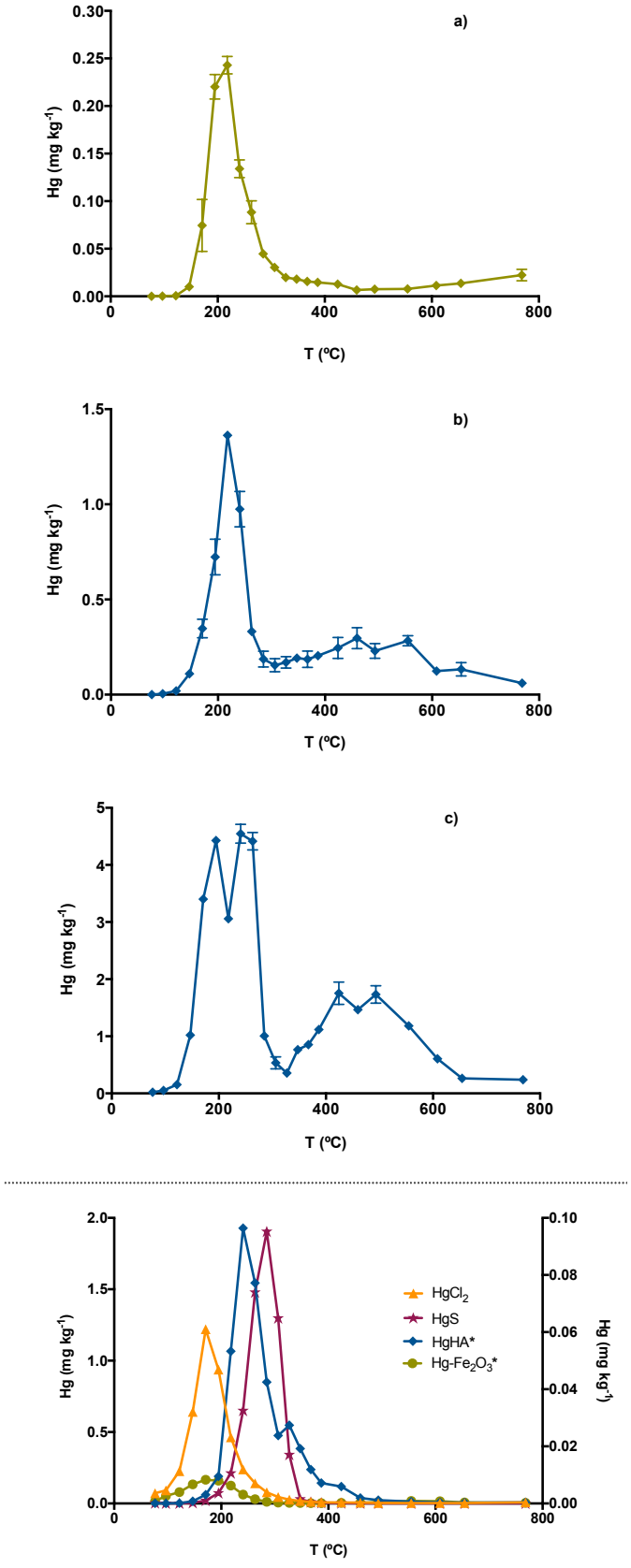

Figure 29. Thermo-desorption of Portuguese soils (mean ± standard deviation, n=3). a) Industrial soil of Estarreja; b) Mine soil of Caveira 1; c) Mine soil of Caveira 2. Bottom: Hg species standards.

The thermo-desorption curves for Spanish mine soils from Asturias and Almadén are presented in Figure 30 a and b, respectively. In the Asturias soil, the position of the first peak suggests that mercury is complexed with organic matter, but also iron; cinnabar was also identified in the sample, but in lower concentration. Soil chemical composition corroborates the results of the thermogram, as Asturias soil is constituted by 9.9% of organic matter and 4.3% of iron, and 0.23% of sulfur, three strong mercury adsorbants. The mercury in Almadén soils seems to be present mainly as cinnabar, as would be predicted considering the source of this sample is a former cinnabar mine.

Comparison of thermo-desorption curves of Portuguese and Spanish soils reveals that higher standard deviations are observed for the latter. This heterogeneity may be related to the variation in the size of cinnabar crystals.

II.7.5.3 Sediment samples speciation

The total mercury vertical profile of the sediment core is shown inset in Figure 31. The higher concentration between 20 and 30 cm corresponds to the years of a chloar-alkali plant effluent discharges. In order to evaluate differences between top and deeper layers, or between less and more contaminated layers, some sections were chosen for speciation analysis. The results of mercury thermo-desorption measurements in the sediment vertical profile show that, with exception of section 0-1 cm, until 20 cm deep the profiles show a thermo-desorption curve with one peak between 140 ºC and 280 ºC that indicates the occurrence of mercury forms associated with mineral components such as iron oxides, chloride ions, or organic matter. Adsorption of Hg^{2+} on sediments is a complex process controlled by a number of parameters, such as pH, temperaure, mercury concentration, composition of sediment and aqueous media, presence of other cations (e.g. Fe^{3+}, Al^{3+}, Mn^{2+}, Ca^{2+}) and anions (such as S^{2-}, SO_4^{2-}) (Pelcová *et al.*, 2010). Vegetated Laranjo sediments contain about 20-40% organic matter and have iron oxide contents that range between 0.50 and 2.6 % (Table 21). Due to the high organic matter content, it would be expected that mercury would be mainly adsorbed to that phase, but this was not the case. The pH in these sediments is close to neutral, varying from 6.1 to 6.9 (Table 21).

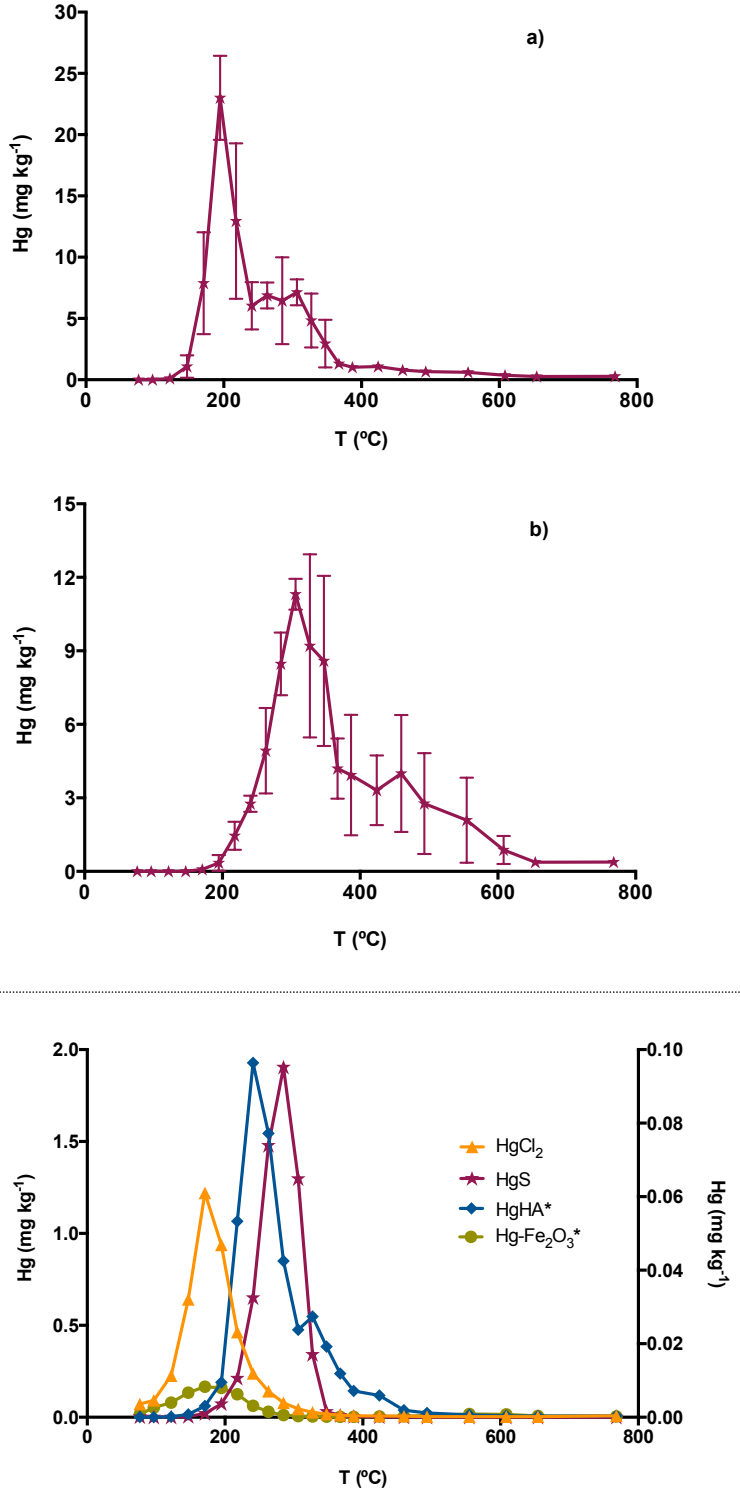

Figure 30. Thermo-desorption of Spanish mine soils (mean ± standard deviation, n=3). a) Asturias; b) Almadén. Bottom: Hg species standards.

Mercury adsorption to sediments is pH-dependent and adsorption to mineral particles is favoured when pH is neutral to alkaline (Gabriel *et al.*, 2004). After 20 cm deep, the maximum mercury release is slightly shifted to higher temperature and is consistent with mercury complexated with organic matter. Speciation of the superficial layer (0-1 cm) also revealed that mercury is mainly present as organic complexes. $HgCl_2$ was not identified, but they can be easily lost to the water column ($HgCl_2$ solubility in water is 7.4 g/ 100 mL, T=20 ºC). Also, mercury adsorption to iron oxides is diminished in the presence of chloride ions (Skyllberg, 2010). A second, smaller peak between 400 ºC and 490 ºC was detected in all layers and is usually attributed to the presence of mercury oxide (Biester *et al.*, 2000).

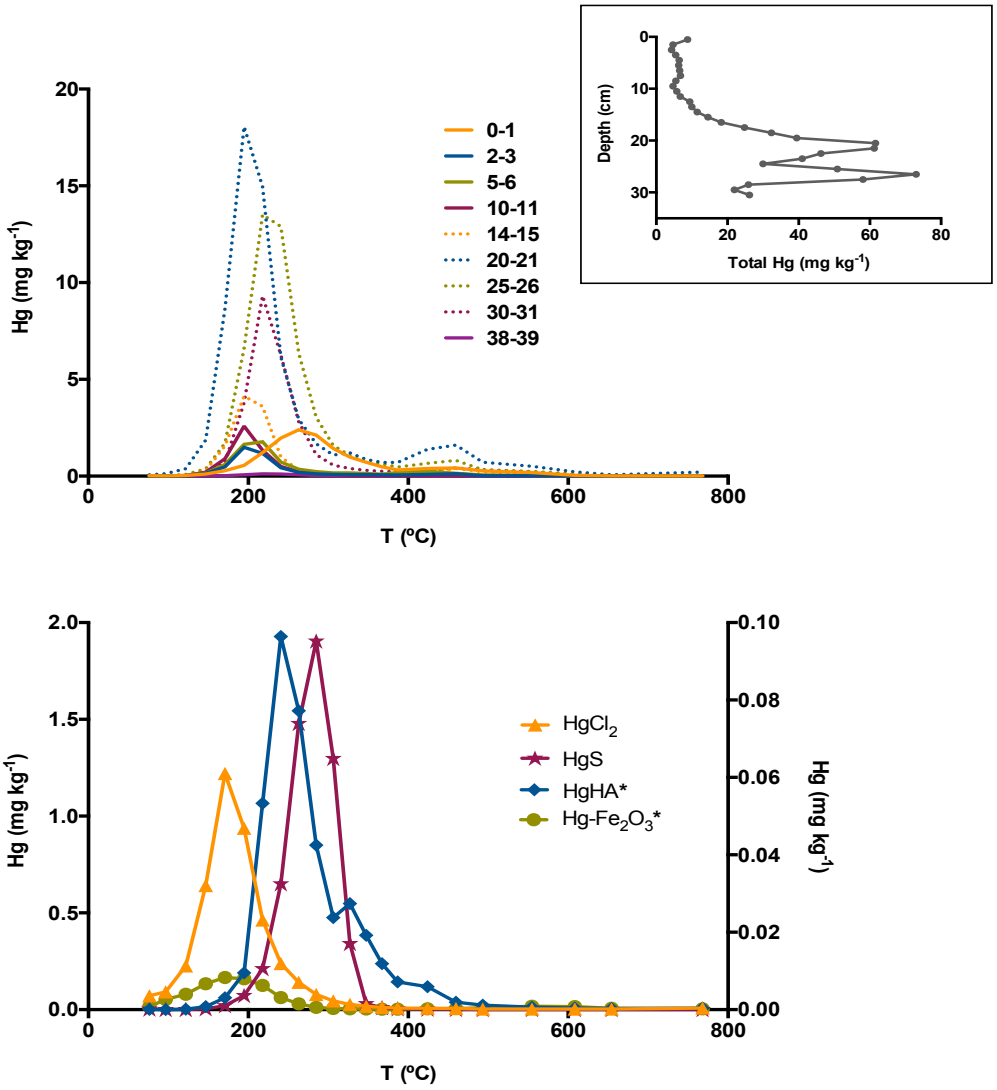

Figure 31. Thermo desorption curves of Laranjo sediments profile (mean ± standard deviation, n=3). Inset: total mercury concentration of the profile. Bottom: Hg species standards.

II.7.5.4 Effect of sample pretreatment and storage

The effects of sample preatment and storage in mercury speciation were studied by analysing the same sample straight from the field, and then the <2 mm fraction after air drying for 24 hours, 96 hours, and 10 days. As the sample was collected in the summer, it dried in less than 24 hours. Figure 32 shows the thermo-desorption curves for each day. Surprisingly, a peak below 100 °C was identified, which should correspond to Hg^0 (Biester *et al.*, 2000). This peak has not been observed previously in samples from the same location. As can be seen in Figure 32, the thermo-desorption curve changes significantly over the 10-day period; the disappearance of the Hg^0 peak is noticeable and it is likely that the species is lost during storage due to its extremely volatile character. All other samples analysed and presented in the first part of this chapter and in the current work were stored for longer periods; therefore, Hg^0 could have been present in the samples but lost before analysis. Another conclusion that can be drawn from Figure 32 is the higher heterogenity of the original sample, as revealed by the higher standard deviations. After drying and sieving, the heterogeneity is reduced, as lower standard deviations were achieved. Among the different species, Hg^0 has a higher associated standard deviation. This may reflect that fact that this species is heterogeneously distributed when it is deposited in soil, as opposed to formed by secondary reduction of Hg^{2+} (Biester *et al.*, 1997b).

It is noteworthy that 10 years after the change of production method from mercury to mebrane-cells in the Estarreja chlor-alkali plant, Hg^0 can still be found in the surrounding environment, confirming the persistence of this element in the environment.

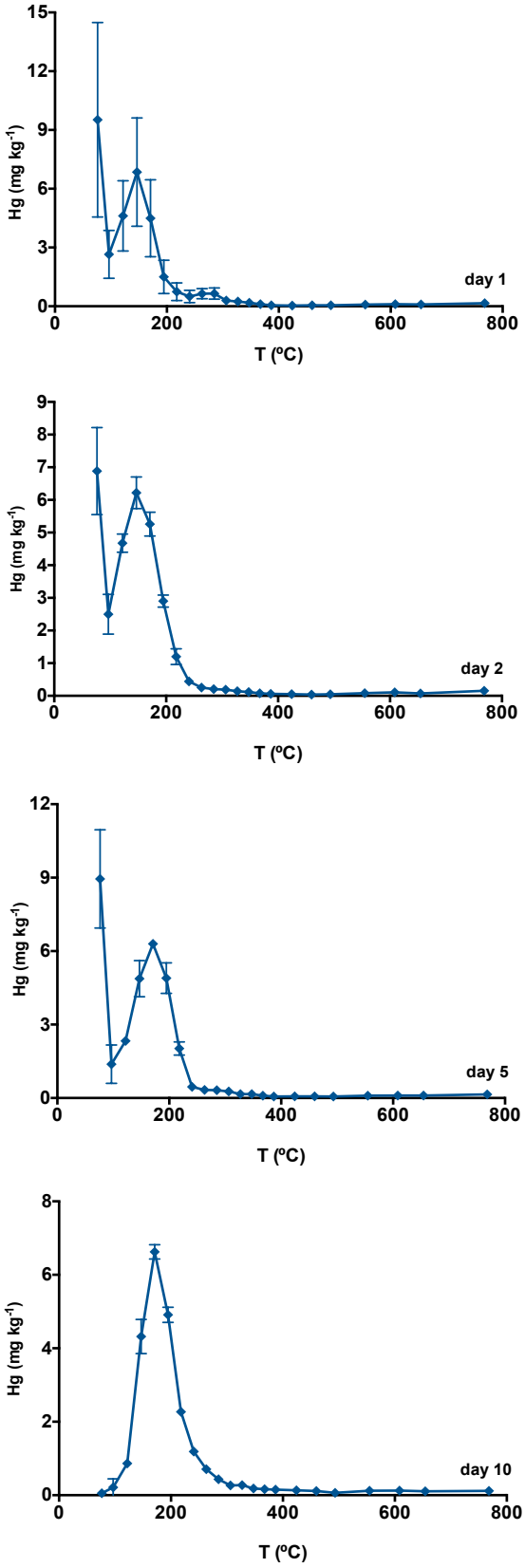

Figure 32. The effects of sample pretreatment and 10-day period storage on the thermo-desorption curve of a soil sample (mean±standard deviation). From top to bottom: day 1, day2, day 5 and day 10.

II.7.6 Conclusion

This study advances mercury speciation in soils and sediments by thermo-desorption. By using only synthetic standards, the peak corresponding to mercury bound to organic matter was able to be separated from other constituents. This is an important step towards differentiation of the mineral and organic fraction, the so-called “matrix-bound” fraction, defined by *Biester et al.* (1997b), and it was possible to identify both fractions in real samples. However, it was still difficult to completely separate all mercury species. Therefore, thermo-desorption cannot be considered a stand-alone tool in mercury speciation analysis. Knowledge of the physico-chemical characteristics of the sample and of whether mercury is of geogenic origin or results from anthropogenic input is essential to complement and interpret the results. Samples where mercury was of geogenic origin showed higher standard deviations between replicates. This was mainly due to the release of mercury in cinnabar, which involves diffusion from inner-sphere sorption sites during the breakdown of the mineral lattice.

This work also proved that samples stored in plastic bags need to be analysed soon after collection, in order to obtain full information on mercury speciation. Longer storage periods can result in loss of volatile Hg^0. After only 10 days the peak corresponding to Hg^0 no longer appeared in the thermogram. Sieving to < 2 mm was beneficial, as homogenation of the sample was improved.

Final considerations

8 FINAL CONSIDERATIONS

Due to the well-known toxicity of mercury and because of growing awareness for risk assessment and remediation of contaminated sites (Ure *et al.*, 2002), interest in mercury speciation/fractionation in soils and sediments has increased in the past decades. But despite numerous researchers dedicating effort to this matter, mercury speciation/fractionation has been proven to be a difficult task.

Mercury speciation and fractionation in soils and sediments is for several reasons challenging. Ligands binding mercury in these matrices are numerous and soils and sediments are naturally heterogeneous, and therefore their structure and association to mercury is difficult to determine. As a consequence, various procedures can be found in the literature to assess mercury speciation/fractionation. Therefore, the first task consisted on an exhaustive review of the methodologies available in literature. It became clear that sequential extraction procedures are the most common choice for mercury fractionation (Bloom *et al.*, 2003b; Fernández-Martínez *et al.*, 2003; Han *et al.*, 2003; Revis *et al.*, 1989; Sakamoto *et al.*, 1992). It also became evident that with many variables involved, such as extractant reagents, time of extraction, or mass:liquid ratio, the results obtained could not be readily intercompared due to the use of different procedures.

Additionally, it was understood that the results obtained by the extraction schemes could be influence by 1) the types of reagents used and the operational conditions to extract each fraction; 2) the matrix from which the metal is to be extracted (matrix effects). For this reason, all procedures were tested in well-characterized samples, where the principal physico-chemical factors that affect mercury speciation in soils and sediments (such as pH, organic matter, Fe, Mn, and sulfur contents, and particle size distribution) were determined. Sample selection criteria took into account a wide range of total mercury concentrations (because total mercury too can influence speciation) and sample origin. It is generally recognized that in anthropogenically-contaminated soils and sediments, mercury is more likely to be present in more labile species (Ratuzny *et al.*, 2009). Considering this, sample assortment allowed studying and understanding not only the influence of sample composition in mercury speciation/fractionation, but also matrix effects.

The first approach consisted of testing some selected sequential extraction procedures, in order to evaluate the information each could provide, the difficulties and challenges associated, and the feasibility of application of the methods in routine analysis.

The *Kingston method* (Han *et al.*, 2003) was chosen and the first to be tested because it differs from other chemical sequential extraction procedures, since it classifies fractions according to their potential mobility - mobile, semi-mobile, and non-mobile. This could be more environmental relevant, particularly the quantification of the mobile fraction. Also, this procedure involved fewer steps than most sequential extraction procedures and, most importantly, was acknowledged by EPA - Method 3200 - (EPA, 2005), as a specific sequential extraction procedure for mercury, which had already been subjected to an inter-laboratory validation (Rahman *et al.*, 2005). Therefore, this procedure seemed to be well underway to become a standard method for mercury fractionation. However, in the literature, the application of this method is not often found. During the course of this work, the application of the Kingston method to soil samples from industrially impacted area of Estarreja and mine area of Caveira yielded good recoveries (sum of fractions in relation to total mercury). There were clear differences between Caveira and Estarreja samples regarding mercury fractionation, and it was possible to study the influence of soil properties on fractionation. Although there were several factors to consider regarding mercury distribution in these soils, which varied with the sample, the results indicated that, in general, manganese and aluminium contents are related to mercury mobility, while organic matter and sulfur retain mercury.

So why is this method not more commonly used? While the results provided were satisfactory, the method presented a few drawbacks. Even though it has only three extraction steps, the fractionation of mercury proved to be time-consuming, difficult and very complex, aspects that limit the procedure robustness. It required two complete full days to obtain the results (one for extraction, one for analysis), and an elevated technical skill to ensure the quality of the results, as corroborated by the inter-laboratory test organized by Rahman *et al.* (2005): "*Most of the laboratories do not routinely perform speciated measurements; this was reflected in the data*.". It should be noted that cross-contamination of samples and mercury losses, for example, can easily occur, if the operator is not sensitized for these problems. These disadvantages could be the reason to constrain its use on a regular basis.

The semi-mobile fraction represented the major portion (between 63 and 97%) in the soils analysed (Reis *et al.*, 2010), but the environmental significance of this fraction is not completely clear. According to Han *et al.* (2003) it includes Hg^0 and Hg^0-metals, some (unspecified) mercury complexes and minor fraction of Hg_2Cl_2, but the presence of the first species is questionable. It was proven that Hg^0 is easily lost, even in short periods of sample storage, due to its high volatility (section II.7.5.4), so it is unlikely that after the vigorous treatment involved in extraction of mobile and semi-mobile fractions, Hg^0 is present in the extracts. In either case, since this fraction was extracted with 1:2 HNO_3:distilled water, it should be mobilised only in extremely acid conditions. The fact that mercury was found in the residue reflects the presence of persistent detrital compounds (e.g. sulfides and the more resistant Fe-oxides) that cannot be mobilised even after the aggressive final extractive stage of the scheme.

Because the complexity of sequential extraction procedures is recognizably high, one could simplify and concentrate on the more reactive and bioavailable fractions, prioritizing environmental relevance. There is extensive evidence that neither total nor dissolved aqueous metal concentrations are good predictors of metal bioavailability and toxicity (Janssen *et al.*, 2000), and the importance of explicitly considering bioavailability in the risk assessment of contaminants has been demonstrated. Accordingly, the more labile, reactive fractions that represent a higher risk for the environment and life need to be accurately determined and quantified. These fractions are the most important in risk assessment; therefore adequate procedures are necessary. Hence, the procedures considered next targeted the water-soluble, exchangeable and generally labile fractions.

Water-soluble fraction procedures found in the literature differed in soil:water ratio and time of extraction. The comprehensive study performed revealed that 1) even in samples with high total mercury content the water-soluble fraction represented a very low quantity (< 0.5 mg kg^{-1}), which is a challenge in mercury quantification; 2) the reaction only reached equilibrium at 24 hours. No procedure considered this time of extraction (Table 8); in the procedures suggested by Renneberg and Dudas (Renneberg *et al.*, 2001), Biester and Scholz (Biester *et al.*, 1997b), Panyametheekul (Panyametheekul, 2004) and Bloom *et al.* (Bloom *et al.*, 2003c), to cite just a few, time of extraction varies between 30 minutes to 18±4

hours. Applying the equations obtained with kinetic fitting, it is possible to determine that in the first hour, less than half of the water-soluble fraction is extracted, hence longer extraction values are needed.

In the study of exchangeable fraction two reagents were considered: 1 mol L^{-1} NH_4Ac and 0.1 mol L^{-1} HCl that release mercury weakly bound electrostatically to organic and inorganic sites by cationic exchange, and by dropping the pH, respectively. Among the salt solutions used in leaching of the exchangeable fraction (e.g. $CaCl_2$, $MgCl_2$), 1 mol L^{-1} NH_4Ac was chosen because the metal complexing power of acetate supposedly prevents readsorption of the release metals (Filgueiras *et al.*, 2002). However, desorption/adsorption phenomenon was observed during the extraction, not only with 1 mol L^{-1} NH_4Ac, but also with 0.1 mol L^{-1} HCl. Considering these results, it is recommendable to always perform kinetic extraction when assessing the exchangeable fraction, to avoid underestimation of the real value.

Extraction with 0.5 mol L^{-1} HCl has been presented as good estimator for the more labile fraction of the metal (Sutherland *et al.*, 2008). With the kinetic extraction, two extraction rates were identified: in the first 10 hours mercury is released at a faster rate and that corresponds to the most labile species among the labile fraction; after that period mercury is released slower and species that are more intricately associated with the matrix, but still labile, are released. Extractions with 0.5 mol L^{-1} HCl never reached equilibrium, which suggests that if the right environmental conditions are prevalent (acidic environments), mercury can be slowly, but continuously released into other environmental compartments.

From a general point of view, kinetic extractions are practical in the sence that they only require one reagent. This is an advantage since many reagents are mercury-contaminated, something that may not be significant in more concentrated fractions, but that can overestimate the results in the less concentrated extracts. The results are not presented, but during the course of this work, the problem of mercury contamination in some reagents was experienced. Kinetic extraction also permitted to understand that the rate of mercury released in the environment is strongly influenced by soil texture. The presence of small particles slows the process, as a diffusion mechanism is involved.

In summary, the results of this work allowed understanding that mercury retention in soil is controlled by its chemical composition (sulfur and organic matter), but the rate of desorption is controlled by its physical properties (particle size).

Although the extent to which laboratory-leaching tests predict mobility in the field under environmental conditions is uncertain, the extraction schemes presented in this study can be useful to assess the potential mobility and bioavailability of mercury. A comparison of the chemical extraction procedures, concerning the most potentially available and labile fractions is presented in Figure 33. The water-soluble fraction was not included in this analysis, because of the extremely low concentration. As can be seen in the figure, the results yielded for the mobile fraction (extracted by the acidic ethanol solution) using the Kingston procedure are similar to the ones obtained using 0.5 mol L^{-1} HCl, confirming that the first step does extract the more labile mercury species. In both cases, mercury extracted is superior to the amount extracted by any of the reagents used for the exchangeable fraction. Both mobile and labile fractions appear to include not only the easily available (water-soluble and exchangeable) mercury, but also fractions of metal that could be mobilized at a particularly acidic pH (pH < 3), such as the metal adsorbed to amorphous iron oxides, to organic matter and to a lesser extent clay. Figure 34 presents the map of soil pH (Atlas of the Biosphere, 1998) and while soils rarely fall into the ultra acid category (pH < 3.5) (Soil Survey Division Staff, 1993), some occurrences such as acid rain, mine spoil, weathering of minerals, plant root activity or high rainfall can lower the soil pH, making it more susceptible to the leaching of labile mercury species.

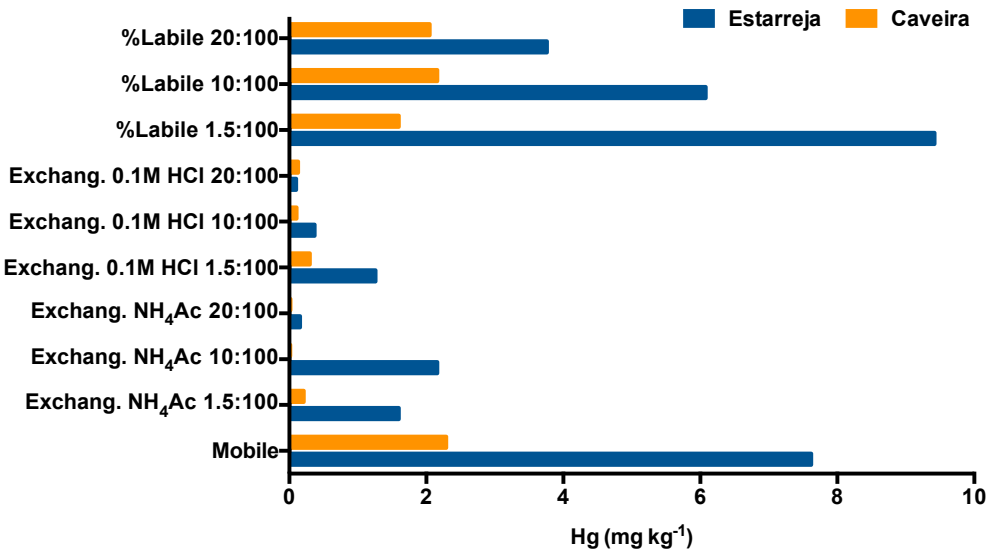

Figure 33. Comparison of mercury extracted (mg kg^{-1}) in the potentially more available fractions, exemplified for Estarreja and Caveira samples.

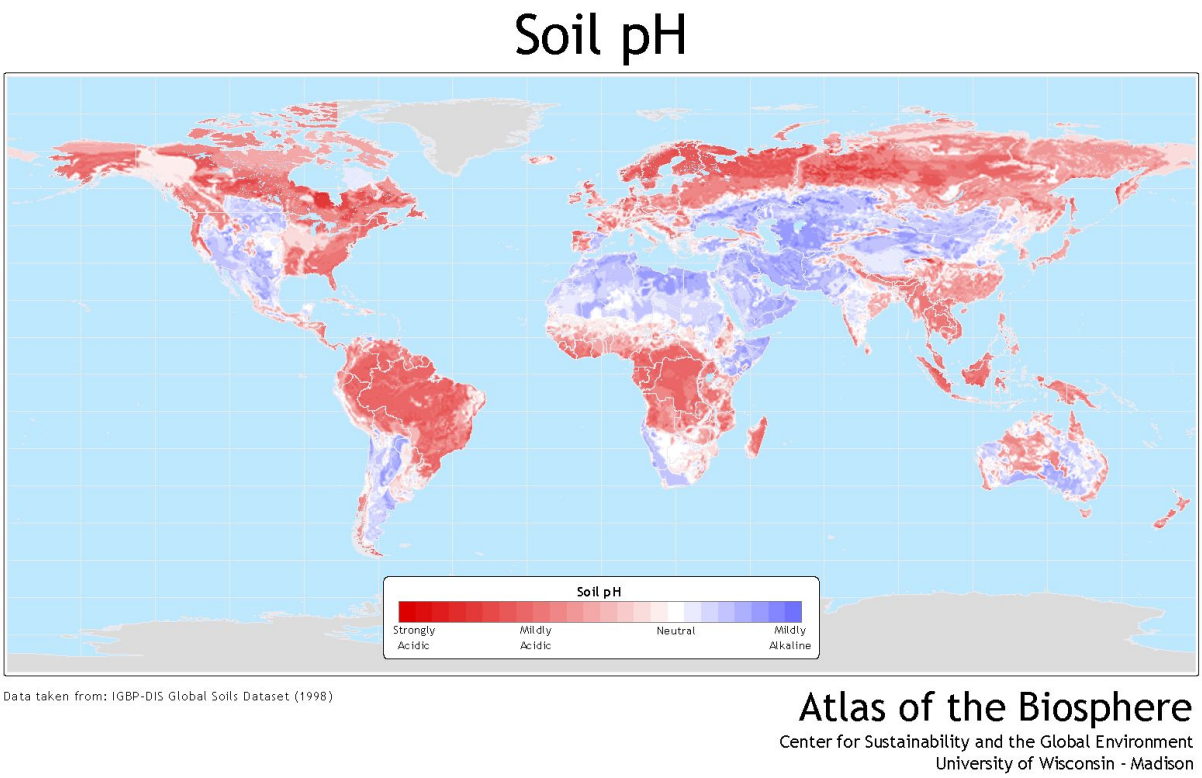

Figure 34. Soil pH map. Image retrieved from Atlas of the Biosphere (Atlas of the Biosphere, 1998).

In terms of the laboratory work involved in both extractions (mobile in the Kingston method vs. 0.5 mol L^{-1} HCl labile), the latter is less labor intensive, and therefore less prone to procedural errors. Although this was not tested, 0.5 mol L^{-1} HCl could be an alternative reagent in extraction of the mobile fraction in Kingston method and it would be interesting, in a future assessment, to consider and study this hypothesis. In either case, a low soil:extractant ratio favors mercury extraction and should be preferred in chemical extractions, as long as sample homogeneity and representativeness are guaranteed.

Crucial in any scheme of speciation/fractionation is the quantification of mercury. The increased awareness for mercury environmental research has resulted in a considerable number of techniques being used in the element quantification. For the purpose of this work, it was important to acknowledge the current status in mercury determination methods, whether they provide similar results, and to identify underlying problems and sources of error. To do so, an inter-laboratory study was organised. Besides soil and sediment test materials, mercury quantification in fish and human hair was also requested. Although mercury determination in organic

matrices is not a goal of this work, both fish and humans are important receptors of environmental contamination. Leaching of mercury in soils and sediments often results in its mobilisation to the water sources where, in turn, fish can bioaccumulate the metal. Humans are the ultimate receptor of mercury present in the environment, with many recognized adverse effects, and in the long term, together with speciation studies is important to understand if the labile and bioavailable mercury species are affecting population.

The most important goal of this inter-laboratory exercise was to study the reproducibility of purposed extraction procedures. The procedures purposed aimed to isolate the bioavailable and organic fractions. These fractions were chosen because their study has received more attention due to their environmental relevance and because they are more likely to be taken up by fish and humans. Simple extraction procedures were chosen, to minimize bias sources. The organometallic extraction procedure purposed is well established (Válega *et al.*, 2006), commonly used in organic matrices (Coelho *et al.*, 2008b; Mieiro *et al.*, 2011), and accurate (Coelho *et al.*, 2008a; Coelho *et al.*, 2008b; Coelho *et al.*, 2006; Mieiro *et al.*, 2012). Therefore, it was purposed for inorganic matrices in order to assess its applicability in inorganic matrices.

The results of this inter-laboratory exercise reveal that total mercury determination is usually satisfactorily performed. In contrast, chemical extraction procedures do not seem to be popular!

The low number of participants performing extractions partially hindered the objective of the inter-laboratory exercise. Still, important conclusions could be acquired. Firstly, the concentrations reported for extractions in inorganic matrices were different among participants. On the other hand, organic mercury extraction in fish yielded more reproducible results than for soil and sediment. Secondly, it appears that there is some reluctance in performing chemical extractions and it is believed that two reasons may be behind this: 1) extractions are labor-intensive, costly and time-consuming; 2) most laboratories are not cognizant with the importance of speciation. Legislation regarding mercury determination in environmental samples only establishes limits for total mercury, which does not contribute to raise awareness of the significance of mercury speciation.

So far, mercury speciation seems to be a matter of research importance, but it is understood that the choice of this PhD theme goes beyond the academic interest.

As said, currently, the majority of the soil and sediment samples submitted for analysis (in risk assessment, for example) request total mercury quantification only, and the resulting concentration is assumed as the "worst case scenario". For example, Portuguese legislation for dredged materials (Portaria n.º 1450/2007) states that sediments with mercury concentration above 10 mg kg^{-1} are considered class 5 (extremely contaminated) and must not be dredged. However, dredging is often required to maintain the depth of navigation channels. If sediments to be removed were analysed for total mercury only, and that concentration was found to be above 10 mg kg^{-1}, two things could happen: 1) sediments would not be dredged, causing disturbance or impeding navigation, or 2) dredged sediments would have to be treated prior to deposition in other locations. In both cases, the implied costs can be high, so it is "financially beneficial" to classify these materials correctly. If mercury speciation analysis revealed that, for example, only 1 mg kg^{-1} of mercury is present as potentially toxic and available species, one could assume that the sediments do not represent such a high risk and could, in fact, be dredged. Speciation analysis costs substantially less than sediment treatment, saving a considerable amount of money and time.

Despite the recognized problems associated with chemical extraction procedures, they provide valuable information for mercury geochemistry interpretation in soils and sediments. Through chemical extractions it was possible to relate a certain fraction to a specific chemical form (e.g., water-soluble, exchangeable, labile) or mobility, which, in turn, allowed making inferences on their reactivity and bioavailability, or response to changes in environmental conditions such as rainfall events or pH changes. Extraction with HCl was particularly useful to infer the effects caused by acid-mine drainage (diminishes pH) in Caveira mine's soils.

However, chemical extractions, even when involving just one step and one reagent, are not feasible on a routine basis. Therefore, a simpler, faster method is required. Speciation by thermo-desorption constitutes an excellent alternative to chemical extraction. This approach is not new and has been explored mainly by Biester and his co-workers, as confirmed by their extensive work (Biester *et al.*, 2000; Biester *et al.*, 1999; Biester *et al.*, 2002a; Biester *et al.*, 1997a; Biester *et al.*, 1997b; Biester *et al.*, 1998). However, Biester's team perform their analysis by adapting atomic absorption equipment. Being obtained under different operational

conditions, the results can be difficult to compare with those of other workers. Thermo-desorption presents many advantages over conventional chemical extraction methods (and x-ray absorption methods too), so it was decided that the technique was worthy to be further explored. Direct mercury analyser equipments (model AMA-254, LECO®), appeared to be a good alternative, as they already use thermal-decomposition for total mercury quantification. Plus, they can be found in many laboratories, as proven by the number of participants of the inter-laboratory exercise using them for total mercury quantification. The advantage of using the direct mercury analyser is that, since the equipment is automated and commercially available, operational conditions are be standardized and results obtained by different laboratories can be easily compared. The use of LECO® AMA-254 for mercury speciation also takes advantage of existing technology, increasing the spectrum of the equipments' applicability, bringing new value to an item of expensive laboratory equipment.

The premise behind mercury speciation by thermo-desorption is that different species are released at different temperature, so adjustments were made in combustion temperature, in order to identify Hg^0, $HgCl_2$, Hg associated with iron oxides, Hg bound to humic acids and HgS. Even though in certain samples it is difficult to completely separate all mercury species, the differentiation of the mineral and organic fraction was achieved. This was a major improvement relatively to previous thermo-desorption methods, since Biester *et al*. were not able to separate mercury associated with mineral components of the soil from mercury species from mercury associated to organic matter, and have settled to include all these species in what they called the "matrix-bound Hg" (Biester *et al.*, 1997b).

Comparing to conventional chemical extraction procedures, the following advantages of speciation by thermo-desorption must be underlined:

- Only a small quantity (<1 g) of sample is required;
- It is selective;
- It is free of cross-contamination;
- It can be applied to a vast range of total mercury concentrations;
- It requires little to no sample treatment, which also prevents the loss of volatile mercury -compounds;

- A complete identification of species present is obtained in less than two hours;
- Based on the work developed, the repeatability of the method is good;
- Mercury losses are almost neglectable;
- It can be considered “clean”, as no residues are produced;
- Since there are not involved any reagents, shaking or any sample manipulation, and the temperature is controlled by the equipments’ software, the worldwide comparison of results obtained is easier and reliable, an important step towards the validation of the method;
- It does not suffer matrix effects, particularly in what concerns sample texture or pH.

The developed technique can be an important contribution for the preliminary screening of the potential risk associated with mercury contamination at a given locale. Even though the complete separation, identification and quantification of all mercury species is sometimes difficult, an indication of how they interact with the matrix is attainable, providing relevant information on the potential mobility and availability of the samples’ mercury species.

To complete this work, in the near future an inter-laboratory exercise to test the thermo-desorption method will be organised. Two main objectives will be purposed for this work: 1) test the validity and reproducibility of the method, which is an important step towards the standardisation of the method; 2) prepare and test an array of materials to develop adequate certified reference materials and an adequate Quality Control/Quality Analysis protocol.

Table 22 provides an overview of the work here presented. Unfortunately, and despite the extensive work developed, there are still no unequivocal methods of distinguishing between different forms of mercury in soil and sediment. However, this does not have to be a drawback! In this thesis, several methods were considered, developed or optimised, which should improve the quality of information obtained in mercury speciation or fractionation. While it is true that many concerns are raised about the validity and appropriate use of extraction techniques for mercury speciation/fractionation, caution is always advocated. However, evidence from any

method should not be disregarded. If applied in an appropriate manner, with correct data interpretation, information is always gained; it is the relative weight applied to the information that should be the driving factor. The choice of method to be used ought to be made depending on the purpose of the study.

As George Bernard Shaw said, *“Science is always wrong. It never solves a problem without creating 10 more”*, and in the case of mercury speciation this seems to be true. It’s a challenge indeed!

Table 22. Overview of the work developed. Procedures are compared for their target species, advantages and disadvantages. General results obtained are also presented.

	Extractability and mobility	Water-soluble fraction kinetic extraction	Exchangeable fraction kinetic extraction	Labile fraction kinetic extraction	Thermo-desorption
Target	•Provides information on Hg mobility (bioavailability).	•Extracts free Hg^{2+} and Hg^{2+} complexed with dissolved OM. •Most mobile and bioavailable fraction.	•Extracts weakly adsorbed Hg retained on the solid surface by weak electrostatic interaction, by ion-exchange processes. •Extremely mobile and bioavailable fraction.	•Extracts the more available species, such as water-soluble, exchangeable, and carbonate associated.	•Hg species and not fractions. •Hg species: Hg^0, $HgCl_2$,Hg associated with Fe, Hg bound to humic acids, HgS.
Advantages and disadvantages of the method.	✔ Fewer steps that other SEP. ✗ Hg easily lost. ✗ Time-consuming.	✗ Concentration is very low and only quantifiable with extremely sensitive analytical techniques. ✔ Water is a cheap extractant.	✔ Only one extraction step and one reagent required. ✔ Cost-effective ✔ Requires less technical skill. ✗ Hg extracted varies with extractant.	✔ Only one extraction step and one reagent required. ✔ Cost-effective ✔ Requires less technical skill. ✗ Doesn't provide geochemical information.	✔ No extraction involved. ✔ Cost-effective. ✔ Requires low technical skill. ✔ No Hg losses. ✗ Requires a mercury analyser.
General results in tested samples.	Hg mostly in semi-mobile fraction. More mobility in Estarreja soils. Hg mobility enabled by Al and Mn contents and inhibited by organic matter and sulfur.	Equilibrium was reached at 24h. Hg removal in two stages (faster t<6h; slower t>6h). Two first-order reaction model fit data. Low % of water-soluble Hg (<2%)	Hg removal in two stages (faster t<10h; slower t>10h). Two first-order reaction and diffusion models fit data. Percentage removed <4%.	Hg removal in two stages (faster t<10h; slower t>10h). Two first-order reaction and diffusion models fit data. Percentage removed up to 30%	Hg^0 and HgS are easily identifiable. Hg species associated with matrix components can sometimes be harder to clearly identify.

Chemical extractions are influenced by:
Sample texture (% sand and % clay);
Method of separation of the extracted solution from the residue.
Results vary with the quantification method chosen.

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