



ISABELA CRISTINA FILARDI VASQUES

**GEOCHEMICAL FRACTIONATION AND
BIOACCESSIBILITY OF MERCURY IN TROPICAL SOILS**

**LAVRAS- MG
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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência do Solo, área de concentração em Recursos Ambientais e Uso da Terra, para a obtenção do título de Doutor.

Prof. Dr. João José Granate de Sá e Melo Marques
Orientador

Prof. Dr. Adélia Aziz Alexandre Pozza
Coorientadora

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Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA,
com dados informados pelo(a) próprio(a) autor(a).

Vasques, Isabela Cristina Filardi.

Geochemical fractionation and bioaccessibility of mercury in
tropical soils / Isabela Cristina Filardi Vasques. – 2019.

84 p. : il.

Orientador: João José Marques.

Coorientadora: Adélia Pozza.

Tese (Doutorado) - Universidade Federal de Lavras, 2019.

Bibliografia.

1. Extração química sequencial. 2. Teores geoquimicamente
reativos. 3. SBET. I. Marques, João José. II. Pozza, Adélia. III.
Título.

ISABELA CRISTINA FILARDI VASQUES

**FRACIONAMENTO GEOQUÍMICO E BIOACESSIBILIDADE DE MERCÚRIO EM
SOLOS TROPICAIS**

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APROVADA em 27 de agosto de 2019.

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**LAVRAS – MG
2019**

AGRADECIMENTOS

Agradeço a Deus por ter seguido comigo essa trajetória.

Agradeço aos meus pais e meus irmãos, família que sempre me incentivou e me apoiou nas decisões. Obrigada, pelos conselhos e pelo amor incondicional.

Ao Diego, pelo amor, companheirismo e compreensão.

À UFLA, pelo ensino de qualidade, ao Departamento de Ciência do Solo, toda sua infraestrutura física e apoio pessoal: a todos os docentes e técnicos, em especial: Geila, Lívia, Mari, Aline, Bethânia e Alexandre.

Às alunas de iniciação científica que me ajudaram na condução dos experimentos, especialmente Luciane e Júlia.

Aos colegas que fizeram essa jornada mais leve: Francielle, Jakeline, Thaisa, Polyana, Rayner, Cynthia, Pedro, Everton e André.

Ao meu orientador, professor João José Marques, pelo ensinamento e apoio.

Às Agências de fomento CAPES, CNPq e FAPEMIG, pelo apoio financeiro.

RESUMO GERAL

A compreensão do comportamento geoquímico do mercúrio, nos solos, incluindo a estimativa de sua fração bioacessível, é de grande relevância, pois facilita a avaliação de sua contaminação. Extrações químicas sequenciais são realizadas com o objetivo de avaliar o fracionamento de Hg, em solos, utilizando diferentes soluções extratoras. Neste trabalho, extrações individuais foram realizadas em amostras de solo tropical, artificialmente enriquecidas com Hg, usando as mesmas soluções aplicadas nas extrações sequenciais bem estabelecidas e correlações entre elas foram feitas. Utilizaram-se amostras enriquecidas com Hg também em análises para quatro testes de bioacessibilidade: utilizando ácido nítrico diluído à temperatura ambiente, ácido nítrico diluído à temperatura corporal, os métodos SBET e a fase gástrica do protocolo IVG. Para todos os procedimentos, avaliou-se a influência dos teores de argila e de matéria orgânica e pH na dinâmica do mercúrio. As extrações sequenciais revelaram que a maioria do Hg nas amostras enriquecidas é propensa a ser bioacessível no estômago humano e outra parte importante são os teores de Hg (0), que é propenso à volatilização. O esquema de extração simples foi uma boa opção para avaliar Hg solúvel em água, Hg bioacessível em pH próximo ao estômago humano, Hg associado a matéria orgânica, Hg reduzido (Hg (0)) e Hg associado a óxidos de Fe e Al (óxidos). Os percentuais de conteúdos bioacessíveis foram em média 20% para os dois primeiros testes e 40%, em média, para o terceiro e quarto testes. O método SBET apresenta menor variação no percentual de conteúdo bioacessível com concentração de Hg. Amostras contaminadas com Hg (0) da cidade de Descoberto provenientes de garimpos de ouro, do século XIX, também foram submetidas aos mesmos testes de bioacessibilidade e, em geral, a metodologia que possuía pH mais baixo, que é a solução de ácido nítrico diluído juntamente com a temperatura corporal estimou maiores teores bioacessíveis em amostras de solo, enquanto o método SBET estimou maiores teores bioacessíveis em amostras de sedimento. Na análise multivariada, dois grupos de testes de bioacessibilidade foram formados: um, com os dois testes com ácido nítrico e outro, com SBET e fase gástrica do protocolo IVG, provavelmente, em razão da adição de compostos orgânicos no último grupo, melhorando a estimativa de teores bioacessíveis de Hg.

Palavras-chave: Extração química sequencial. Teores geoquimicamente reativos. SBET. Protocolo IVG.

GENERAL ABSTRACT

Understanding mercury geochemical behavior in soils, including the estimation of its bioaccessible fraction is of high relevance because it makes easier the evaluation of its contamination. Sequential chemical extractions are conducted in order to evaluate the fractionation of Hg in soils using different extracting solutions. In this study, single extractions are performed in tropical soil samples, spiked with Hg, using the same solutions applied in the well established sequential extractions and correlations among them are made. Spiked soil samples were also used and four bioaccessibility tests were used: with dilute nitric acid at room temperature, with dilute nitric acid at body temperature, the SBET methods and the gastric phase of the IVG protocol. For all procedures the influence of clay contents, organic matter content and pH on mercury dynamics is evaluated. Spiked samples revealed that the majority of Hg is prone to be bioaccessible in human stomach and another major part is reduced Hg (Hg (0)) which is prone to volatilization after sequential extraction. The single extraction scheme was a good option in order to evaluate water soluble Hg, bioaccessible Hg at pH near human stomach, Hg associated with organic matter, reduced Hg (Hg (0)) and Hg associated to Fe and Al hydr(oxides). Bioaccessible contents percentages were higher than 20% on average for the two first tests and higher than 40% on average for the third and fourth tests. The SBET method has a lower variation in bioaccessible contents percentage with Hg concentration. Samples contaminated with Hg (0) in Descoberto after artisanal mining operations in XIX century were also submitted to the same bioaccessibility tests and in general, the methodology with the lowest pH, which is the dilute nitric acid solution together with body temperature, estimated higher bioaccessible contents in soil samples while the SBET method estimated higher bioaccessible contents in sediment samples. At the multivariate analysis, two groups of bioaccessibility tests are formed: one with the two tests with nitric acid and the other with SBET and gastric phase of IVG protocol, probably due to the addition of organic compounds in the last group, better estimating bioaccessible contents of Hg.

Keywords: Sequential chemical extraction. Geochemically reactive contents. SBET. IVG protocol.

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PRIMEIRA PARTE

1 INTRODUÇÃO

No solo, mercúrio é um elemento encontrado em baixas concentrações, mas maiores concentrações podem ser encontradas em rochas sedimentares sedimentos argilosos e ainda materiais ricos em matéria orgânica (KABATA-PENDIAS, 2001). As mais importantes propriedades do Hg são: capacidade de formar ligações fortes com S, a capacidade de formar compostos organomercuriosos relativamente estáveis em meio aquoso e ainda capacidade de volatilização do Hg elementar (KABATA-PENDIAS, 2001).

Os efeitos danosos do Hg no meio ambiente e na saúde humana são bem conhecidos, e entre eles estão efeitos no sistema nervoso, central e periférico (WORLDHEALTH ORGANIZATION - WHO, 2007). A maior preocupação são as crianças, mais vulneráveis à exposição, e podem estar diretamente expostas, por meio do consumo de peixes contaminados com Hg (WHO, 2007). No solo, sabe-se que os efeitos deletérios do Hg na microbiota dependem das características do solo, especialmente do teor de argila que tem capacidade de reter parte do Hg bioacessível, o que reduz seus efeitos tóxicos (FROSSARD; HARTMANN; FREY, 2017).

Com o objetivo de estudar a interação do Hg com as partículas sólidas do solo e determinar suas espécies principais, estudos de fracionamento geoquímico podem ser conduzidos. Estudos de fracionamento geoquímico sequencial utilizam soluções com forças extratoras crescentes em sucessivas extrações químicas. Não há descarte de amostras e a mesma amostra é submetida a todas as extrações. O fracionamento sequencial tem algumas desvantagens como a falta de especificidade de algumas soluções empregadas (AUDRY; BLANC; SCHAFER, 2006), a baixa eficiência de extração de algumas soluções e a precipitação de novas fases minerais durante o procedimento (BACON et al., 2008). Para evitar esses tipos de problemas, a extração individual pode ser uma alternativa, diminuindo os erros associados ao procedimento realizado de forma sequencial, mas estudos de correlação entre extrações simples e sequenciais são ainda necessários.

No fracionamento geoquímico, pode-se avaliar quais são os “pools” ambientais mais importantes do Hg e é possível construir uma hipótese sobre o comportamento desse elemento no solo. As fases mais associadas ao Hg, em estudos de fracionamento geoquímico, variam entre tipos de solos e podem ser sulfetos (FERNÁNDEZ-MARTÍNEZ et al., 2019), matéria orgânica (BLOOM et al., 2003) e óxidos de Fe, Al e Mn (DURÃO JÚNIOR, 2010).

Outra avaliação importante no estudo do comportamento de Hg, no solo, é a bioacessibilidade. Existem vários testes diferentes que avaliam os teores bioacessíveis de elementos traços no solo, usando soluções extratoras com ou sem adição de substâncias orgânicas. Alguns testes de bioacessibilidade já possuem correlação estabelecida com testes de biodisponibilidade, testes que são realizados “*in vivo*” com animais e são portanto, mais robustos (RODRIGUEZ et al., 1999). O interesse em se estudar o conceito de bioacessibilidade tem se intensificado, já que, em avaliações de risco e manejo de solos contaminados, os efeitos tóxicos induzidos por elementos-traço têm se relacionado melhor com o teor bioacessível desses elementos (KUMPIENE; GIAGNONI; MARSCHNER, 2017).

Neste trabalho, objetivou-se estudar os fatores que afetam o fracionamento geoquímico de Hg e os seus teores bioacessíveis em solos tropicais. Variações nos teores de matéria orgânica, argila e pH são estudados. Na primeira parte do trabalho, apresenta-se um referencial teórico, seguido pelos artigos 1 e 2. O artigo 1, refere-se ao fracionamento geoquímico de Hg, em solos tropicais e o artigo 2 refere-se à avaliação da bioacessibilidade de Hg em solos tropicais.

2 REVISÃO DE LITERATURA

2.1 Mercúrio no solo

Mercúrio no solo pode estar presente de várias formas em três estados de oxidação: Hg (0) (mercúrio elementar), Hg (I) (mercuroso) e Hg (II) (mercúrico). Entre essas três espécies, o Hg (II) é mais usualmente encontrado no solo, porque o Hg (0) é volátil e mais comumente encontrado na atmosfera e o Hg (I) é instável na natureza (O'CONNOR et al., 2019). Maiores concentrações de Hg, no solo, podem ser encontradas, em decorrência de atividades antrópicas como indústrias de cloro-soda (FERNÁNDEZ-MARTÍNEZ et al., 2019), minerações artesanais de ouro (MARRUGO-NEGRETE et al., 2015) também conhecidos como garimpos, que utilizam o mercúrio para formar amálgamas e ainda as próprias minerações de Hg, como as localizadas na Espanha (FERNÁNDEZ-MARTÍNEZ et al., 2019).

As transformações das espécies de Hg, no solo, são muito dinâmicas. As espécies orgânicas de Hg são mais tóxicas que as espécies inorgânicas e têm o potencial de bioacumular em tecidos vivos. A metilação é, então, um processo que pode aumentar a toxidez dos compostos com Hg e está diretamente relacionada com o Hg associado com a matéria orgânica (BLOOM et al., 2003). A metilação é realizada por bactérias, especialmente as bactérias redutoras de sulfato, e pode ser afetada por vários fatores como a complexação de Hg (SI et al., 2014), atividade bacteriana e especiação de Hg (MAZRUI et al., 2016).

Outra transformação importante de Hg nos solos é relacionada com os processos de oxidação-redução desse elemento. Soares et al. (2015) revelaram que, em uma atmosfera saturada com Hg (0), ocorre a oxidação do Hg em Hg (II) na interface solo-ar e esse se liga à matéria orgânica na sua forma oxidada (Hg⁺²), contribuindo com a retenção do Hg no solo. De acordo com do Valle et al. (2005), a cinética de oxidação do Hg em solos é favorecida em comparação com a redução. Windmüller et al. (2015) afirmam que a redução do Hg (II) em solos se dá em duas etapas: inicialmente ocorre a redução de Hg (II) a Hg (I) e, posteriormente, a redução Hg (I) a Hg (0).

O potencial de volatilização do Hg é preocupante e deve ser profundamente estudado em áreas contaminadas. Hg pode ser volatilizado da interface solo/ar como Hg (0) ou ainda como outros compostos. De acordo com Windmüller et al. (2015), a volatilização ocorre mais rapidamente em temperaturas mais elevadas. Mesmo assim, a velocidade de oxidação é favorecida em comparação à de redução. De acordo com Osterwalder et al. (2019), solos

contaminados com Hg (0) podem se tornar uma fonte de emissão de Hg para a atmosfera, em longo prazo e comunidades microbianas podem ser as maiores responsáveis pela reação de redução desse elemento, especialmente considerando os solos oxídicos estudados por esses autores.

Mercúrio em solos tropicais ainda exige mais pesquisas, a fim de desvendar o comportamento desse elemento, nesse tipo de solo que, geralmente, apresenta altos teores de óxidos de Fe, Al e Mn e é bastante intemperizado. Semu, Singh e Selmer-Olsen (1987) estudaram solos tropicais e a adsorção de Hg e mostraram que em solos tropicais, a adsorção de Hg inorgânico é mais governada pela matéria orgânica do solo, enquanto que a adsorção de Hg orgânico é governada mais por frações minerais do solo. Roulet et al. (1998) estudaram solos tropicais da Amazônia central e afirmaram que a ausência de uma camada bem desenvolvida de matéria orgânica aliada à sua rápida degradação, limitam a acumulação de Hg na superfície do solo. Portanto, a textura argilosa é um fator essencial determinando elevada ou baixa concentração de Hg no solo e é o teor de óxidos de Fe e Al associada com a textura do solo que irão então governar o acúmulo de Hg em solos tropicais (ROULET et al., 1998).

O solo é capaz de reter ou mobilizar Hg a depender das suas propriedades e da significativa contribuição de cada uma na composição do solo. Teores de matéria orgânica são considerados os maiores responsáveis pela adsorção de Hg no solo e sua oxidação. De acordo com Soares et al. (2015), diferentes profundidades de solos são responsáveis por diferentes níveis de retenção de Hg, o que foi atribuído ao aumento de matéria orgânica no horizonte superficial. Os grupos funcionais da matéria orgânica hidroxílicos e os -SH (principalmente esse último), são os grupos responsáveis por essa capacidade de adsorção em solos (SOARES et al., 2015).

De acordo com Wallschlager et al. (1998), tanto os ácidos fúlvicos quanto os ácidos húmicos da matéria orgânica contribuem para a retenção de Hg em solos. As moléculas, com maior massa molar de ácidos húmicos, se mostraram os ligantes mais significativos em áreas alagadas, sendo inertes e constituindo interações irreversíveis, mesmo em condições redutoras. As substâncias húmicas exerceriam grande influência na especiação de Hg e nas transformações desse elemento no solo, afetando a sua disponibilidade e volatilização para a atmosfera (WALLSCHLAGER et al., 1998).

A textura do solo, ou seja, a composição granulométrica da parte sólida, exerce papel fundamental na retenção e mobilização de Hg pelo perfil. Granulometrias mais finas tendem a ter mais sítios de adsorção, em razão da maior superfície específica dessas partículas. De

acordo com Rózański, Castejón e Fernández (2016), formas mais disponíveis de Hg estão relacionadas com solos com menores teores de minerais de argila. Em solos ricos em húmus e minerais de argila, os cátions positivamente carregados de Hg são adsorvidos na superfície negativamente carregada da argila e da matéria orgânica (RÓZAŃSKI; CASTEJÓN; FERNÁNDEZ, 2016).

A fase mineral do solo, como óxidos de Fe, Al e Mn é outra importante contribuição significativa na retenção de Hg no solo. A partir de espectros formados com a estrutura fina de absorção de raios-X estendidos (EXAFS) Kim, Rytuba e Brown (2004) concluíram que a interação entre Hg e Fe na superfície de óxidos de Fe como a goethita, se dá a partir de complexos de esfera interna, que é uma ligação química direta entre o Hg (II) e a superfície do substrato. Esse tipo de ligação também se dá entre Hg e óxidos de Al, como a bayerita.

A adsorção de Hg^{+2} pelos solos é dependente do valor de pH (YIN et al., 1996). Em ambientes aquosos ácidos ($\text{pH} < 3$), há a predominância de Hg^{2+} e HgCl^+ . O aumento de pH do solo promove a formação de $\text{Hg}(\text{OH})_2$ e em $\text{pH} > 6$, essa espécie é favorecida. De acordo com Miretzky et al. (2005), em um estudo de adsorção em solos tropicais, a máxima adsorção se deu em pH entre 3 e 5, devido a forte natureza ácida de Lewis do Hg (II). Em valores de pH mais básicos, a dissolução da matéria orgânica e sua complexação com Hg (II) diminuem a adsorção de Hg(II) pelo solo (MIRETZKY et al., 2005). Um estudo feito por Park et al. (2018) revelou que a fonte de Hg introduzida no sistema afeta como o pH influenciará a retenção de Hg pelo solo. Nos dados de Park et al. (2018), o aumento do pH do solo favoreceu a adsorção de Hg. A adição de Hg como HgCl_2 indica que pode haver favorecimento de adsorção de Hg com o aumento de pH (PARK et al., 2018; SEMU; SINGH; SELMER-OLSEN, 1987). A adição de Hg como nitrato [$\text{Hg}(\text{NO}_3)_2$] mostrou que o aumento de pH decresce a adsorção de Hg em solos, depois de atingir um máximo de adsorção entre valores de pH 3,0 e 5,0 (YIN et al., 1996).

A presença de outros componentes na solução do solo afeta a adsorção de Hg a medida que esses outros componentes podem competir com Hg pela adsorção. De acordo com Semu, Singh e Selmer-Olsen (1987), o aumento da força iônica na solução em um experimento de adsorção diminuiu a capacidade de adsorção de Hg pelo solo. A presença do Ca^{2+} afetou mais significativamente quando comparado a outros cátions, como o K, mas os dois reduziram a adsorção de Hg. A maior eficiência do Ca em reduzir adsorção do Hg se deve a sua divalência e aos íons acompanhantes que, no estudo de Semu, Singh e Selmer-Olsen (1987), era o Cl. Cloretos (Cl^-) formariam mais complexos HgCl_2 , forma que é menos adsorvida pelos solos, especialmente as fases inorgânicas (MIRETZKY et al., 2005). A

influência da força iônica na adsorção de Hg em solos pode sugerir as várias formas pelas quais o Hg pode adsorver aos solos tropicais, não só por complexos de esfera interna.

Para estimar os teores de Hg que são disponíveis para absorção de plantas, muitos métodos podem ser empregados. Lima et al. (2018) estudaram a correlação de teores disponíveis no solo com teores biodisponíveis encontrados nas plantas e concluiu que o melhor método para esse elemento seria o método USEPA 3051A que utiliza ácido concentrado (HNO_3) e temperatura elevada em digestor de micro-ondas de laboratório. Outras soluções que são conhecidas por determinar de teores disponíveis de elementos, como Mehlich-1, Mehlich-3 e DTPA não se mostraram tão eficientes para o Hg. A pior correlação de teores no solo e teores biodisponíveis na planta se deu com a avaliação de extração com água, e isso se deve ao fato de que as condições de extração da água destilada não refletem as condições naturais de extração da solução do solo, que tende a ser levemente ácida.

A fim de estabelecer valores seguros para o teor de Hg nos solos, os valores de referência podem ser derivados e originados de estudos estatísticos. Estudos ecotoxicológicos podem ser realizados, utilizando espécies de animais ou plantas e doses conhecidas do contaminante são aplicadas durante o seu crescimento. É importante que os valores de referência estabelecidos pelos órgãos de proteção ambiental garantam a saúde do solo e o desenvolvimento adequado da microbiota. Segundo Lima et al. (2019), a microbiota do solo é muito sensível às concentrações de Hg e uma derivação de valores de prevenção comprovou que o valor atual de prevenção usado pelo Conselho Nacional de Meio Ambiente (BRASIL, 2009), que é de $0,5 \text{ mg kg}^{-1}$, é protetivo o suficiente para a saúde do solo.

2.2 Fracionamento de mercúrio em solos

O fracionamento de Hg em solos tem como principal objetivo avaliar o comportamento geoquímico do Hg. Em geral, as primeiras extrações se relacionam com as fases mais solúveis de Hg e as últimas, às mais estáveis. Várias metodologias de fracionamento geoquímico são usadas e algumas extrações sequenciais têm objetivos específicos dependendo das condições ambientais. Neculita, Zagury e Deschênes (2005), por exemplo, propuseram um procedimento a ser realizado em locais contaminados com plantas de fabricação de cloro-soda. Portanto, compreender a condição do ambiente é a primeira abordagem para escolher o procedimento correto. Condições não tão específicas foram estipuladas por Revis et al. (1989) para extrações sequenciais. Neste estudo, as diferentes frações de mercúrio avaliadas foram: mercúrio total, mercúrio elementar e sulfetos de

mercúrio em solo com contaminação conhecida, proveniente de poluição industrial. Com a extração sequencial, concluiu-se que 85% do Hg do solo foram sulfetos, menos solúveis que os demais e, portanto, menos preocupantes em termos de intoxicação.

Com o objetivo de avaliar diferentes procedimentos sequenciais para Hg, Barnett et al. (1995) compararam três procedimentos já estabelecidos na literatura: Miller (1993), Revis et al. (1989) e Sakamoto, Tomiyasu e Yonehara (1992), em cinco diferentes solos naturais. A diferença entre metodologias para avaliar as composições das frações de mercúrio foi significativa. Por exemplo, para o Hg associado aos sulfetos, a porcentagem média de extração para os cinco solos com os três procedimentos foram: 46%, 25% e 83%, respectivamente determinados pelo método de Miller (1993), Revis et al. (1989) e Sakamoto, Tomiyasu e Yonehara (1992). Esse resultado provou que mais pesquisas sobre esse assunto são necessárias e que a especificidade para as determinações de Hg ainda são questionáveis.

Em razão da falta de especificidade para Hg, Bloom et al. (2003) desenvolveram outro método para extrações sequenciais de Hg em solos e sedimentos. Esse método visa a determinar as cinco frações de maior importância geoquímica para o Hg: Mercúrio solúvel (F1), Mercúrio solúvel em pH próximo ao estômago humano (F2), Hg orgânico (F3), Hg elementar (F4) e sulfetos (F5). Bloom et al. (2003), ao final do experimento, fizeram uma correlação dessa extração proposta com outros procedimentos de extração bem estabelecidos: o teste de lixiviação (TCLP) e o teste in vitro gastrointestinal (IVG). Essa correlação mostrou que a soma das duas primeiras etapas do procedimento proposto por Bloom et al. (2003) teve uma grande correlação com TCLP e IVG, com pequena variação em razão das diferenças na razão solo: solução, tempo de extração e temperatura. Isso significaria que o teor de Hg extraído pelas duas primeiras etapas da extração sequencial representaria o teor mais disponível para lixiviação e mais disponível considerando o pH do estômago humano.

Vale ressaltar que a escolha do procedimento sequencial também depende do objetivo geral do seu estudo, às vezes metodologias com menos frações conseguem fazer o fracionamento desejado em menos tempo. Exemplo disso é a proposta de extração sequencial feita por Fernández-Martínez e Rucandio (2013) no que foi definido como um esquema de quatro etapas que seriam relacionadas com as fases e espécies mais importantes do Hg. Essa proposta avalia menos fases que a proposta de Bloom et al. (2003) e de acordo com essa proposta, as quatro etapas avaliadas seriam: espécies mais lábeis de Hg, complexos com ácidos fúlvicos e húmicos, mercúrio elementar, e espécies de mercúrio refratário e associados a sulfetos. Para avaliar as formas mais lábeis de Hg, que incluem as formas mais solúveis, fracamente adsorvidas e espécies livres de Hg inorgânico, soluções levemente ácidas podem

ser a opção (FERNÁNDEZ-MARTÍNEZ; RUCANDIO, 2013). A primeira extração, apenas com água, também é usada em alguns procedimentos (BLOOM et al., 2003).

As formas de Hg mais bioacessíveis ao estômago humano podem ser definidas como as que seriam solubilizadas com o pH ácido próximo ao que é encontrado no sistema digestivo. Existem testes próprios para avaliar a bioacessibilidade de elementos-traço, mas uma boa estimativa é dada com uma solução de ácido acético e ácido clorídrico, com pH 2 (BLOOM et al., 2003). Como em solos tropicais o Hg é significativamente retido em óxidos de Fe, Al e Mn (DURÃO JÚNIOR, 2010), ele será mobilizado em pH ácido, pela dissolução ácida de óxidos de Fe e Al, e então pode-se admitir que a maioria do Hg retido no solo se tornará bioacessível, ao passar pelo estômago, sendo possível em alguns casos desprezar as outras fases digestivas, como a fase intestinal. De acordo com Ruby et al. (1999), as formas minerais tendem a ser mais estáveis em condições próximas a seu ambiente de formação, sendo assim, as que se formam em ambientes ácidos tendem a ser mais estáveis no estômago, e são menos bioacessíveis. Fases minerais que se formam em ambientes alcalinos bioacessíveis, no estômago, em razão da perda de estabilidade a esse pH.

As espécies de Hg associadas à matéria orgânica são muito importantes, visto que as interações com matéria orgânica são as mais responsáveis por conter a mobilização de Hg no solo. Para quantificar os teores de Hg associados à matéria orgânica, podem ser usadas soluções ácidas e extremamente oxidantes, como uma solução de H_2O_2 em pH 2 (TESSIER; CAMPBELL; BISSON, 1979) e seu uso se justifica porque essa solução formaria um ambiente oxidante e a matéria orgânica se degradaria, liberando os elementos-traço solúveis associados a ela. Nesse caso, é necessária uma nova solução no processo que impeça a re-adsorção de elementos já extraídos em decorrência de ambiente oxidante. Soluções básicas também podem ser empregadas como uma solução de NaOH a um pH de 13 e $Na_4P_2O_7$ a um pH entre 10–13. A solução básica se justificaria baseada na extração seletiva da matéria orgânica que estaria associada com os elementos-traço, mas de acordo com Fernández-Martín e Rucandio (2013), a solução de $Na_4P_2O_7$ ainda seria melhor.

Bloom et al. (2003) utilizaram uma solução de HNO_3 para estimar o potencial de volatilização de Hg, por meio da presença de Hg (0). Para verificar a correlação entre os teores extraídos pela solução de HNO_3 e os teores de mercúrio elementar, foi realizado um experimento anterior utilizando a redução com $SnCl_2$. De acordo com Montoya, Lena e Windmüller (2019), a formação de Hg (0) no solo pode ser influenciada por teores de matéria orgânica, que tendem a diminuir a taxa de redução desse elemento e promovem a oxidação de Hg^{+2} .

A extração sequencial proposta por Bloom et al. (2003) não possui uma etapa que seja específica para determinar Hg associado aos óxidos de Fe, Al e Mn. Considerando que, em solos tropicais, esses óxidos podem ser componentes importantes para a retenção de Hg no solo, incluir uma etapa que avalie especificamente essa fração é muito importante. Em solos contaminados com mercúrio elementar provenientes da ação de garimpeiros na cidade de Descoberto, Minas Gerais, a maior parte do Hg estaria associada a óxidos de Fe, Al e Mn (DURÃO JÚNIOR, 2010). Para chegar a essa conclusão, Durão Júnior (2010) inseriu uma etapa na extração sequencial já estabelecida por Bloom et al. (2003) com o uso de uma solução de HCl (6 mol L^{-1}) antes da extração que avaliaria as formas mais estáveis de Hg (sulfetos) com uma solução forte de água régia.

Nas últimas etapas das extrações sequenciais, são empregadas soluções ácidas e oxidantes concentradas que desestabilizam até as fases mais estáveis de Hg (sulfetos) e que não foram extraídas nas etapas anteriores. Em áreas de mineração de Hg, com a presença de cinábrio (sulfeto de Hg) as extrações sequenciais tendem a revelar maiores teores nas últimas extrações, que avaliam as formas mais estáveis de Hg. De acordo com Fernández-Martínez et al. (2019), em um estudo de extração sequencial em áreas contaminadas com plantas de cloro-soda e em uma área de mineração de cinábrio, pode ser mostrado que, mesmo em maiores concentrações de Hg nas áreas de mineração, as formas de Hg que predominavam eram sulfetos, que eram muito estáveis. Já nas plantas industriais de cloro-soda, as formas de Hg estão mais disponíveis e mostram que há maior risco de serem solubilizadas e movimentadas pelo perfil de solo.

2.3 Bioacessibilidade de mercúrio em solos

De acordo com Galán et al. (2019), estudar as frações solúveis e biodisponíveis de elementos tóxicos deveriam governar a avaliação de risco em solos contaminados. Mahbub et al. (2018) também ressaltaram a importância de se avaliar teores biodisponíveis de Hg no solo, estimados pelas frações mais solúveis de Hg, e a partir desses derivar valores de referência que fossem mais apropriados. É sugerido ainda que, a predição da toxicidade de solos contaminados por Hg apenas com a avaliação dos teores totais de Hg não poderia ser válida. Portanto, estimar teores biodisponíveis e bioacessíveis de Hg confiáveis é muito importante.

No trato digestivo, os elementos-traço que estão associados com as partículas do solo podem passar por uma série de processos que permitem sua mobilização. As diversas

transformações pelas quais os elementos podem passar quando estão em solução são oxidação e redução, metilação e demetilação, ou ainda podem interagir com moléculas ou macromoléculas. Essas transformações são resultados da interação de enzimas digestivas no lúmen e do metabolismo pré sistêmico que resultam da ação da microbiota e das células epiteliais da mucosa gastrointestinal (VÁZQUEZ et al., 2015).

Existem métodos “*in vivo*” de avaliação de biodisponibilidade de elementos e que utilizam animais para estimar os teores que são absorvidos pelo corpo. Para esses testes, podem ser feitas determinações dos elementos nas urinas de animais (RODRIGUEZ et al., 1999) e amostras de sangue (RUBY et al., 1996). Com o objetivo de superar algumas dificuldades impostas por métodos “*in vivo*”, correlações dessas metodologias com metodologias “*in vitro*” que estimam o teor bioacessível são bastante usadas. Essa correlação já foi feita para As (RODRIGUEZ et al., 1999; RUBY et al., 1996) e para Pb (RUBY et al., 1996).

Os testes de bioacessibilidade podem usar enzimas ou aminoácidos, como é o caso do teste Simplified Bioaccessibility Extraction Test (SBET) que utiliza glicina, um aminoácido presente no trato digestivo e já foi largamente empregada em testes de bioacessibilidade, mas originalmente empregada para estimar teores bioacessíveis de Pb (UNITED STATES ENVIRONMENTAL PROTECTION AGENCY – USEPA, 2008). Além disso, materiais como pancreatina e bile de porco também são usadas para tentar aproximar ainda mais os testes de bioacessibilidade dos testes de biodisponibilidade, sendo que esses últimos só são estimados por testes “*in vivo*” (RODRIGUEZ et al., 1999).

Rodriguez et al. (1999) propuseram o protocolo In vitro gastrointestinal (IVG), um teste de bioacessibilidade em duas fases: uma que simularia a fase gástrica e outra que estimaria a fase intestinal, mas originalmente para arsênio. Estudos com o protocolo IVG já foram feitos para Hg em solos contaminados com indústrias de cloro-soda, e resultados da porcentagem bioacessível de Hg na área variaram entre 1,1 % a 4,5%. As propriedades desses solos que mais influenciaram os teores de bioacessibilidade foram os teores de enxofre e sulfetos nos solos, refletida no efeito da solubilidade desses compostos nos teores bioacessíveis de Hg (WELFRINGER; ZAGURY, 2009).

Um grande esforço tem sido feito para diminuir a complexidade de testes de bioacessibilidade, considerando a grande importância em realizá-los. Barnett e Turner (2001) propuseram uma forma de avaliar a bioacessibilidade de Hg utilizando solução de HCl diluído com pH 2,5. Por meio desse estudo, os autores mostraram que a avaliação de risco de locais contaminados deve considerar a espécie de Hg do local, para que os valores de referência de

solos não sejam superestimados ou subestimados, já que espécies diferentes mostram grande variação na solubilidade a pH ácido, e portanto, na bioacessibilidade.

Outra tentativa de se estabelecer testes de bioacessibilidade mais simples foi feita por Rodrigues et al. (2018). Para isso, foram usados vários tipos de solos, em várias condições ambientais, e foram feitas correlações entre métodos já estabelecidos na literatura como o SBET (USEPA, 2008) com outro método já estabelecido, mas que tinha como objetivo avaliar teores geoquimicamente reativos. Esse método usa ácido nítrico diluído, um reagente não tão difícil de ser encontrado, o que representa a possibilidade de maior adesão desses testes em avaliações de risco de solos contaminados.

A maioria dos testes de bioacessibilidade utiliza a temperatura corporal como uma condição experimental, com o objetivo de simular o que ocorre no estômago mais realisticamente. De acordo com Kim et al. (2012), a temperatura pode aumentar o fluxo de Hg do solo para a atmosfera, o que pode significar um aumento dos teores biodisponíveis quando se avalia a temperatura do corpo humano, comparado ao mesmo procedimento realizado a temperatura ambiente.

Em geral, encontram-se porcentagens bioacessíveis de Hg em solos bem baixos. De acordo com Safruk et al. (2015), solos contaminados com mercúrio inorgânico no Canadá mostraram uma porcentagem de bioacessibilidade média de 1,2 %, considerando a simulação apenas da fase gástrica em um teste de bioacessibilidade conhecido como “physiological based extraction test” (PBET). Similar ao protocolo IVG, o teste também estima os teores bioacessíveis de elementos-traço utilizando as duas fases gastrointestinais.

2.4 Contaminação por Hg em Descoberto- MG

No Brasil, casos de contaminação de solo por Hg já foram reportados em locais da Amazônia com a mineração de ouro representando uma grande parcela de emissão de Hg na natureza nessa região (CASTILHOS et al., 2015). Podem ser encontrados teores de Hg naturalmente adicionados no sistema, provenientes de material revolvido da movimentação de partículas do solo, a partir da erosão e de processos de formação do solo, como a podzolização (MIRETZKY et al., 2005; ROULET et al., 1998). Fora da região Amazônica, casos de contaminação com Hg são mais raros, mas ainda são encontrados.

Na região do quadrilátero ferrífero, especialmente, em razão da presença significativa de garimpos, ainda são encontradas concentrações de Hg expressivas no solo e na água. De acordo com Rhodes et al. (2018), concentrações totais de mercúrio chegaram a $1207 \mu\text{g kg}^{-1}$

em sedimentos do riacho Gualaxo do Norte. O potencial de metilação nessa região foi também correlacionada com o teor de matéria orgânica, assim como em outros estudos (BLOOM et al., 2003; MENDES et al., 2016), e a baixa velocidade da água no riacho foi ressaltada como um acelerador desse processo. Vale ressaltar que a metilação é o processo que torna formas menos tóxicas (inorgânicas) em formas mais tóxicas (orgânicas), constituindo um importante processo na avaliação de risco na exposição de Hg.

A cidade de Descoberto está localizada no Estado de Minas Gerais e se expandiu com a descoberta de ouro na região e, por isso, a cidade leva esse nome. O mercúrio é usado em minerações de ouro porque esses dois elementos juntos formam uma amálgama, facilitando a separação do Au nos sedimentos de rios, o que justifica a mancha de contaminação por Hg na zona rural da cidade (DURÃO JÚNIOR, 2010). Um dos primeiros estudos na área mostrou que a maior parte do Hg presente no solo é Hg⁺² e pode chegar a teores de até 161 mg kg⁻¹ (DURÃO JÚNIOR et al., 2009).

Na região, foram instalados caixas e tanques de sedimentação, sendo que esses últimos são maiores e possuem maior capacidade, ambos com a intenção de reter as partículas de solo que adsorvessem Hg e dissipassem a contaminação. De acordo com Durão Júnior et al. (2009), concentrações elevadas de Hg também foram encontradas nessas estruturas de contenção, o que evidencia o transporte de particulados e que pode ser fonte de contaminação para o curso de água que corre nas cotas mais baixas do terreno.

A dissipação da contaminação de Hg associada às partículas de solo foi evidenciada por Mendes et al. (2016) que encontraram concentrações de Hg nos sedimentos do ribeirão do Grama entre 5.8 a 266 µg kg⁻¹. Esse ribeirão passa nas cotas mais baixas do terreno que está com a mancha de contaminação de Hg. Além disso, Mendes et al. (2016) ressaltaram a importância de se monitorar a área, especialmente em razão da potencial de metilação do Hg na região, que se correlaciona com os teores de matéria orgânica (BLOOM et al., 2003) e com os teores de S no solo (MENDES et al., 2016), possivelmente, em decorrência das ligações entre grupos sulfídricos da matéria orgânica e Hg.

De acordo com Windmoller et al. (2015), a área em Descoberto-MG pode ser um exemplo de área contaminada com Hg (0). As emissões de Hg (0) nessa área e a consequente adsorção de Hg (0) pelo solo foram estudadas por Montoya, Lena e Windmüller (2019), juntamente com outros solos, com diversas fontes de contaminação e diferentes concentrações de Hg. De acordo com Montoya, Lena e Windmüller (2019), os óxidos de Mn são importantes protagonistas na retenção de Hg (0) explicada, principalmente, pela formação de HgO a partir do contato dos óxidos de Mn e Hg (0). A utilização de vários solos em diversas condições de

contaminação por Montoya, Lena e Windmüller (2019) permite extrapolar esse resultado para além das áreas de Descoberto-MG.

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SEGUNDA PARTE - ARTIGOS**ARTIGO 1 – MERCURY GEOCHEMICAL FRACTIONATION IN TROPICAL
SOILS****(VERSÃO PRELIMINAR)**

Artigo redigido conforme normas do periódico científico *Science of the Total Environment* (ISSN: 0048-9697). Este artigo é uma versão preliminar e, portanto poderão surgir alterações para adequá-lo. Os gráficos e tabelas foram inseridos no decorrer do texto para facilitar a leitura.

Abstract

Understanding mercury geochemical behavior in soils is of high relevance because it makes easier the evaluation of its contamination. Sequential chemical extractions were conducted in order to evaluate the fractionation of Hg in soils using different extracting solutions. However, single multiple extractions are more appropriate because it is less time consuming and precipitation of new mineral phases are avoided. Therefore, single extractions were here performed in tropical soil samples, spiked with HgCl_2 , using the same solutions applied in the well established sequential extractions and correlations among them were made. An adsorption experiment was performed in order to spike samples and high adsorption percentage was observed for all treatments. Influence of clay and organic matter content and pH adjustment with lime application on mercury fractionation was evaluated. Spiked samples revealed that the majority of Hg is prone to be bioaccessible in human stomach and the other major part is reduced Hg ($\text{Hg}(0)$) which is prone to volatilization. The single extraction scheme was a good option in order to evaluate water soluble Hg, bioaccessible Hg at pH near human stomach, Hg associated to organic matter, Hg extracted by HNO_3 - elemental Hg ($\text{Hg}(0)$) and Hg associated to Fe and Al hydr(oxides). Pearson correlation revealed that the individual extraction that assess Hg associated with Fe, Al and Mn oxides can be overestimated, suggested by the lack of correlation among Hg, Fe and Al contents in the single extraction. Addition of organic matter in soils and liming is of paramount importance because reduction rates of Hg could be slowed and also the potential of volatilization, increasing rates of adsorption in soils and reducing risks of contamination.

Keywords: sequential chemical extraction, discrete chemical extraction, bioaccessibility.

1. Introduction

Mercury is a toxic element and its toxicity is related to its species, being the most toxic species the organic ones, such as methylmercury (Du et al., 2014). Even being known for low translocation factors (Patra and Sharma, 2000), mercury can be absorbed by plants at different rates, depending on organic acids, bacteria, fungi and protozoa in soil (Liu et al., 2018), roots anatomy (Wang et al., 2015), overall plants' capacity (Fernández-Martínez et al., 2015) and soil characteristics. Li et al. (2017) have already evaluated that the long-term consumption of food that is produced in soils contaminated with Hg is deleterious to human health. The consequent contamination of foods with heavy metals can lead to limiting recommendations

for the consumption of locally produced foods with the objective of protecting human health, but reducing the food options of rural communities (Tirado et al., 2010). This jeopardizes the world's food security, a recurring concern of FAO, since adequate nutrition and food security have been depreciated in almost all African and South American regions (Food and Agriculture Organization, 2018).

In Brazil, Hg contamination is already known in some Amazonian sites (Bastos et al., 2006) and especially affects miners called “garimpeiros” (Castilhos et al., 2015) who deal directly with the amalgamation of gold and mercury. Although more concentrated in the Amazon, another site that revealed high levels of Hg in soils is Descoberto, in the State of Minas Gerais. The city, which arose precisely with the gold search in the 19th century, reports concentrations of Hg up to 160 mg kg⁻¹ (Durão Jr. et al., 2009). In that area, concentration of Hg in sediments from a small river close to the area ranges from 5.8 and 266 µg kg⁻¹, which endangers the health of people who lives there (Mendes et al., 2016). One important concern regarding Hg contamination is the capability of Hg to be volatilized and volatilization can be a global risk concerning Hg contamination because Hg vapors can reach long-range transportation (Yan et al., 2019).

The fate of Hg in tropical soils is of important relevance but is still poorly studied. According to Lima et al. (2019), the adsorptive characteristics of tropical soils are capable to reduce toxicity levels in soil microbiota. According to Carvalho et al. (2018), tropical and highly weathered soils are able to adsorb high contents of metal ions, including Hg contents, especially due to the clay/fine texture and even more important, the association between Fe and Mn oxides with Hg accumulation. Goethite, hematite and gibbsite (in a lesser extent) and in particularly the amorphous oxides (Rózański et al., 2016) play an important role governing Hg. In addition to that, studies concluded that organic matter is crucial to explain high Hg contents in the surface layer (0-0.2m) in different soils (Wallschlager et al., 1998; Rózański et al., 2016). According to Rózański et al. (2016), in soils enriched in humus and clay minerals, due to adsorption processes of positively charged mercury cations on the negative charge promoted by humus compounds and clay minerals, water soluble Hg complexes Hg-humus-clay minerals may be formed and the soil solid phase has a paramount role on Hg retention. The organic matter can also be important to understand Hg⁰ assimilation from atmosphere by soils (Soares et al., 2015).

Considering that many factors can influence Hg availability in soils, identifying the main responsible fractions for Hg adsorption is of high relevance. Sequential extractions procedures (SEP) can be performed with representative soil samples and increasing strength of extraction solutions. The geochemical behavior of Hg can be predicted by using SEP and therefore, it helps the prediction of the fate of Hg in the environment (Bloom et al., 2003). Fractionation of mercury was already performed using sequential extractions in flooded soils for rice (Li et al., 2014), in wetland sediments contaminated with Hg (Ramasamy et al., 2012) and in soil contaminated with Hg in mining areas (Varejão et al., 2009; Durão Jr., 2010; Malehase et al., 2016), therefore, in a variety of environmental conditions.

This study aims to understand how Hg behaves in a short-time range contamination scenario, conducting a sequential chemical extraction and comparing it with single extractions, which could represent greater efficiency in studies of fractionation of Hg in soils. The sequential chemical extraction evaluated the biogeochemically relevant fractionation of Hg in the environment of tropical soils. The influence of soil type, soil depth and lime application was assessed during the geochemical fractionation. Also, the relation among Hg geochemical fractions was highlighted.

2. Methods and Materials

2.1. Sample collection

Soil samples of two soils were collected under minimal anthropogenic influence, in the city of Lavras, as follows: a Rhodic Acrudox (RhAcx) collected at the geographic coordinates 21°13'30" S /44°57'38" W and the Typic Hapludox (TyHpx) collected at 21°09'18" S/45°05'55" W. These soils are largely present in Brazil, in a wide range of climatic conditions. Soil chemical characterization is shown in table 1. Two soil layers were collected: at 0-20 cm (soil surface) and 20-40 cm (soil sub-surface).

Table 1: Soil chemical properties of RhAcx and TyHpx at two soil depths (0-20 and 20-40 cm).

	Soil	pH	K	P	Ca	Mg	Al	H+Al	t	BS	OM	m	V
	Layer	mg dm ⁻³			cmol _c dm ⁻³						dag kg ⁻¹	%	
RhAcx	0-20	4.8	63.93	2.02	0.37	0.13	2.04	13.72	2.70	0.66	4.69	75.56	4.62
	20-40	5.0	25.82	0.38	0.20	0.10	1.02	7.05	1.39	0.37	2.28	73.38	4.94
TyHpx	0-20	5.2	107.14	0.89	1.06	0.36	0.47	4.99	2.16	1.69	2.09	21.76	25.37
	20-40	4.8	33.10	0.41	0.26	0.10	0.96	4.42	1.40	0.44	1.36	68.57	9.15

pH in water soil:solution 1:2.5; Ca - Mg- Al- Extractant: KCl - 1 mol L⁻¹ ; OM; Organic matter oxidized by Na₂Cr₂O₇ (4N) + H₂SO₄ (10N); P- K-Extractant Mehlich 1; t: effective exchange cation capacity; BS: base saturation; m: aluminum saturation index; V: base saturation index

Soil physical characterization was made according to the Bouyoucos' method and it revealed that RhAcx has a clay content of 65% in both soil layers and TyHpx has 27 and 31% of clay content in the surface and at the subsurface, respectively. Samples were air-dried and sieved through < 50µm for the fractionation study. Half of the samples had their pH adjusted with lime (CaCO₃) to reach a base saturation of 60%. Soil samples were allowed to settle for three weeks with CaCO₃ until geochemical fractionation was performed in these samples.

Background values for mercury concentrations in soils were accessed with air-dried and sieved samples (< 2 mm) using a Direct Mercury Analyzer (DMA-80, Milestone, Sorisole, Italy) due to its low limit of detection. Table 2 shows these values.

Table 2: Mercury natural concentration in RhAcx and TyHpx at both soil depths (0-20 and 20-40 cm).

	Soil Layer	Hg (mg kg ⁻¹)
RhAcx	0-20	0.07
	20-40	0.06
TyHpx	0-20	0.03
	20-40	0.03

Method Detection Limit (MDL): 0.022 mg kg⁻¹; Method Quantification Limit: 0.109 mg kg⁻¹

2.2. Adsorption study

In order to spike the samples with Hg, soil samples were submitted to a batch adsorption procedure. An amount of 0.4 g of soil samples were added in centrifuge tubes with 20 mL adsorption solution. The adsorption solution constituted of a background electrolyte solution

with $0.1 \text{ mol L}^{-1} \text{ NaNO}_3$ as performed by Tong et al. (2013) and different concentrations of Hg as HgCl_2 : 3 mg L^{-1} and 6 mg L^{-1} . The tubes were shaken on an end-over-end shaker (180 r min^{-1}) at 25°C for 24 h and then centrifuged at 3000 r min^{-1} for 15 min. According to Lima et al. (2019), who studied the same tropical soils from this study, 24h is enough to reach equilibrium between Hg and binding sites of the soil. The concentration of Hg adsorbed by the soil was calculated by the difference between the initial concentration and the final concentration at the supernatant. The adsorption experiment was performed with four replicates for each treatment.

2.3. Chemical extractions

The sequential extraction performed was suggested by Bloom et al. (2003) and adapted by Durão Jr. (2010) and it was already recognized as the most used SEP among researches (Bacon et al., 2008). The adaptation of Durão Jr. (2010) is the addition of one step focusing on Hg associated to Fe and Al oxides, approaching more closely the soil properties of tropical soils. According to Bloom et al. (2003), a soil:solution ratio of 1:100 is the most appropriate to be used in this experiment, and therefore, 0.4 g of soil was used with 40mL of extraction solution for all steps as follows.

- Step 1 – Water soluble Hg

An amount of 0.4 g of soil was shaken with 40 mL of ultrapure water in centrifuge tubes for $18\text{h} \pm 2$ at 120 rpm. The extract was separated by centrifugation at 3000 rpm for 20 min and then the supernatant was removed for analysis. For the rinsing step, 40 mL of the same extractor was added to the sample, the tubes were vigorously shaken, centrifuged and filtered again. This solution was combined to the previous obtained one.

- Step 2 – Hg soluble at pH near human stomach (acid)

This step aims to simulate the pH close to human stomach. An aliquot of 40 mL of CH_3COOH (1 mol L^{-1}) + HCl ($0,01 \text{ mol L}^{-1}$) (pH=2) was added to the sample and then shaken for $18\text{h} \pm 2$ at 120 rpm and centrifuged as Step 1. Again, the rinse step was conducted as Step 1, but this time, with this solution.

- Step 3 – Hg associated to organic matter

The residue from step 2 was shaken with 40 mL of KOH (1 mol L^{-1}) and then centrifuged as the earlier steps. The operational conditions were the same as the previous one.

- Step 4 – Hg extracted by HNO₃ (12 mol L⁻¹) (Elemental Mercury)

The residue from step 3 was shaken with 40 mL of a HNO₃ solution (12 mol L⁻¹). Additional 40 mL of this solution was used as the rinse step after centrifugation, like the previous steps.

- Step 5- Associated to Fe, Al and Mn (hydr)oxides

An aliquot of 40 mL of HCl (6 mol L⁻¹) solution was added to the sample residue, at the same operational conditions mentioned before regarding shaking and centrifugation.

- Step 6 – Residual fraction

To the residue from step 5, 10 mL of concentrated HCl and 3 mL of concentrated HNO₃ were added to the tubes, which were left in room temperature for 12 h and then separated by centrifugation at 3000 rpm for 20 min.

According to Baig et al. (2009), a well-established sequential extraction could be performed as multiple single extractions, aiming to analyze arsenic fractions in sediments. These authors performed this extraction scheme as discrete extractions (it will be also treated here like this) and he found positive and significant correlations among both extractions scheme. In order to make the same comparison, single extractions were performed with the same extraction solutions of SEP proposed by Bloom et al. (2003) adapted by Durão Jr. (2010), and the same operational conditions, but without the rinse step and discharging sample after each step. All extraction procedures were conducted with four replicates.

An aliquot of 1 mL from the solution of BrCl (0.2 mol L⁻¹) was added in the extracts from steps 1, 2 and 4 and an aliquot of 10 mL was added to step 5, in both schemes: sequential and discrete. The methodology to produce BrCl was mentioned by Durão Jr. (2010) and it was prepared right before being used in order to oxidize Hg prior to analysis, as suggested by Bloom et al. (2003). Mercury concentrations in supernatants were determined using cold vapour atomic absorption spectrometry (CV-AAS) (Penha et al., 2017). The detection limit was determined according to the American Public Health Association (2012). The method detection limit was accessed by using seven blanks in each batch of analysis and it varied from 0.01 to 0.64 µg kg⁻¹.

A last single extraction was performed as a semi-total digestion (USEPA 3051A) (U.S. Environmental Protection Agency, 1998). Samples were spiked likewise an adsorption test as previously described for the adsorption test and then the soil was transferred to Teflon tubes, with 4 mL of HNO₃ and were taken to microwave for 15 minutes. Then the extracts were

filtered and analyzed at the atomic absorption spectrometer (CV-AAS) to quantify Hg. In this same extract, iron and aluminum contents were analyzed at the ICP-OES in order to evaluate the efficiency of the extraction and analyze the role of Fe and Al in retaining Hg in tropical soils. For comparison purposes, the single extraction with HCl, which intends to determine Hg that is associated to Fe and Al oxides was also analyzed for Fe and Al concentration at the ICP-OES. In each digestion batch, one reference “NIST SRM2710a -Montana Soil” was added in order to ensure the method efficiency. Recoveries of the standard sample were above 80%. Only analytical grade reagents were used in the whole experiment.

2.4. Statistical analysis

In each methodology (sequential and single extractions) and Hg concentration, the data set of fraction studied had their models adjusted using the stats package (R Development Core Team, 2018) and the residuals of the variance analysis for each model were tested for their normality, independence, and homogeneity. When the assumptions of Anova were not attended, the data set was transformed using square root or logarithm aiming the best adjustment of the data set using the base package (R Development Core Team, 2018). If even after the transformations of the data set, the assumptions of Anova were not attended, each model was tested for the possibility of influential values (Outliers) through the outlierTest function of the car package (Fox and Weisberg, 2011), and then outliers values were removed from each model according the Bonferonni test ($p < 0.05$). When the assumptions were attended, variance analysis (Anova) was carried out on the data, and when statistical difference was noticed ($p < 0.05$), the means of the variables were compared using the Tukey's test ($p < 0.05$). The Anova was performed using the car package (Fox and Weisberg, 2011) and the Tukey's test was performed using the emmeans package (Lenth, 2018).

Models were adjusted to predict each fraction studied in sequential method by discrete method using the linear models, the intercept was fixed in 0, and significance of regression and the mathematical equation parameters was performed using the base package (R Development Core Team, 2018) and to verify the accuracy of each model was calculated the root-mean-square error using the Metrics package (Hamner and Frasco, 2018). A Pearson correlation matrix was performed for each soil type using the corrplot package (Wei and Simko, 2017), aiming to evaluate the correlations among Al, Fe and Hg semi total contents and Al, Fe and Hg contents extracted in fraction 5 at the discrete method, and Al:Hg and

Fe:Hg ratio contents in semi total, and Al:Hg and Fe:Hg ratio extracted in fraction 5 by discrete method.

The multivariate analysis was carried out using the principal component analysis (PCA) method, generating a biplot graph with the PCA functions from the FactoMineR package (Le et al., 2008) and fviz_pca_var function from the factoextra package (Kassambara and Mundt, 2017), together with the assistance of tidyverse (Wickham, 2017). In the multivariate analysis was studied the behavior between the fractions separately for concentrations of 3 and 6 mg L⁻¹. The fractions 1, 2, 4 and 5 were plotted in biplot graph in discrete method due to the high and positive correlation between sequential and discrete methods for these fractions and the fractions 3 and 6 were plotted both for discrete and sequential method because these fractions did not show any positive correlation between the methods.

3. Results and Discussion

3.1. Sequential extractions

The adsorption experiments revealed that both soils at both surfaces were able to adsorb more than 99% of Hg, except for one treatment, which means Hg contents in soils of approximately 150 mg kg⁻¹ and 300 mg kg⁻¹ for contaminating solutions of 3 and 6 mg L⁻¹, respectively (Table 3). The treatment with lower adsorption capacity of Hg is the one at TyHpx, sub-surface without lime application after spiking samples with 6 mg L⁻¹. In order to evaluate the extracted contents, the total amount considered is the total adsorbed for each treatment individually.

Table 3: Average percentage of Hg adsorption and the standard deviation for the following conditions: Rhodic Acrudox (RhAcx) and the Typic Hapludox (TyHpx) without application of lime (No-Lime) and with lime, at surface and subsurface, after spiking samples with 3 and 6 mg L⁻¹.

Soil	Soil depth	Spike concentration	Lime application	Average adsorption percentage	Standard deviation
RhAcx	Surface	3 mg L ⁻¹	No-Lime	99.85	0.05
			Lime	99.81	0.05
	Subsurface	3 mg L ⁻¹	No-Lime	99.91	0.01
			Lime	99.88	0.00
		6 mg L ⁻¹	No-Lime	99.95	0.01
			Lime	99.95	0.00
TyHpx	Surface	3 mg L ⁻¹	No-Lime	99.88	0.04
			Lime	99.90	0.00
	Subsurface	3 mg L ⁻¹	No-Lime	99.92	0.03
			Lime	99.93	0.04
		6 mg L ⁻¹	No-Lime	99.89	0.04
			Lime	99.95	0.02
		6 mg L ⁻¹	No-Lime	96.69	0.13
			Lime	99.94	0.01

Results for the sequential extraction can be seen in figure 1. There is a clear difference among mercury extracted at background contents (Figure 1A) and mercury extracted after spiking samples (Figure 1B and 1C). The most significant fractions in samples with no addition of Hg are Fraction 4 and Fraction 6, corresponding to elemental mercury and residual mercury, respectively in both soils at both soil surfaces (Figure 1A). Fraction 4 and Fraction 6 revealed similar extraction potential, except at the surface of RhAcx without lime application, in which Fraction 4 has higher contents of Hg. The significant amount of Hg extracted by fraction 6 was expected, as they represent the most resistant form of Hg as sulfides, less prone to be bioavailable (Ram et al., 2009) and being related to natural occurrence of Hg in soils. This also means that dry deposition of Hg (0) can occur in the soil samples studied and there is a volatilization potential when Hg is not stable as sulfides, which consists a bigger environmental problem, even in low contents. In general, type of soil, soil depth and liming application did not influence the geochemical fractionation of natural Hg in tropical soils because in all treatments Fraction 4 and 6 remained to be the most significant fractions.

According to Bloom et al. (2003), soils with naturally low levels of Hg, which is our case, are supposed to have up to 80%, 95% of total Hg associated to organic matter, represented by the

the third fraction evaluated. This could be true due to the high affinity among organic matter and Hg, already related elsewhere (Wallschlager et al., 1998). The third fraction represents the second fraction in significance (Figure 1A), considering that our soils are highly weathered from tropical areas and organic matter is not so expressive comparing to temperate soils. What could also have happened is that the elevated pH of the solution employed at the fraction 3 precipitated some Hg and at the next solution, which is a highly acidic one (HNO_3), the Hg was dissolved, contributing to the higher contents of Hg (0) at the fraction 4. Therefore, the assumption that all Hg extracted by fraction 4 is reduced Hg is not so evident.

Spiking samples with 3 mg L^{-1} and 6 mg L^{-1} (Figure 1B and 1C) have changed the pattern of mercury distribution compared to background values. For samples spiked with 3 mg L^{-1} , the most significant fractions turned out to be Fraction 2 ($> 60 \text{ mg kg}^{-1}$) and Fraction 4 ($> 6 \text{ mg kg}^{-1}$) while fraction 6 losses its magnitude, extracting the lowest contents of Hg, which is expected due to the short time involved in contamination. Species of Hg that are rapidly developed and therefore, not so stable, are favored in this scenario, which make them more bioaccessible for human stomach (Fraction 2).

Higher Hg contents found in Fraction 2 under all the conditions shown in figure 1B is due to the low pH of the extraction solution, since pH values of 2 makes the soil surface positive, therefore, adsorption is not favorable for the cationic species Hg^{2+} and HgCl^+ , which are dominant species in the solution at this pH value (Miretzky et al., 2005). According to Fernández-Martínez et al. (2015), another extracting solution, a slightly acidic solution with HNO_3 , could be also used to evaluate the most labile species of Hg, which could be associated to our most soluble species (Sum of Fraction 1 and Fraction 2). Reis et al. (2016) stated that the most mobile fractions of Hg in soils could be sorbed to soil by weak electrostatic bonds and can release Hg to solution by ion-exchange processes and species coprecipitated with carbonates.

For the 4th fraction, which was supposed to evaluates Hg (0), it could be seen that TyHpx extracted higher contents of Hg compared to RhAcx, which could be attributed to the lower clay contents and organic matter that allowed higher bioaccessibility (Rózański et al., 2016). Hg (0) was also a significant pool in another soil with low organic matter and clay contents studied by Varejão et al. (2009) after fractionation performed in artisanal mining sites in Brazil. According to Windmüller et al. (2015), the presence of organic matter avoids the

reduction of Hg (II), which could explain the lower extraction of this fraction in the RhAcx. However, higher contents of Hg were extracted in this phase at the surface, with high organic matter contents, compared to the sub-surface, at both soil types. It is good to highlight that differences in the composition of the organic matter can also arise differences in mobilizing Hg in soils, and this result can show this behavior (Wallschlager et al., 1998). However, from what have being seen, the specificity of this solution to remove only Hg (0) is questionable.

According to Fernández-Martinez et al. (2013), the solution employed at the fourth fraction here (HNO_3 12 mol L^{-1}) was also used to assess Hg associated to crystalline oxides, which in our case, is assessed by fraction 5. This lack of specificity could be observed because fraction four is more significant than fraction five in all treatments. Therefore, even the fourth fraction aiming to assess reduced Hg and conclusions about percentage of reduced mercury in samples were already made based on this extraction by other authors (Ramasamy et al., 2012), it must be noticed that other forms of Hg must be extracted after this extraction.

According to Gabriel and Williamson (2004), elemental mercury became less prone to volatile loss in presence of organic matter contents, especially because, organic matter adsorbs Hg (0) better than minerals. This result suggests that soil managements that keep organic matter in soil are appropriate to lessen reduction and consequently, volatilization of Hg (0). The third fraction, which assess Hg associated to organic matter, revealed higher contents of Hg when extracted at the surface, for RhAcx, while at the TyHpx, the sub surface extracted higher amounts. This was unexpected because the subsurface of TyHpx has almost half of the organic matter contents than the surface. This suggests that a further study in the types or organic matter responsible for Hg mobilization in tropical soils must be conducted in order to better assess the role of organic matter.

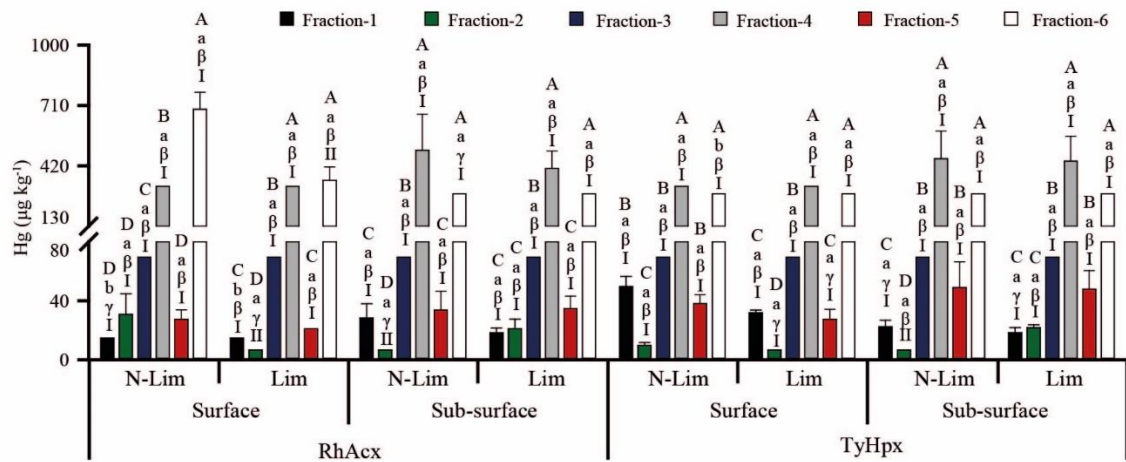
According to studies of Hg fractionating by Bloom et al. (2003) the most expressive fractions in mine contaminated soils and sediments is Fraction 3 and Fraction 6. In our spiked soils, the third fraction is also the third in significance, in comparison with other fractions, in only one treatment: RhAcx at the surface. Other treatments revealed lower contents of Hg associated to organic matter in comparison with the water extractable Hg, therefore, being the fourth fraction in significance. This is an evidence of differences between short and long time contamination and temperate and tropical soils. The long time contamination allows the presence of more stable species of Hg, which are extracted by fraction 6, and the higher

organic matter contents, in general found in temperate soils, allows Hg to be more binded to this geochemical pool.

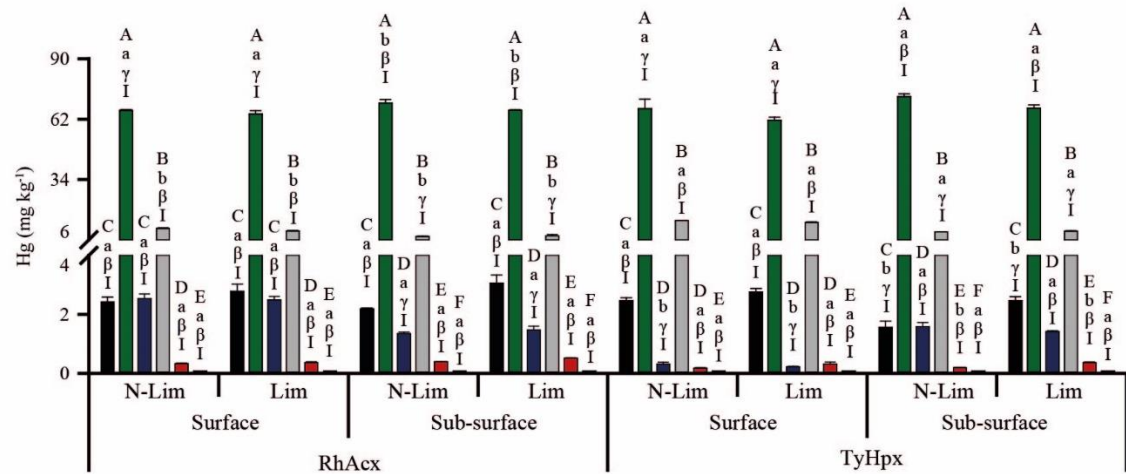
This same fractionation procedure proposed by Bloom et al. (2003) was conducted in tropical soils contaminated with Hg (0) after artisanal mining operation (Durão Jr., 2010) and revealed that the major part of Hg was associated to Fe and Al oxides. Soil samples had Hg total contents of 71 mg kg^{-1} and of this, 50 mg kg^{-1} were associated with Fe, Al and Mn oxides. In the present study, less than 1 mg kg^{-1} of Hg from 150 mg kg^{-1} approximately was noticed to be associated with those particles. Considering that RhAcx and TyHpx also have potential to bind Hg and oxides, this results highlights the difference between long and short time contamination.

Additional differences in Hg fractionation were found by Fernández-Martinez et al. (2019) who revealed that soils with industrial contamination and soils from mining with the natural presence of Hg have different levels of methylation capacity and metallic Hg contents, which are both higher in sites with industrial contamination. The reason for that is the higher stability of Hg species in mining sites, especially sulfides of Hg that is of natural occurrence (Varejão et al., 2009). In our case, the least significant pools of Hg were Fe and Al oxides and Hg sulfides (fraction 5 and 6).

(A)



(B)



(C)

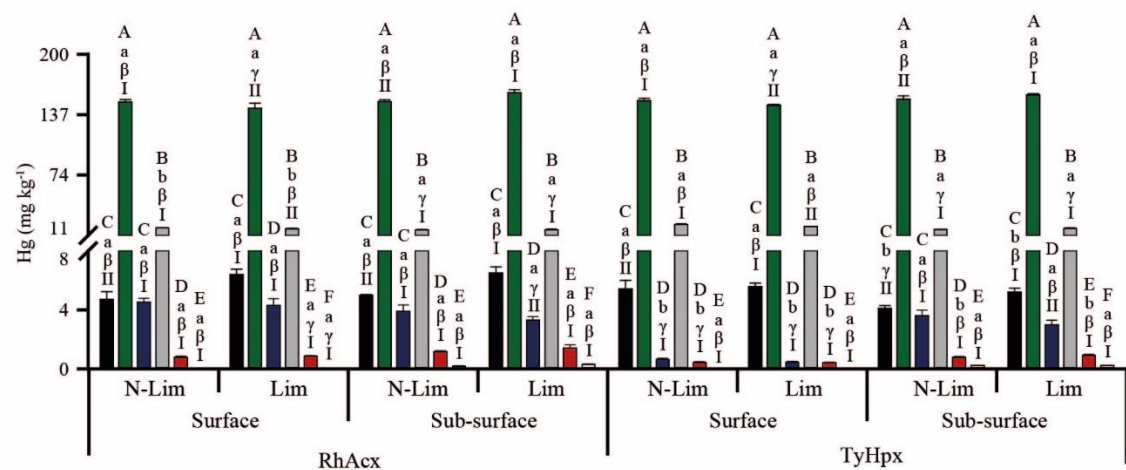


Figure 1: Comparison among Hg contents (mg kg^{-1}) extracted by sequential chemical extractions with background contents of Hg (A), after spiking soils with 3 mg L^{-1} (B) and with 6 mg L^{-1} (C) in Rhodic Acrudox (RhAcx) and Typic Hapludox (TyHpx) at both soil depths: surface (0-20 cm) and sub-surface (20-40 cm), with no-limed (N-Lim) and limed (Lim) treatments. F1: Water soluble Hg; F2: Hg soluble at pH near human stomach; F3: Hg associated to organic matter; F4: Hg extracted by HNO_3 -Elemental Hg; F5: Hg associated to Fe, Al and Mn (hydr)oxides; F6: Residual Hg. Upper-case letters compare contents extracted by fractions, lower-case letters compare soil types, Greek letters compare soil depths and roman numbers compare liming.

When spiking samples with 6 mg L^{-1} , (Fig. 1 C) the order of magnitude kept the same, being fraction 2, 4 and 1 the most important, in this order, similarly to what happened for samples spiked with 3 mg L^{-1} (Fig. 1 B). One difference is that while liming did not result in significant differences after spiking samples with 3 mg L^{-1} , treatments with lime resulted in higher extracted contents of water soluble Hg (Fraction 1) at both soil types and soil depths, being more retained at no-lime treatments. Also, influence of liming on Hg contents extracted from organic matter was found on samples from sub-surface, in which lime reduced contents of Hg.

Liming seemed to increase Hg extractability by water (Fraction 1) in both soil types and soil depths. This means that increasing the soil pH promoted higher solubility of Hg in tropical soils. One reason for that probably is associated to the displacement of Hg^{+2} promoted by Ca^{+2} . However, the bioaccessible fraction (fraction 2) had different behavior after lime application depending on soil depth, lime application was responsible for lower Hg contents at soil surface and higher contents at the sub-surface. Fraction 4 had lower contents of Hg after lime application, but only at the surface of both soils. According to Miretzky et al. (2005), an increase in pH favors the formation of the $\text{Hg}(\text{OH})_2$ over other species such as HgOH^+ and HgClOH and the soil surface becomes less repulsive to cationic species, thus increasing adsorption. We can therefore conclude that the action of liming on mobilizing Hg depends on the fraction studied. Considering that the amount of Hg extracted by pure water hardly express the natural conditions in the environment, fraction 2 and fraction 4 are of paramount relevance in understanding Hg retention in soils, and one can state that liming could immobilize Hg in tropical soils either by decreasing bioaccessibility, decreasing volatilization or increasing adsorption processes, concerning these soil types. According to Yan et al. (2019), alkaline conditions are better for retaining Hg in a mine area, which corroborates with our study, especially because due to H^+ ions removing and replacing metal ions (Gabriel and Williamson, 2004).

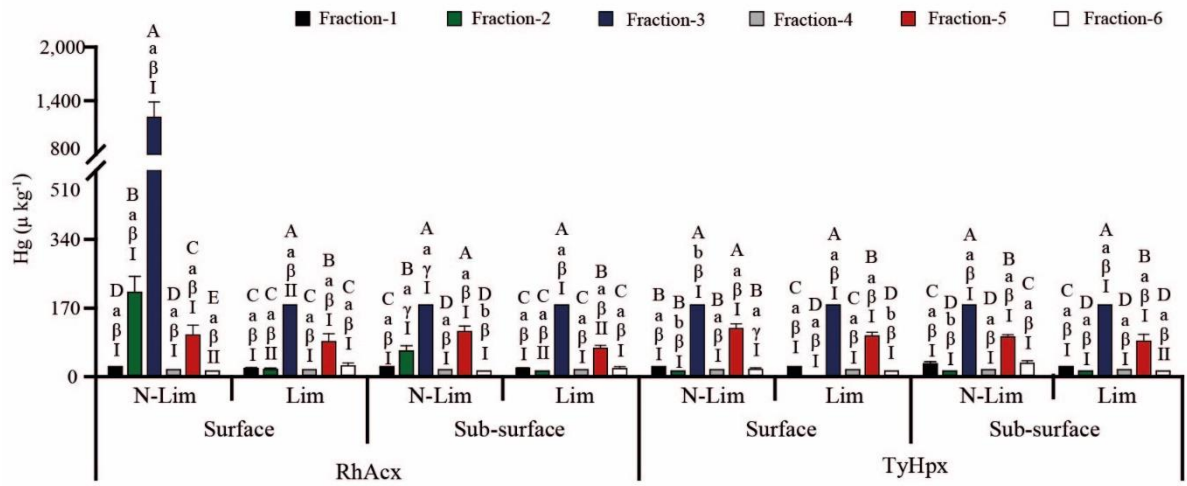
The most significant fraction, Fraction 2, was not affected by soil type, but was affected by soil depths at treatments with lime application, in which sub-surface extracted higher amounts of Hg, again showing the importance of organic matter to reduce bioaccessible contents. The effect of soil type was observed in the fraction 4 only at surface, being TyHpx responsible for a higher mobilization of Hg compared to RhAcx. However, at the sub-surface, this effect was not observed. In summary, spiking samples with different Hg concentrations led to

differences on effects of soil types, soil depths and lime application, but the magnitude of Hg geochemically pools were the same.

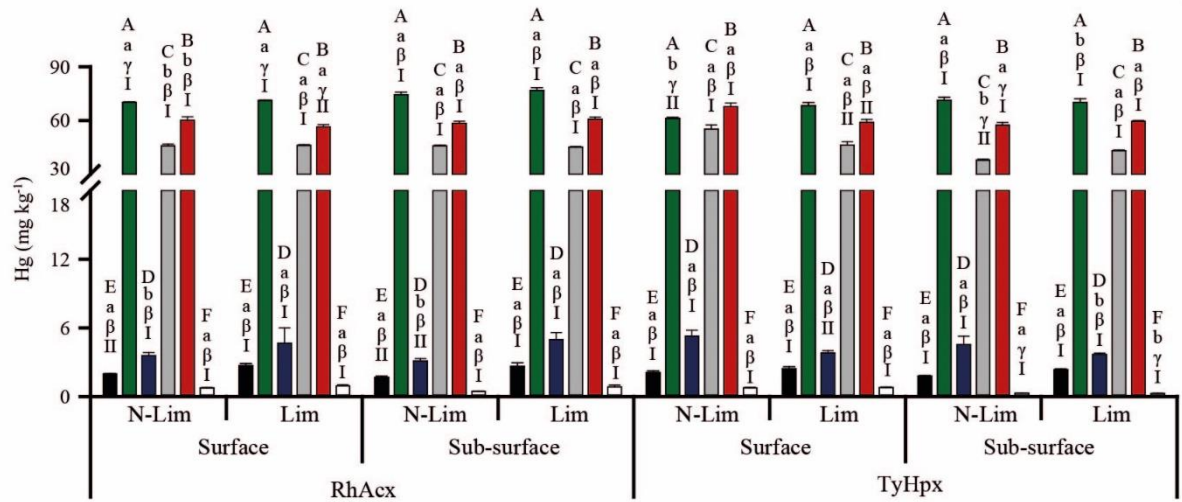
3.2. Single extractions

Figure 2 shows the comparison among treatments for discrete extractions. Again, similarly to what was observed for sequential extractions, differences in concentrations of spiking were not so significant, as contaminating soils with either 6 or 3 mg L⁻¹ revealed the same pattern of mercury distribution. However, the pattern of extraction has clearly changed. The first observation is that Fraction 4 and Fraction 6 loses their importance in background values of Hg and fraction 3 and 5 increase importance as the most significant fractions (Figure 2A). This could suggest that in the sequential extractions scheme, the Hg contents that are here related to fraction 3 (Hg associated to organic matter) and to the fraction 5 (Hg associated to Fe and Al oxides) were altered by previous solutions, suggesting a lack of selectivity of the solutions employed. According to Piccolo et al. (1996), the organic acids, as acetic acid, can alter the structures of humic substances of organic matter, producing a degree of permanent alteration into the hydrophobic arrangement of humic micelles. These alterations promoted by the solution of fraction 2 could have been responsible for altering the pattern of distribution of Hg associated to organic matter when comparing sequential and discrete scheme.

(A)



(B)



(C)

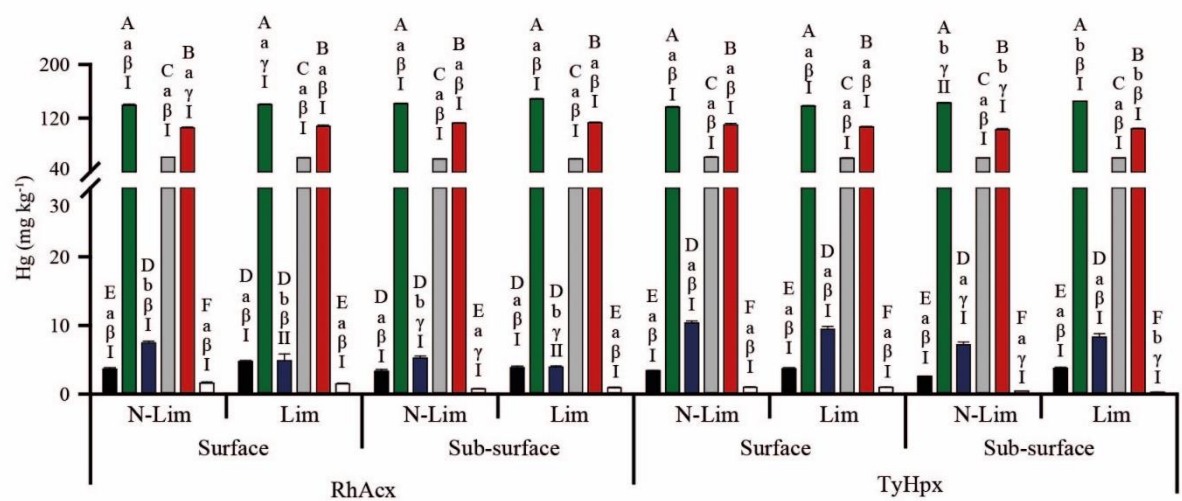


Figure 2: Comparison among Hg contents (mg kg^{-1}) extracted by individual chemical extractions with background contents of Hg (A), after spiking soils with 3 mg L^{-1} (B) and with 6 mg L^{-1} (C) in Rhodic Acrudox (RhAcx) and Typic Hapludox (TyHpx) at both soil depths: surface (0-20 cm) and sub-surface (20-40 cm), with no-limed (N-Lim) and limed (Lim) treatments. F1: Water soluble Hg; F2: Hg soluble at pH near human stomach; F3: Hg associated to organic matter; F4: Hg extracted by HNO_3 -Elemental Hg; F5: Hg associated to Fe, Al and Mn (hydr)oxides; F6: Residual Hg. Upper-case letters compare

contents extracted by fractions, lower-case letters compare soil types, greek letters compare soil depths and roman numbers compare liming.

For spiked samples, single extractions revealed the following order for extracted contents: fraction 2 > fraction 5 > fraction 4 > fraction 3 > fraction 1 > fraction 6. Fraction 5 in single extractions increased importance compared to the sequential extraction, extracting contents up to 60 mg kg⁻¹ (Figure 2B) and 105 mg kg⁻¹ (Figure 2C) in a total of 150 mg kg⁻¹ and 300 mg kg⁻¹, respectively. This means that the Hg that was extracted by the fifth step on the sequential extraction was extracted earlier by previous step, which corroborates with Fernández-Martínez and Rucandio (2013) who revealed that fraction 4 can also extract Hg adsorbed to crystalline oxides. However, the second fraction remained to be the most important, as in the sequential extraction. This reinforces the risk of a short time contamination and the contents of Hg that could be adsorbed by stomach, which increases the risk depending on the route-exposure scenario.

At 3 mg L⁻¹ (Figure 2B), the second most important fraction in this scheme, the Hg associated to Fe and Al oxides, did not reveal a pattern of effect of soil types and soil depths. However, liming resulted in higher extracted contents in limed treatments at surface, in both soil types, compared to the same treatments at sub-surface. When spiking samples with 6 mg L⁻¹, this effect of liming was not observed anymore, but there is an effect of soil type, when comparing the soil sub-surface, RhAcx extracted higher contents of Hg compared to TyHpx, which is in agreements with the higher clay content of this soil type.

The short extraction content for the last fraction which assesses Hg that is strongly bounded to soil even at the single extraction shows that maybe aqua regia at room temperature may be not the best extractor for Hg. Ramasamy et al. (2012), who performed the same extraction procedure proposed by Bloom et al. (2003) in sediments revealed contents lower than the detection limit for the fraction with aqua regia at room temperature. According to Malehase et al. (2016), who also performed this extraction procedure, heating samples in digester blocks would be necessary to facilitate the digestion procedure. Borghesi et al. (2016) also heated samples in order to evaluate digestion efficiency with aqua regia. Therefore, heating samples would be mandatory in order to assess Hg more stable in soil.

In summary, as observed for sequential extractions, different concentrations of Hg arise differences in the effects of soil depth, soil type and lime application, but not in the magnitude of the Hg pools in soil. However, differences in the fractions of single and sequential extractions were important to discuss the selectivity of each solution and the role of each pool in retaining Hg in tropical soils.

3.3. Comparing Sequential and Single extractions

The main advantage of performing single extractions instead of sequential extraction is that if you want to assess one specific fraction of Hg in soils, it is not necessary to pass along with all the previous steps. In addition, one-step extractions are generally fast, cost-effective, and require less technical knowledge (Reis et al., 2016). On table 3 it is possible to see that Fractions 1, 2, 3, 4 and 5 are correlated with the contents extracted in the single extraction. The correlation of Fraction 1 in sequential and single extractions are quite obvious because it does not have a previous step to make them different, except for the cleansing step that is required in the sequential extraction and can, therefore, extract more Hg.

The main advantage in this correlation is related to step 4 and 5, because they require respectively 3 and 4 steps before assessing the aimed fraction. It is possible to assess Hg associated to elemental Hg and Hg binded to Fe and Al oxides without performing the whole sequential procedure, by using the correlation equation. According to Bacon et al. (2008), chemical extractions conducted sequentially are relatively complicated, time-consuming and expensive, which encourages the use of single extractions. Audry et al. (2006) also tried to perform multiple single extractions using a well-established sequential one in order to assess trace metals fractionation and concluded that issues regarding selectivity in the extraction for most mobile phases could not be observed in the sequential scheme, which is an advantage of using it as individual extractions.

Differences found in the single and sequential extractions could be associated to lack of specificity that is not identified in the sequential extractions (Audry et al., 2006) and incomplete extraction in sequential extractions (Bacon and Davidson, 2008). Also, potentially toxic elements, like Hg, can form, during the extraction process, new mineral phases that were not originally present in the sample, and according to Scheckel et al. (2005) who studied Pb

fractionation in soils and different evaluation methods, this only happens during sequential chemical extractions. Due to these reasons and to the possibility of evaluating the geochemical reactive pools of Hg in soils in a short time, single extractions would be better than a sequential scheme.

Table 4: Correlation equations among sequential chemical extractions and single multiple extraction for the six geochemical fractions.

Fraction	Equation	R ²	RMSE (mg kg ⁻¹)
1	Hg (mg kg ⁻¹)= 1.3781 ^{**} x Hg in discrete method (mg kg ⁻¹)	0.98 ^{**}	0.5068
2	Hg (mg kg ⁻¹)= 1.0466 ^{**} x Hg in discrete method (mg kg ⁻¹)	0.99 ^{**}	5.6617
3	Hg (mg kg ⁻¹)= 0.3361 ^{**} x Hg in discrete method (mg kg ⁻¹)	0.61 ^{**}	1.3370
4	Hg (mg kg ⁻¹)= 0.2796 ^{**} x Hg in discrete method (mg kg ⁻¹)	0.97 ^{**}	2.1593
5	Hg (mg kg ⁻¹)= 0.0073 ^{**} x Hg in discrete method (mg kg ⁻¹)	0.87 ^{**}	0.1990
6	Hg (mg kg ⁻¹)= 0.1170 ^{ns} x Hg in discrete method (mg kg ⁻¹)	0.12 ^{ns}	0.2260

** : significant (p<0.01); * : significant (p<0.05); ns: non-significant (p<0.05); R²: regression coefficient; RMSE: root-mean-square error. 1: Fraction of water soluble Hg; 2: Fraction of Hg soluble at pH near human stomach; 3: Fraction of Hg associated to organic matter; 4: Fraction Hg extracted by HNO₃ (elemental Hg) ; 5: Fraction of Hg associated to Fe, Al and Mn (hydr)oxides; 6: Fraction of residual Hg.

3.4. Role of Fe and Al on Hg immobilization

Due to the importance of Fe and Al oxides in retaining Hg (Roulet et al., 1998; Carvalho et al., 2018; Montoya et al., 2019) especially in tropical soils, a more deep study in the extraction step proposed by Durão Jr. (2010) was made. According to Durão Jr. (2010), a fractionation in tropical soils contaminated with Hg after artisanal mining operations revealed that the major pool of Hg is associated to Fe, Al and Mn oxides. Also, in our study, concerning short time contamination scenario, this same fraction was only more significant when the procedure was performed as single multiple extractions. According to Windmüller et al. (2015), soil contaminated with Hg was suggested to have complexes of Hg (II) formed with inorganic and organic sulfur ligands and/or nonspecific adsorption onto oxides of Fe, Al and/or Mn which explains the close association with these colloids and Hg.

These facts encouraged us to perform this deep evaluation. In figure 3 there is a correlation study among the semi total contents extracted by nitric acid and heating samples with

microwave (USEPA 3051A) and the contents extracted by fraction 5 in the single extraction (HCl 6 mol L⁻¹), concerning both soil types.

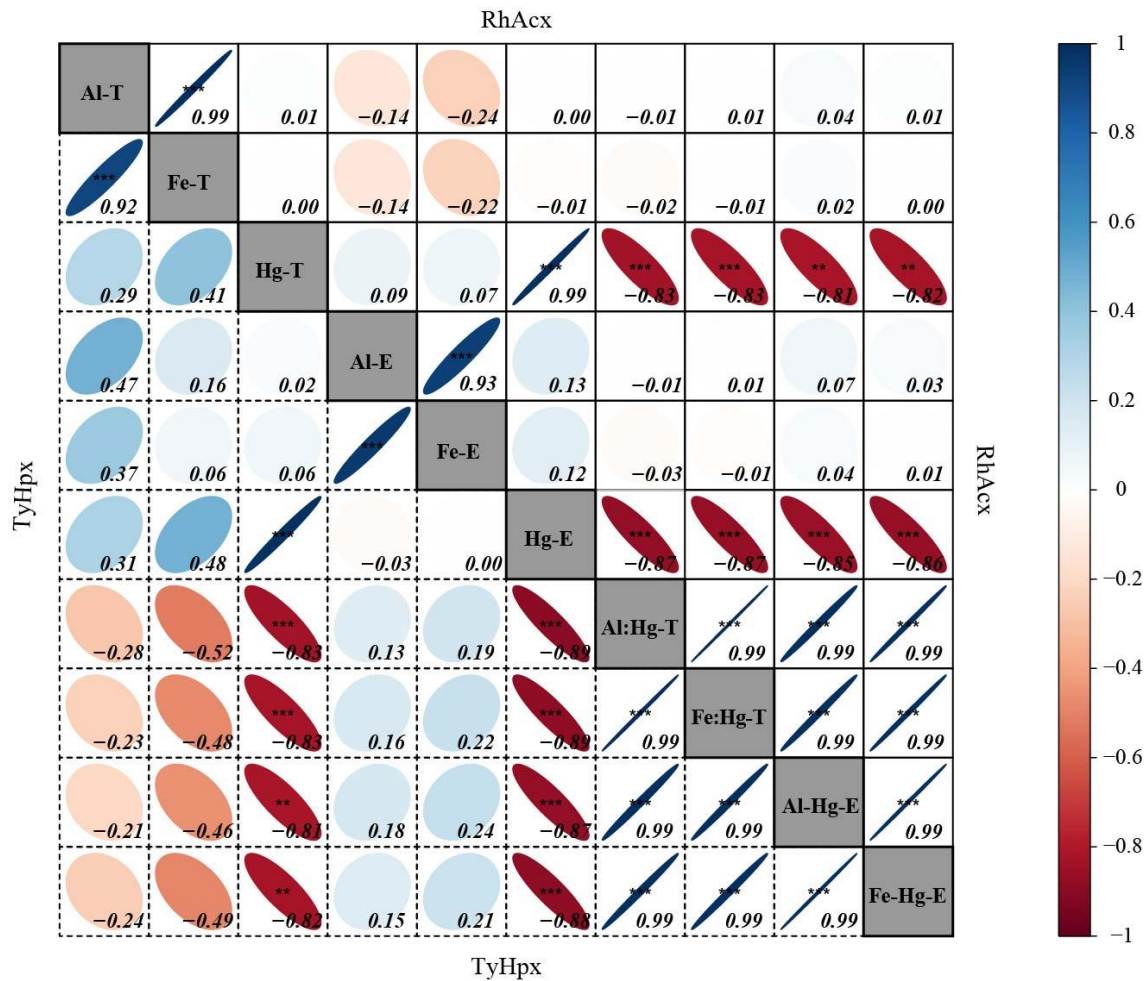


Figure 3: Pearson correlation matrix for both soil types: Rhodic Acrudox (RhAcx) and Typic Hapludox (TyHpx). Al-T : Aluminum semi-total content; Fe-T: Iron semi-total content; Hg-T: Mercury semi-total content, Al-E: Aluminum contents extracted by HCl 6 mol L⁻¹; Fe-E: Iron contents extracted by HCl 6 mol L⁻¹; Hg-E: Mercury contents extracted by HCl 6 mol L⁻¹; Al:Hg-T: Proportion of aluminum semi-total contents and mercury semi-total contents; Fe:Hg-T: Proportion of iron semi-total contents and mercury semi-total contents; Al:Hg-E: Proportion of aluminum contents extracted by HCl 6 mol L⁻¹ and mercury contents extracted by HCl 6 M; Fe:Hg-E: Proportion of iron contents extracted by HCl 6 mol L⁻¹ and mercury contents extracted by HCl 6 mol L⁻¹.

It can be seen that there is a positive and significant correlation between Al and Fe semi-total contents in both soils, which is closely associated to Fe and Al oxides contents in tropical soils. The correlation is also positive and significant in contents extracted by HCl 6 mol L⁻¹. Mercury semi-total contents is correlated to mercury contents in the extraction with HCl 6 mol L⁻¹, which means that higher Hg contents in soil, promoted higher extracted contents by HCl. This validation can only be made if the increase in Hg semi total contents in soil is from the same source of Hg: HgCl₂ (in this case).

Mercury contents extracted by HCl (6 mol L^{-1}) (Hg-E) did not show significant correlation with contents of Fe and Al in this extraction. The lack of correlation among the extracted contents revealed that some Fe that was not binded to Hg was also released during this extraction and the opposite, mercury that was not binded of Fe could have been also released. Negative and significant correlations were found between Hg-E and the proportions Al:Hg-T, Fe:Hg-T, Al:Hg-E and Fe:Hg-E. This result means that while Hg contents in the extraction procedure increases, the proportions Al:Hg-T, Fe:Hg-T, Al:Hg-E and Fe:Hg-E decreases, this was true as the semi total contents of Al and Fe are somehow constant for both soils, and the increases in Hg concentration make these proportions to be smaller. Positive correlations were found for Al:Hg-T, Fe:Hg-T, Al:Hg-E and Fe:Hg-E among themselves, this is, while one proportion increases, also the other increases, which is expected as the higher is the semi total content, the higher is the extracted content.

The rationale for the chemical extraction with HCl (6 mol L^{-1}) could be explained by the acid dissolution of Fe and Al oxides, a dissolution mechanism that may dissolve Hg that is bounded to oxides after forming complexes of Cl with Fe and Hg. In addition, the excessive protonation at the surface of oxides displace the adsorbed Hg. The lack of correlation between Hg, Fe and Al contents in the extracted solution ($\text{HCl } 6 \text{ mol L}^{-1}$) is explained by the simultaneous extraction of weakly bound Hg that in the sequential procedure would be extracted prior to the fraction 5. This result, together with table 3 shows that is possible to have an estimation of the contents of Hg adsorbed in Fe and Al oxides in the studied tropical soils in a wide range of conditions: with liming and in both soil depths and soil types by performing the individual extraction, but for a better approach, would be suitable to ensure extracting of Hg weakly sorbed to soil prior to fraction 5 extraction.

3.5. Principal component analysis (PCA)

The extraction procedures were plotted (Figure 4) at a principal component analysis: for the sequential extractions that were positive correlated with the discrete scheme we have plotted the discrete scheme (Fraction 1, 2, 3, 4 and 5), and for the one without correlation, both procedures were plotted (Fraction 6).

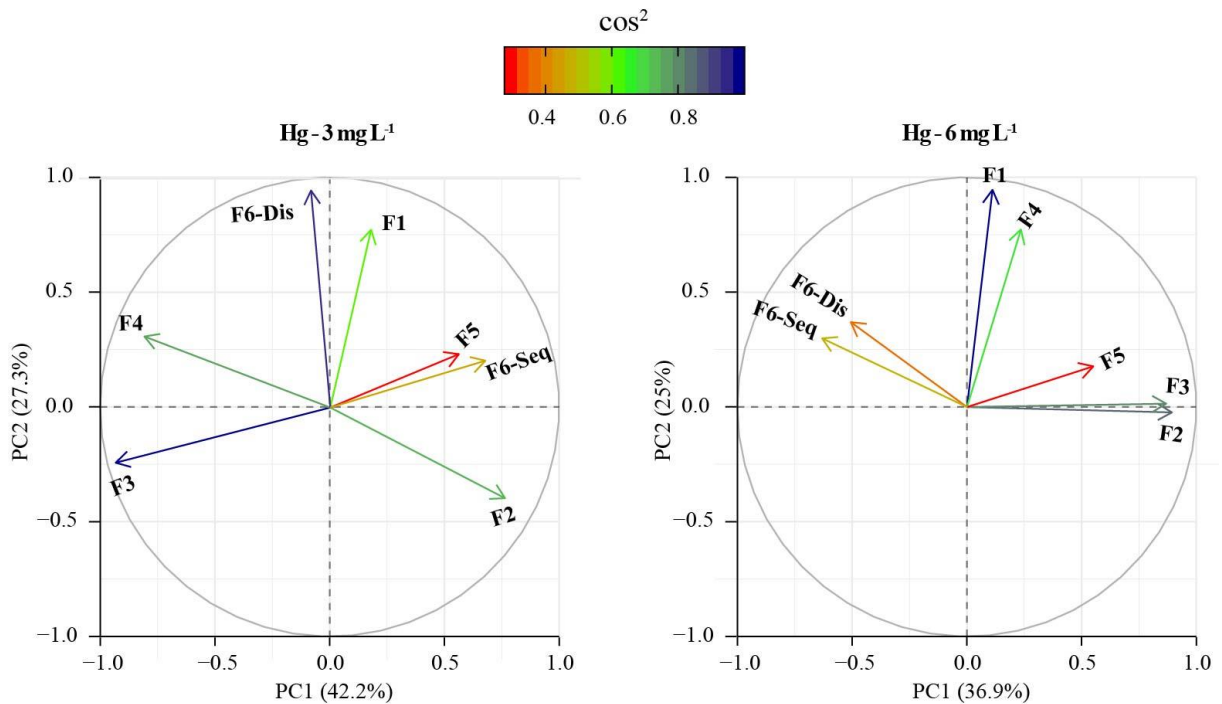


Figure 4: Principal component analysis for chemical extraction procedures after spiking with 3 and 6 mg L⁻¹ solutions of Hg. F1: Water soluble Hg at the discrete extraction procedure; F2: Hg soluble at pH near human stomach (acid) at the discrete extraction procedure; F3-Seq: Hg associated to organic matter at the sequential extraction procedure; F3-Dis: Hg associated to organic matter at the discrete extraction procedure; F4: Elemental Hg at the discrete extraction procedure; F5: Hg associated to Fe and Al oxides at the discrete extraction procedure; F6-Seq: Residual Hg at the sequential extraction procedure; F6-Dis: Residual Hg at the discrete extraction procedure.

The different extractions are differently associated depending on the concentration used to spike samples. At 3 mg L⁻¹ it is revealed that while contents extracted by fraction 2 increases, fraction 4 decreases. This shows that increasing volatilization of mercury, or, increasing reduction of Hg, stomach bioaccessibility goes in an opposite direction and increases. This is due to the low solubilization of the species Hg (0) (Windmüller et al., 2015) assessed by fraction 4, which decreases Hg bioaccessibility, an ability highly dependent on solubility (Ruby et al., 1996).

According to Bloom et al. (2003), after performing this same extraction procedure in sediments contaminated with Hg, fraction 3 which evaluates Hg binded to organic matter, revealed a negative correlation with fraction 4 and fraction 6, which assess reduced Hg and more stable phases of Hg, respectively. In our results, the negative correlation was observed only for the fraction 6, at the sequential scheme. Fraction 3 has also a negative correlation with fraction 5.

However, spiking samples with 6 mg L^{-1} , this is, in soils with more severe contamination compared to the 3 mg L^{-1} , correlation among fractions revealed another pattern, especially for fraction 2 and fraction 3 which assess bioaccessible Hg and Hg associated with organic matter. Increasing Hg concentration in the form of HgCl_2 promoted higher association between Fraction 2 and fraction 3. This close association between bioaccessible contents of Hg and Hg associated with organic matter is explained by the reduction of bioaccessible contents when Hg is close associated to organic matter.

4. Conclusion

Short time contamination revealed to have a great potential in increase Hg bioaccessibility and Hg volatilization as Hg (0), which is of great relevance in risk assessment studies, but the organic matter contents, clay contents and lime application revealed a trend to retain more Hg in these geochemical pools. Individual extractions that assess water soluble Hg, Hg soluble at pH near human stomach, Hg associated to organic matter, elemental Hg and Hg associated to Fe and Al oxides revealed a positive and significant correlation to the sequential extraction scheme, which needs to be highlighted specially with the advantages of single extractions over sequential ones. The role of Fe and Al oxides in retaining Hg in tropical soils is not so emphasized considering short time contamination scenario, only in the single extraction scheme, suggesting an overestimating in this last procedure. However, critics to this procedure can be made, especially regarding the specificity on removing Hg species. The fraction that assess bioaccessible contents of Hg (Fraction 2) and fraction that assess reduced forms of Hg (Hg (0)) have an opposite pattern in soils due to the low solubility of Hg (0).

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Supplementary

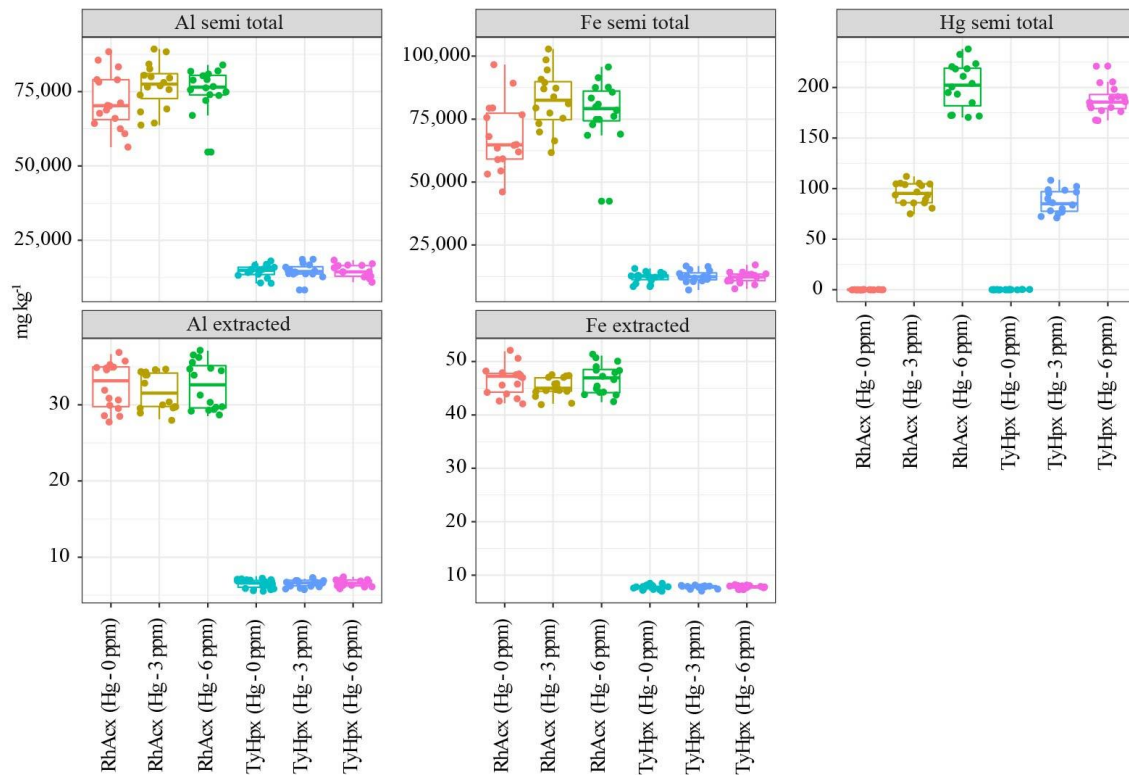


Figure 5: Boxplot of contents semi-total of Fe, Al and Hg extracted by USEPA 3051A and Al and Fe contents extracted by HCl (6 mg L^{-1}) at two soil types: Rhodic Acrudox (RhAcx) and the Typic Hapludox (TyHpx) and three spiking concentrations: 0, 3 and 6 mg L^{-1} .

ARTIGO 2 - BIOACCESSIBLE CONTENTS OF HG IN TROPICAL SOILS

(VERSÃO PRELIMINAR)

Artigo redigido conforme normas do periódico científico *Science of the Total Environment* (ISSN: 0048-9697). Este artigo é uma versão preliminar e, portanto poderão surgir alterações para adequá-lo. Os gráficos e tabelas foram inseridos no decorrer do texto para facilitar a leitura

ABSTRACT

Estimating bioaccessible contents in soils is essential to evaluate the real risks of soils contaminated with potentially toxic elements. Mercury is one of these elements and hotspots with high contents of Hg in soil can be found in Brazil and elsewhere and therefore, studying its bioaccessibility represents a better understanding of its dynamics in the environment. In this study, spiked soil samples with HgCl₂ were used and four bioaccessibility tests were used: with dilute nitric acid at room temperature, with dilute nitric acid at body temperature (37°C), the SBET methods and the gastric phase of the IVG protocol. Effects of liming, organic matter, pH and Hg concentration were assessed for all tests. Bioaccessible contents percentages were higher than 20% on average for the two first tests and higher than 40% on average for the third and fourth tests. The SBET method has a lower variation in bioaccessible contents with Hg concentration. Samples from Descoberto, a city with a well-known record of Hg (0) soil contamination were also submitted to the same bioaccessibility tests and total contents of Hg in soils and sediment samples differed from the bioaccessible percentage. In general, the lower pH of the dilute nitric acid estimated higher bioaccessible contents in soil samples while the SBET method estimated higher bioaccessible contents in sediment samples. At the multivariate analysis, two groups of bioaccessibility tests are formed: one with the two tests with nitric acid and the other with SBET and gastric phase of IVG protocol, probably due to the addition of enzymes or amino acids in the last group, which estimates more realistic Hg bioaccessible contents.

Keywords: geochemically reactive contents, SBET, IVG protocol

1. Introduction

Considering the high toxicity of mercury, soil contamination with this element is worrisome. In order to evaluate different effects of Hg in human body, bioavailability tests can be conducted and they represent Hg contents in soils that are prone to reach the circulatory system, but they require “*in vivo*” studies, with animals and plants (Koch et al., 2007). In order to overcome difficulties associated to the conduction of these tests, bioaccessibility tests are a suitable alternative evaluating the capability of a contaminant to be released and promptly absorbed by gastrointestinal tract and expressing a potential cause for health

disturbances, also called oral bioavailability (Ruby et al., 1999). These bioaccessibility tests can be performed “*in vitro*” and huge efforts have been made in order to get them closer to a good correlation with real conditions in animals (“*in vivo*”) (Ruby et al., 1996, 1999; Rodriguez et al., 1999).

In vitro gastrointestinal test proposed by Rodriguez et al. (1999) is one well established procedure, validated for As and it was conducted in parallel with *in vivo* tests (with piglets). The test is conducted in two sequential phases: one that simulates gastric phase with a low pH solution and the second simulates the intestinal phase, with a high pH solution. Aiming to simplify the estimation of bioaccessible test, Barneet and Turner (2001) proposed a test that uses only pH adjustment with acid or base and it was used to evaluate Hg in floodplain soils. This preconized bioaccessibility test also uses two steps: one that simulates the stomach conditions and other that simulates small intestine conditions. This procedure was used to conclude that samples of mercury sulfides were not so bioaccessible when compared to mercuric chloride, which was 100% bioaccessible, representing a higher risk regarding Hg contamination. This fact suggested that, if the reference dose for Hg contents in soils was calculated based on mercury chloride, as it really was in the studied area, the risk can be overestimated, suggesting the importance of using bioaccessibility tests in risks assessment in contaminated soils.

The majority of metals are bioaccessible after passing through stomach due to the acidic conditions and therefore, some studies are been conducted in one single step. A more recent study from Ciminelli et al. (2018) assessed As oral bioaccessibility with a glycine solution in an acid condition, a test called Simple Bioaccessibility Extraction Test (SBET). This methodology is also used for Pb (U. S. Environmental Protection Agency, 2008) with good correlation with bioavailability “*in vivo*” studies and considering its simplicity, reveals many advantages. Ciminelli et al. (2018) concluded that the risk associated to As contamination was low after assessing its bioaccessible contents in a mining area, even this area showing high total contents of this potentially toxic element. Therefore, bioaccessibility tests can identify many gaps in the investigation of contaminated sites. This test was already used for Hg bioaccessibility by Bloom et al. (2003).

Rodrigues et al. (2018) tried to validate also a single step extraction procedure to reach good results for bioaccessibility of some potentially toxic elements in soils. Aiming that, the authors

used a dilute solution of HNO_3 in 240 soil samples from Brazil, Portugal and Netherlands and compared the results with well-established bioaccessibility tests such as: SBET (U. S. Environmental Protection Agency, 2008) and Unified Barge Method (UBM) (Ruby et al., 1996). Rodrigues et al. (2018) used many soil types, soil properties, parent materials and climatic conditions and reached good correlation among the compared procedures, confirming the feasibility of these studies. However, they did not use Hg, which represents a gap in bioaccessibility studies. All experiments performed by Rodrigues et al. (2018) considered soil particles $< 250 \mu\text{m}$, which, according to Ciminelli et al. (2018), is the soil fraction more susceptible to accidental ingestion due to its easily capacity to stick to hands.

In Brazil, Hg contamination is already known at high levels in soils from Descoberto, in Minas Gerais State. The contamination in that area has its origin with the gold artisanal mines from XIX century and it is justified by the use of mercury on amalgam formation with gold, making easier the separation with river sediments (Durão Jr., 2010). Studies of quantification and speciation were conducted in that area, giving important information about oxidation process (Windmüller et al., 2015), methylation capacity (Mendes et al., 2016) and leaching potential (Durão Jr. et al., 2009). So, a study of bioaccessibility of Hg is of high relevance in this area, evaluating the real risk associated to the exposure of this contamination.

This study aims to assess bioaccessible contents of Hg in an area in Descoberto-MG, which could help decision makers develop more effective risk assessment studies in that area. Also, spiked samples were used in order to evaluate the role of soil characteristics such as pH, clay and organic matter contents in the bioaccessibility of Hg, which would help us better understand the behavior of Hg in tropical soils.

2. Material and methods

2.1. Soil samples characterization

Soil samples used to evaluate bioaccessible contents of Hg were collected under minimal anthropogenic influence, in the city of Lavras, being the Rhodic Acrudox (RhAcx) collected at the geographic coordinates $21^{\circ}13'30'' \text{ S} / 44^{\circ}57'38'' \text{ W}$ and the Typic Hapludox (TyHpx) collected at $21^{\circ}09'18'' \text{ S} / 45^{\circ}05'55'' \text{ W}$, with the following characteristics shown at table 1 for both soil layers collected (Soil Survey Staff, 2010). These soil samples were used for the spiking experiments and for the evaluation of the influence of soil properties on

bioaccessibility tests. The effect of pH was evaluated by adding lime (CaCO_3) in soil samples in order to achieve the 60% of base saturation, which is the common for soils to have crops installed. Soil samples were allowed to settle for three weeks with CaCO_3 . Mercury contents in soils were accessed with air-dried and sieved samples (< 2 mm) and determined by a Direct Mercury Analyzer (DMA-80, Milestone, Sorisole, Italy).

Table 1: Soil chemical properties and texture of RhAcx and TyHpx at both soil depths (0-20 and 20-40 cm).

		RhAcx		TyHpx	
		0-20 cm	20-40 cm	0-20 cm	20-40 cm
Hg	mg/kg	0.07	0.06	0.03	0.03
pH		4.8	5.0	5.2	4.8
K	mg/dm ³	63.93	25.82	107.14	33.10
P		2.02	0.38	0.89	0.41
Ca	cmol _c /dm ³	0.37	0.20	1.06	0.26
Mg		0.13	0.10	0.36	0.10
Al		2.04	1.02	0.47	0.96
H+Al		13.72	7.05	4.99	4.42
T		2.70	1.39	2.16	1.40
BS		0.66	0.37	1.69	0.44
OM	dag/kg	4.69	2.28	2.09	1.36
M		75.56	73.38	21.76	68.57
V		4.62	4.94	25.37	9.15
Clay	%	65	65	27	31
Silt		14	19	14	12
Sand		21	16	59	56

pH in water soil:solution 1:2.5; Ca - Mg- Al- Extractant: KCl - 1 mol L⁻¹ ; OM; Organic matter oxidized by Na₂Cr₂O₇ (4N) + H₂SO₄ (10N); P- K-Extractant Mehlich 1; t: effective exchange cation capacity; BS: base saturation; m: aluminum saturation index; V: base saturation index.

Bioaccessibility tests were also conducted in soil samples with a long term contamination, located in the city of Descoberto, Minas Gerais. The area is a private property, located in the countryside and the area is used for cattle breeding. According to Windmoller et al. (2015), the area is an example of an area contaminated with Hg (0), from artisanal gold mine operation. Soil samples and sediments from Descoberto were characterized according to chemical and physical properties, as describing at Table 2. Contaminated soil was classified as an Inceptsol, according to the Soil Taxonomy System (Soil Survey Staff, 2010) and it was sampled at both soil layers (0-20 and 20-40 cm) and sediments were sampled from a sedimentation tank and from two sedimentation boxes. The tank was located below the boxes

and has a higher storage capacity. The area has a significant slope and these structures were built in order to avoid translocation of soils samples to the water stream that passes down the hotspots.

One sample of soil (surface and sub-surface) was also sampled as a reference sample. Table 3 shows the coordinates of the samples and the average content of Hg in each one. To assess total concentration of Hg in samples, USEPA 3051A (U.S. Environmental Protection Agency, 1998) method of digestion was applied. An amount of 0.5 g of soil was added in Teflon tubes with 5 mL of nitric acid (HNO₃) and digested in microwave. The certified sample “NIST SRM2710a -Montana Soil” was used to ensure the quality of the results and the recovery was 90%. The extracts were analyzed at the Atomic Absorption Spectrometry (CV-AAS).

Table 2: Chemical and physical properties of both soil layers of an Inceptisol collected in Descoberto.

		0-20 cm	20-40 cm
pH		5.2	5.10
K	mg/dm ³	36.82	32.34
P		1.14	1
Ca	cmol _c /dm ³	2	1.46
Mg		1.23	1.34
Al		0.04	0.35
H+Al		5.16	4.62
T		3.36	3.23
BS		3.32	2.88
M		1.19	10.84
V		39.20	38.44
Clay	%	32	40
Silt		13	15
Sand		55	45

Table 3: Geographic coordinates and average Hg concentration on samples after USEPA 3051A digestion.

Samples	Coordinates (UTM coordinates, fuse 23 K)		Average Hg concentration (mg kg ⁻¹)
Reference area (0-20 cm)	712082	7626922	0.16
Reference area (20-40 cm)	712082	7626922	0.23
Contaminated soil (0-20 cm)	715716	7632335	6.96
Contaminated soil (20-40 cm)	715716	7632335	3.56
Sedimentation box 1	715704	7632339	3.08
Sedimentation box 2	715703	7632345	25.87
Sedimentation tank	715691	7632363	2.72

2.2. Bioaccessibility tests

Previous study of these soils has shown that their adsorption capacity of Hg is really high. So, a batch adsorption study was conducted in order to spike samples more homogeneously as possible. The spiking solutions were made with HgCl_2 in a background electrolyte solution of $0.1 \text{ mol L}^{-1} \text{ NaNO}_3$ (Tong et al., 2013) in a soil solution ratio of 1:10. For the two first bioaccessibility tests, 3g of soils were spiked with 30 mL of either 120 or 60 mgL^{-1} Hg solution and for that, centrifuge tubes were shaken for 24 h, centrifuged for 20 minutes at 3000 rpm and the supernatant was analyzed for soluble Hg concentration. This soil/solution ratio was chosen because this was the suggested ratio to perform the posterior bioaccessibility test. Total Hg in soil samples were calculated after each adsorption trial, individually for each sample, considering that the total adsorbed was the difference among the initial concentration and the final concentration of Hg in solution.

The first bioaccessibility test performed was suggested by Rodrigues et al (2018) and authors have developed this bioaccessibility test using HNO_3 0.43 mol L^{-1} . After soil samples have adsorbed the Hg, soil samples were shaken for 2h at 120 rpm with the extracting solution to estimate bioaccessible contents. After that, the suspensions were filtered at $0.45 \mu\text{m}$ pore size filter papers and bioaccessible contents of Hg were analyzed at Atomic Absorption Spectrometry (CV-AAS) with a quartz cell (Model AAnalyst 800 from Perkin Elmer).

Many bioaccessibility tests are conducted trying to simulate the corporal temperature (37°C) as it may influence the release of Hg from soil. Therefore, the same bioaccessibility test before described was repeated, but this time, after adding the solution of HNO_3 , suspensions were transferred to erlenmeyers and they were shaken in a water bath at 37°C and 120 rpm, for the same two hours. The suspensions were also filtered at $0.45 \mu\text{m}$ pore size filter papers and analyzed as the previously test.

For the third and fourth test, the soil:solution ratio suggested is 1:100. Therefore, in order to spike samples for these tests, 300 mg of soil were shaken with either 12 or 6 mg L^{-1} of Hg. After shaking for 24h, the centrifuge tubes were centrifuged and the supernatant was analyzed at Atomic Absorption Spectrometry (CV-AAS) with a quartz cell (Model AAnalyst 800 from Perkin Elmer) for Hg concentration. After that, different solutions corresponding to the following bioaccessibility testes were applied.

The third test performed was the SBET test. For this test, a solution of 0.4 mol L⁻¹ glycine was used and the solution pH must be adjusted with HCl to 1.5 ± 0.05 in order to simulate gastric solution (U.S. Environmental Protection Agency, 2008). Suspensions with the previously spiked 0.3 g of soil and 30 mL of glycine solution were then transferred to an Erlenmeyer and shaken for one hour at a water bath at 37 °C at 120 rpm. After that, suspensions were filtered at 0.45 µm pore size filter paper and Hg bioaccessible contents were assessed at Atomic Absorption Spectrometry (CV-AAS) with a quartz cell (Model AAnalyst 800 from Perkin Elmer).

The fourth bioaccessibility test that was performed was the first step that simulates gastric phase of the “In vitro Gastrointestinal” test (Rodriguez et al., 1999). Even having two steps, the use of only one phase from the IVG protocol was based in the assumption that the majority of potentially toxic elements are released under acidic pH, this is, the gastric phase. The solution that simulates the gastric phase is 1% of pepsine in a solution of 0.15 mol L⁻¹ of NaCl. The gastric solution was acidified with concentrated HCl (37%) until pH= 1,80 ± 0.05. The tubes with 0.3 g of previously spiked soil sample and 30 mL of gastric solution tubes were allocated in water bath at 37°C in order to approach body temperature and then shaken for 1 h at 120 rpm.

The percentage of bioaccessible contents for all tests was calculated as:

$$\text{Bioaccessible Hg (\%)} = \frac{\text{Hg contents after the bioaccessibility tests}}{\text{Total Hg in samples}}$$

In addition to the spiked samples, all bioaccessibility tests mentioned in the before section were also conducted in samples from Descoberto, using the same soil:solution ratio and procedures previously described, but without the spiking step. Seven blank samples were performed in each analysis batch and the overall detection limit of Hg determination at the Atomic Absorption Spectrometry ranged from 0.25 µg L⁻¹ until 9.63 µg L⁻¹, determined according to the American Public Health Association (2012). All reagents were of analytical grade.

2.3. Statistical analysis

In each methodology for assessing bioaccessible contents, the data set of fraction studied had their models adjusted using the stats package (R Development Core Team, 2018) and the residuals of the variance analysis for each model were tested for their normality, independence, and homogeneity. When the assumptions were attended, variance analysis (Anova) was carried out on the data, and when statistical difference was noticed ($p < 0.05$), the means of the variables were compared using the Tukey's test ($p < 0.05$). The Anova was performed using the car package (Fox and Weisberg, 2011) and the Tukey's test was performed using the emmeans package (Lenth, 2018).

The multivariate analysis was carried out using the principal component analysis (PCA) method, generating a biplot graph with the PCA functions from the FactoMineR package (Le et al., 2008) and fviz_pca_var function from the factoextra package (Kassambara and Mundt, 2017), together with the assistance of tidyverse (Wickham, 2017). In the multivariate analysis was studied the behavior between the total bioaccessible contents of Hg, the percentage of bioaccessible contents extracted by each of the four methodologies and the total contents of Hg in soil samples from Descoberto.

3. Results and Discussion

3.1. Spiked samples and bioaccessibility tests

The spiked samples and the bioaccessibility tests conducted were shown at Figure 1. For the first and second test, the average Hg retention during the spiking procedure varied from 45% at the subsurface of TyHpx at the lowest Hg concentration, without lime application until 99.93% at the surface of RhAcx at the highest Hg concentration and at the subsurface, at the lowest concentration, both with lime application (Table 4). The third and fourth tests showed higher retention of Hg, with average retention percentages greater than 99% for all treatments (Table 5). The difference among them is essentially the concentration of Hg and the soil:solution ratio, and this last could have promoted a higher interface contact between soil and solution in a ratio of 1:50 (for third and fourth methodology) compared to 1:10 (for first and second methodology), increasing the soil retention percentage at the former.

Table 4: Average percentage of Hg adsorption and the standard deviation for the following conditions: Rhodic Acrudox (RhAcx) and the Typic Hapludox (TyHpx) without application of lime (No-Lime) and with lime, at surface and subsurface, after spiking samples with 60 and 120 mg L⁻¹.

Soil	Soil depth	Spike concentration	Lime application	Average adsorption percentage	Standard deviation
RhAcx	Surface	60 mg L ⁻¹	No-Lime	99.81	0.06
			Lime	99.72	0.14
		120 mg L ⁻¹	No-Lime	93.44	0.09
			Lime	99.93	0.02
	Subsurface	60 mg L ⁻¹	No-Lime	92.75	0.19
		120 mg L ⁻¹	No-Lime	82.81	0.39
TyHpx	Surface	60 mg L ⁻¹	Lime	99.93	0.04
			Lime	99.93	0.04
		120 mg L ⁻¹	No-Lime	82.81	0.39
			Lime	95.30	0.03
	Subsurface	60 mg L ⁻¹	No-Lime	98.90	0.10
			Lime	99.90	0.01
		120 mg L ⁻¹	No-Lime	81.98	0.37
			Lime	98.81	0.06
Subsurface	60 mg L ⁻¹	No-Lime	66.82	0.62	
		Lime	99.27	0.03	
	120 mg L ⁻¹	Lime	45.26	1.52	
		No-Lime	81.20	0.23	

Table 5: Average percentage of Hg adsorption and the standard deviation for the following conditions: Rhodic Acrudox (RhAcx) and the Typic Hapludox (TyHpx) without application of lime (No-Lime) and with lime, at surface and subsurface, after spiking samples with 6 and 12 mg L⁻¹.

Soil	Soil depth	Spike concentration	Lime application	Average adsorption percentage	Standard deviation
RhAcx	Surface	6 mg L ⁻¹	No-Lime	99.90	0.02
			Lime	99.94	0.01
		12 mg L ⁻¹	No-Lime	99.86	0.02
			Lime	99.77	0.12
	Subsurface	6 mg L ⁻¹	No-Lime	99.89	0.06
		12 mg L ⁻¹	Lime	99.92	0.08
TyHpx	Surface	6 mg L ⁻¹	No-Lime	99.67	0.06
			Lime	99.78	0.03
		12 mg L ⁻¹	No-Lime	99.82	0.17
			Lime	99.55	0.27
	Subsurface	6 mg L ⁻¹	No-Lime	99.87	0.12
			Lime	99.83	0.13
		12 mg L ⁻¹	No-Lime	99.59	0.25
			Lime	99.74	0.06
Subsurface	6 mg L ⁻¹	Lime	99.68	0.13	
	12 mg L ⁻¹	No-Lime	99.81	0.08	

Figure 1 aims to evaluate how liming, soil depth, soil type and Hg concentration affected the determination of the four methodologies for bioaccessibility tests. For the first and second method, with dilute nitric acid at room temperature and body temperature, respectively, the bioaccessible percentage varied from 20% in general until 60% and for the third and fourth tests they had a lower variation amplitude, maintaining bioaccessible percentages in the range of 50%. The bioaccessible contents of Hg can be affected by the source of Hg in soils, especially noncinnabar forms tends to be more soluble in water and therefore, more soluble (Bavec and Gosar, 2016). In the present study, the source of Hg is very soluble (HgCl_2), which explains the high bioaccessible content compared to other studies.

Rodrigues et al. (2014) stated that more soluble forms of Hg, as HgCl_2 , the one used in this study, are more related to industrial contaminated areas. However, the range variation of bioaccessible contents percentages in an industrial area in Portugal contaminated with chlor-alkali plant activity was 0.13% to 0.67%, really lower compared to our bioaccessible contents in spiked samples. This could be due to the short time involved in spiking samples compared to older contaminations, decreasing the stability of Hg and soils bindings. High bioaccessible contents percentage similar to ours were found by Zaguri et al. (2009) in reference materials (44.3%) and the total Hg contents in this sample was $99.8 \pm 31.7 \text{ mg kg}^{-1}$.

At the first test, proposed by Rodrigues et al. (2018) (Figure 1 A), it can be seen that the effect of concentration of Hg is of great relevance. Higher contents of Hg in soils revealed lower percentage of bioaccessible contents. This could be due to some precipitation of secondary phase compounds due to the higher concentration of Hg, decreasing the solubility and therefore, bioaccessibility of Hg. The precipitation of secondary phase minerals during extraction procedures conducted as “batch methodologies” was already mentioned by Bacon et al. (2008).

The soil with higher clay contents and higher organic matter contents (RhAcx), revealed in general, lower bioaccessible contents of Hg compared to TyHpx. Mercury is more bounded to finer particles (Fernández-Martínez et al., 2014) like clay, and in natural contaminated soils, Hg contents tend to increase with decreasing soil particle. This is due to the higher specific surface area of smaller soil particles and greater number of adsorption sites, consequently promoting lower bioaccessibility of trace elements (Kumpiene et al., 2017). These soil types were already studied by Lima et al. (2018) and the higher availability of Hg in TyHpx were

noticed, being ascribed to the lower organic matter and clay contents. Therefore, the higher availability of Hg already reported is associated to the higher bioaccessibility in the TyHpx. In addition to the effect of soil that highlighted the effect of clay particles and organic matter contents, the effect of the surface also highlighted this effect. In general, sub-surface had higher bioaccessible contents compared to the surface contents. The effect of organic matter accumulation in soil surface is here reinforced. According to O'Connor et al. (2019), Hg tends to be more retained at the surface, where organic matter has a significant effect, establishing strong bindings with Hg (Gabriel and Williamson, 2004; Soares et al., 2015) especially with Hg^{+2} . The effect of liming in the first methodology had a large variation with Hg concentrations. At the lowest Hg concentration, liming tended to increase bioaccessible contents and at the highest, liming tended to decrease bioaccessible contents.

The input of body temperature in the methodology proposed by Rodrigues et al. (2018) (Figure 1B) kept the greater bioaccessibility in samples spiked with lower concentration of Hg, except at the sub-surface for the TyHpx, in which higher Hg concentration promoted higher bioaccessible content percentage. The same behavior of soil types in Figure 1A was shown in 1B, as RhAcx having lower bioaccessible contents of Hg due to the reasons mentioned. The effect of soil depth is more pronounced on RhAcx, where the surface again retains more contents of Hg. The effect of lime application here is more defined and differences between Hg spiking concentrations is not significant for it. Lime application decreased bioaccessible contents in the treatments that it was a significant influence. According to Miretzky et al. (2005), the increase in soil pH decreases the surface positive charge onto soil particles, favoring the adsorption of cationic species, likewise Hg. Differences between soil depths are here more pronounced and sub-surface revealed higher bioaccessible contents.

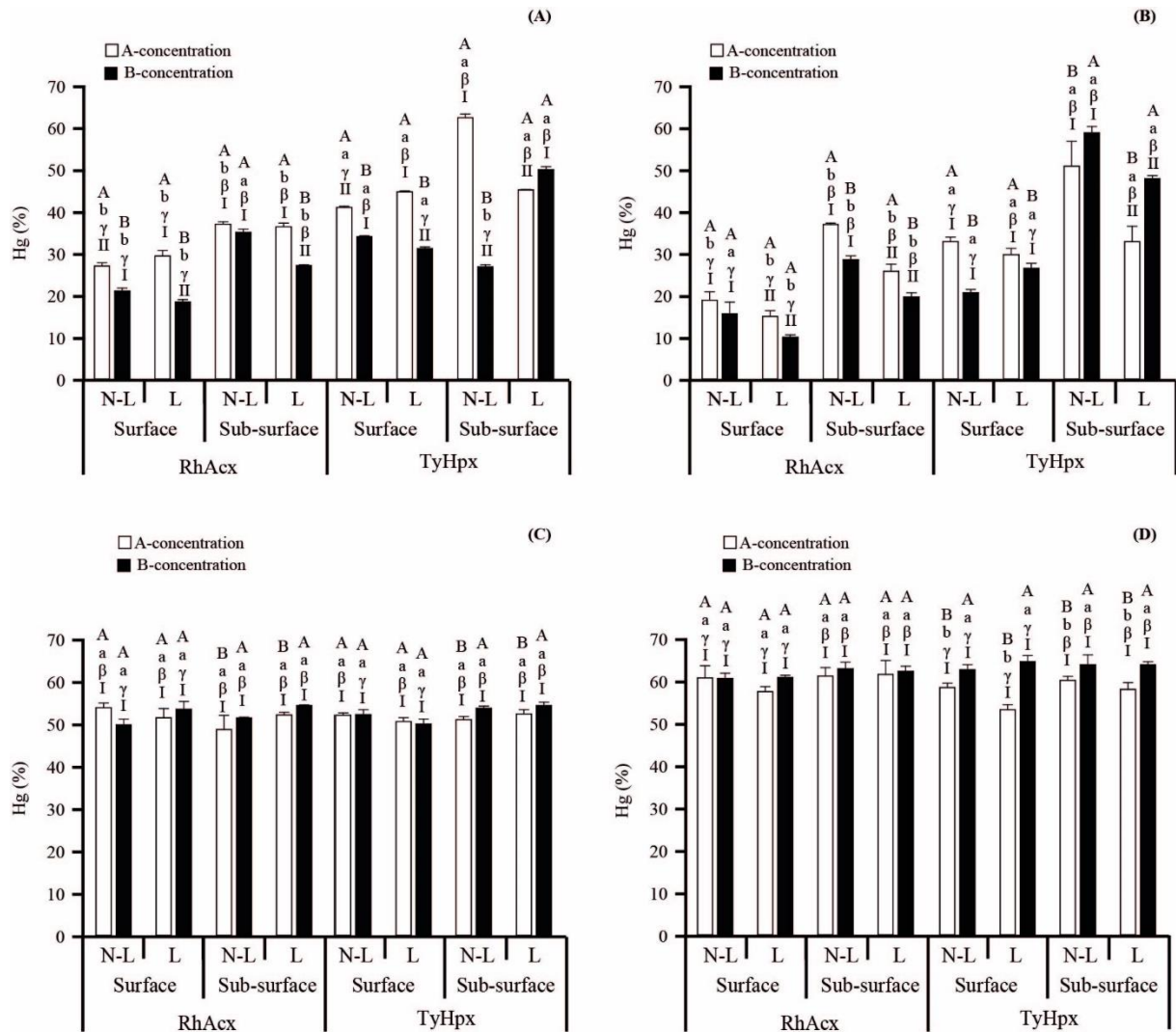


Figure 1: Bioaccessibility tests performed in spiked soil samples of Rhodic Acrudox (RhAcx) and Typic Hapludox (TyHpx). (A) Bioaccessibility test proposed by Rodrigues et al. (2018) with dilute nitric acid at room temperature with spiking solutions of A-60 mg L⁻¹ and B- 120 mg L⁻¹, (B) Bioaccessibility test proposed by Rodrigues et al. (2018) modified with dilute nitric acid at 37° C with spiking solutions of A-60 mg L⁻¹ and B- 120 mg L⁻¹ (C) Bioaccessibility test proposed by U. S. Environmental Protection Agency (2008) with spiking solutions of A-6 mg L⁻¹ and B- 12 mg L⁻¹ (D) Gastric phase of the IVG protocol proposed by Rodriguez et al. (1999) with spiking solutions of A-6 mg L⁻¹ and B- 12 mg L⁻¹. Upper-case letters compare Hg concentrations, lower-case letters compare soils types, greek letters compare soil depth and greek numbers compare the effect of liming (N-L – without lime application and L- with lime application).

The third methodology revealed that differences in spiking concentrations influenced differences in bioaccessible percentage only in sub-surface, for both soils, and higher spiking concentration showed higher bioaccessible percentage (Figure 1C). Greater content of organic matter at the surface and its significant cationic exchange capacity (CEC) at the common soil pH could be responsible for retaining more Hg, especially because Hg in soil is positively correlated with CEC (Kabata-Pendias, 2001), making the influence of Hg spiking

concentration irrelevant. Soil depth was significant for both soil types, but only at higher spiking concentration of Hg and in this case subsurface had higher bioaccessible contents of Hg. Probably, at lower Hg concentration, the organic matter present at the sub-surface was enough to retain mercury at the same extent of the surface. The smooth variation between treatments has shown that the effect of the addition of glycine, an organic substance, may have diminished the influence of pH variation, clay and organic matter contents, as it was seen at the previous tests. It is expected that, if the source of Hg is the same and the soil properties are the same, bioaccessible percentage does not vary between different Hg spiking concentrations, which is an advantage for estimating bioaccessible percentage of Hg in soils.

The fourth test, which simulates the gastric phase of the digestive tract, reveals different bioaccessible percentage with different spiking concentration, but only for TyHpx (Figure 1D). At RhAcx, bioaccessible percentage was not influenced by Hg spiking concentration, probably due to the adsorption capacity of clay and organic matter contents in this soil type, making Hg less soluble in solution (Lima et al., 2018). The effect of soil type was only noticed at lower concentration, in which TyHpx extracted lower bioaccessible percentage of Hg. Higher extractability capacity is seen at the sub-surface, at both soil types, which is explained by the strong bindings between organic matter and Hg. Likewise the methodology three, the addition of organic compounds, here represented by pepsine, seemed to promote a smooth variation in bioaccessible percentage compared to methodology one and two.

Even being composed of two phases (gastric and intestinal phase), the IVG protocol performed here only considered the gastric phase. According to Welfringer and Zagury (2009), the IVG protocol proposed by Rodriguez et al. (1999) was performed using pure compounds of HgCl_2 and revealed that 100% of this compound is bioaccessible at the gastric phase and 90% at the intestinal phase. This reinforces the fact that analyzing the gastric phase of the IVG protocol is enough to determine Hg bioaccessible contents in soil in this situation, because soil samples were also spiked with HgCl_2 . However, for soils with a long term contamination, the intestinal phase was not so negligible. Sources of Hg that are less soluble likewise mercury sulfides and $\text{Hg}(0)$ could require the evaluation of the intestinal phase.

3.2. Descoberto-MG samples

Figure 2 shows the comparison among soil samples and their Hg contents. As it was expected, the reference area revealed the lowest content for Hg. The greatest Hg content is found in a sedimentation box, a result of erosion of soil particles with Hg being adsorbed on them. The movement of soil particles in this area was already reported by Mendes et al. (2016), who found elevated concentration of Hg in the river that is close to the area, highlighting the importance of soil monitoring in that area. For some reason, the Box B1 did not receive such concentration of Hg, compared to the B2. According to Tinôco et al. (2010), diffuse sources of pollution, such as atmospheric deposition, could have contributed to the increased Hg levels in sediments from this area.

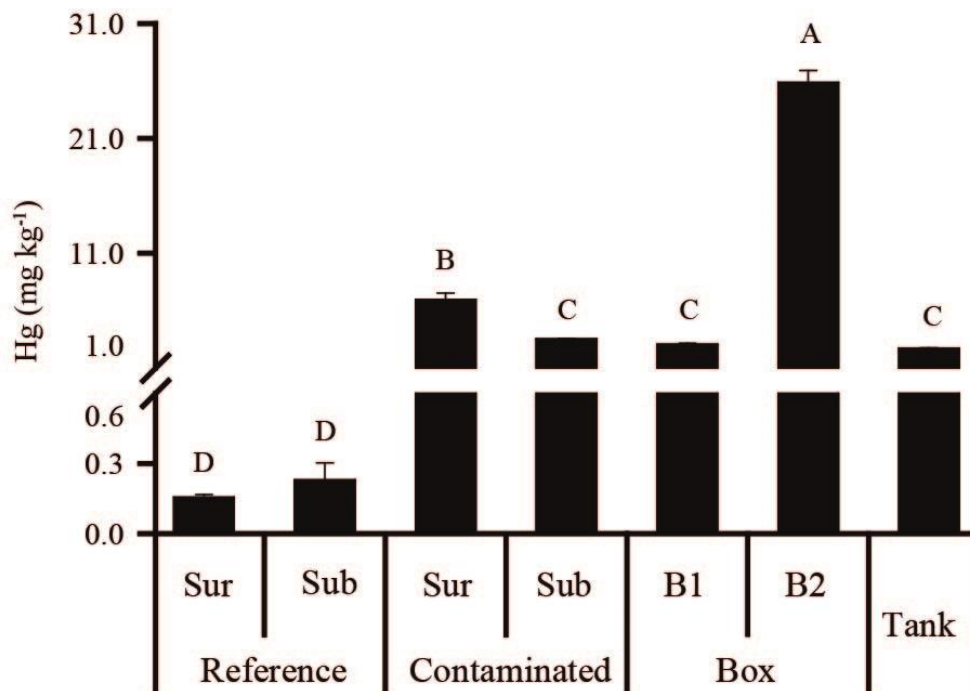


Figure 2: Total contents of Hg in samples and sediment samples from a reference area both surface (Sur) and subsurface (Sub), a contaminated area, both surface (Sur) and subsurface (Sub), two sedimentation boxes (B1 and B2) and sedimentation tank.

The surface soil revealed higher contents of Hg compared to the sub-surface in the contaminated area. The surface has high organic matter contents which is responsible for retaining more Hg through strong bindings. According to Kabata-Pendias (2001), accumulation of Hg is related to contents of C and S and therefore, concentration of Hg in surface soils can reach several times the concentration of Hg at the sub-surface.

According to Durão Jr. et al. (2009), contents of Hg in that area were in the range of 0.0371–161 mg kg⁻¹. However, Tinôco et al. (2010) revealed contents in this same area with a lower magnitude (260–550 µg kg⁻¹). These values are also different from the ones found in this present study. This variation of contents of Hg could have been promoted to differences in soil sampling and proves the heterogeneity of contamination in that area. Also, the dynamics of Hg volatilization is constantly acting and promoting changes on Hg concentration. According to Osterwalder et al. (2019) soils could be a source for Hg(0) emissions to the atmosphere for a long time, which would decrease Hg concentrations in soils as time passes by.

The quality reference value for contents of Hg in soil of Minas Gerais is 0.05 mg kg⁻¹ and the prevention value is 0.5 mg kg⁻¹ (Conselho Estadual de Política Ambiental, 2011). This means that soil and sediment samples from Descoberto are polluted because these contents are higher than the prevention value suggested. However, is important to emphasize that a more realistic background value for this area is necessary. According to Guevara et al. (2018), at the area of the Rio Doce Basin, also in Minas Gerais, a proposed quality reference value ranged from 0.27 to 0.4, depending on the statistical method used. This last variation range proposed by Guevara et al. (2018) could be more appropriate to the conditions in Descoberto due to the concentration in Hg in the reference area. This study reinforces the idea that regional studies for background values of trace elements are needed, including areas close to Descoberto.

Figure 3 compares bioaccessibility tests conducted in samples from soils and sediments. In general, the bioaccessible percentage is very low. Durão Jr. (2010) performed sequential chemical extraction in this same area, Descoberto. The procedure, proposed by Bloom et al. (2003) and modified, has one step that aims to evaluate the bioaccessible Hg at pH near human stomach. Durão Jr. (2010) found that bioaccessible contents were very low and the bioaccessible fraction at pH near human stomach and the water soluble fraction could represent the leaching potential. Therefore, Durão Jr. (2010) stated that the risk of Hg leaching in that area was minimal.

There is a considerable difference in total contents of Hg (Figure 2) and bioaccessible contents percentage. This reinforces the importance of evaluating the bioaccessible contents of potentially toxic elements and not only the total contents (Rodrigues et al., 2014). It is good to highlight that the highest bioaccessible content percentage is observed at the soil subsurface

and is approximately 7% , which corresponds to 0.2 mg kg^{-1} , lower than the proposed prevention value for soils in Minas Gerais. So, even being polluted, the risk associated to the bioaccessible contents of Hg may be considered low.

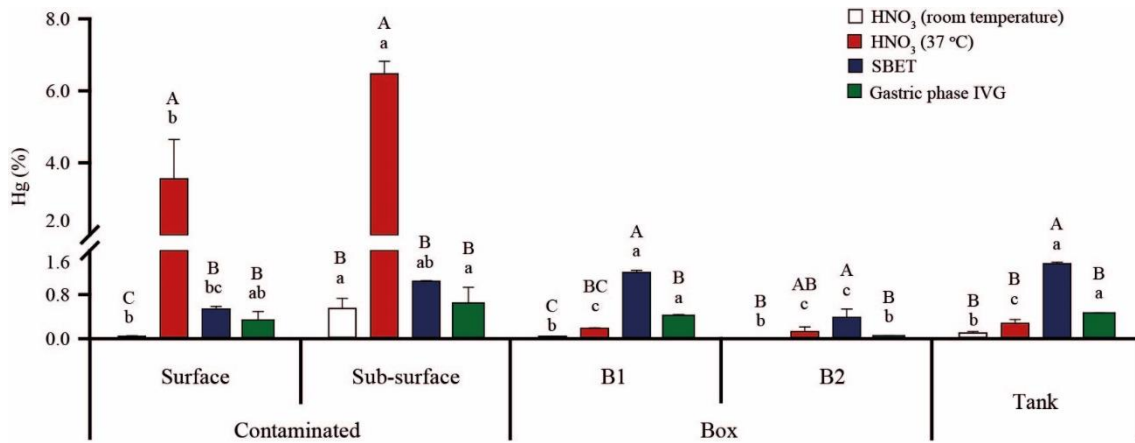


Figure 3: Comparison of bioaccessibility tests in soils (both surface and subsurface) and sediments (Boxes B1 and B2 and sedimentation tank). Uppercase letters compare methods and lower case letters compare samples.

Among soil and sediment samples, it was seen that there is a variation in samples with the greatest bioaccessible percentage for each methodology. Considering the first method, the soil sub- surface had the greater bioaccessible percentage related to the total content of Hg. Concerning the second methodology, sub-surface also had the greatest percentage of bioaccessible Hg. The third method estimated higher contents for sediments at B1 and the tank and the fourth method estimated similar contents for soil and sediment samples, except for the box B2, with the lowest content.

It can be highlighted that in general, the lowest bioaccessible content percentage was found in the first extraction methods, with HNO₃ at the room temperature. For soil samples, the highest bioaccessible percentage was obtained using the second method. However, for sediments the third methodology was responsible for the highest bioaccessible percentage. According to Groenenberg et al. (2017), the use of dilute HNO₃ expresses the geochemically reactive contents of elements in soil. According to this author, elements that established strong bindings with organic matter, likewise Hg, could have their contents extracted underestimated by dilute nitric acid, especially if the concentration of Hg in soil is low. That is a reason why the first method extracted the lowest contents in contaminated samples. However, the use of body temperature in the procedure number 2 has significantly increased the percentage of bioaccessible Hg. The reason for that could be remaining Hg (0) in these soils, from the

artisanal mining operations, and the consequently oxidation with HNO_3 which was enhanced with temperature.

According to Safruk et al. (2015), differences of procedures that uses organic compounds likewise methodologies 3 e and 4, in which glycine and pepsine are used, respectively, tend to produce higher bioaccessible contents, especially because they consider body temperature. According to Welfringer and Zagury (2009), bioaccessible contents percentage of the gastric phase of the IVG protocol were higher than the methodology Camp Dresser and McKee (CDM), which uses only pH adjustment with HCl in order to approach stomach pH (Barnett and Turner, 2001). This CDM methodology can be associated to the first extraction method, as it does not use body temperature and organic compounds.

The effect of organo-chelated compounds on bioaccessibility tests was also highlighted by Rodrigues et al. (2014). Soils from urban, industrial and mining areas were evaluated and two phases of bioaccessible contents were assessed, simulating the gastrointestinal fluid and lung fluid. The lung fluid estimated higher bioaccessible contents and one reason for that was the greater organo-complexes in the simulating fluid of this phase.

In addition to the differences on organo-compounds added to the extracting solutions of bioaccessibility tests, there is a pH variation among them as described in the methodology. The first and second methodology with dilute nitric acid uses a solution with pH approximately 0.8-0.95 (Rodrigues et al., 2018), the third solution has a pH of 1.5, adjusted with HCl and the forth, has a pH of 1.8, also adjusted with HCl. Solutions with HNO_3 employed in the first and second method was also used to displace Hg from crystalline Fe and Al oxides in sequential extractions methodologies (Fernández-Martínez and Rucandio, 2013) and therefore, with an enhancement promoted by the temperature, the second method was responsible for extracting more Hg from Fe and Al oxides, which are the most significant pool of Hg in soils from that area (Durão Jr., 2010). This fact could explain higher extraction capacity of the second methodology for soils and means that an overestimation can be happening.

In general bioaccessible content percentages are very low and matches with previous studies. Bavec and Gosar (2016) who studied the urban area close to mercury mines in Spain found bioaccessible content percentages ranging between 0.002%–3.3% in urban soils and 0.02% - 2.04% in garden soils. A slightly higher bioaccessible content percentage was found by

Safruk et al. (2015) who found up to 3 % in an area contaminated at a Canadian smelter Community. Soil management can influence the gastric bioaccessibility of Hg, especially because of the input of organic matter in some conservative practices (Bavec and Gosar 2016).

3.3. Multivariate analysis

Figure 4 shows the interaction between the many variables in this study. It can be seen two different groups: the bioaccessible contents estimated by the HNO_3 at room temperature and at body temperature and the other group formed by the estimation methodologies that use organic compounds (SBET and IVG protocol). This lack of correlation may suggest that the method proposed by Rodrigues et al. (2018) and the modification proposed with the body temperature are not suitable for assessing Hg bioaccessible contents.

The strong association between total bioaccessible contents and percentage of bioaccessible contents estimated by the several methodologies is explained as when the former increases, the latter increases as it represents a ratio between bioaccessible contents and total Hg contents. However, for the SBET methodology it can be seen the close association between the estimation of bioaccessible contents and the total content of Hg in soil, being the methodology most associated with this variable. However, this does not mean that this is the best methodology, as it was shown that increasing total contents of Hg not always means increasing bioaccessible contents.

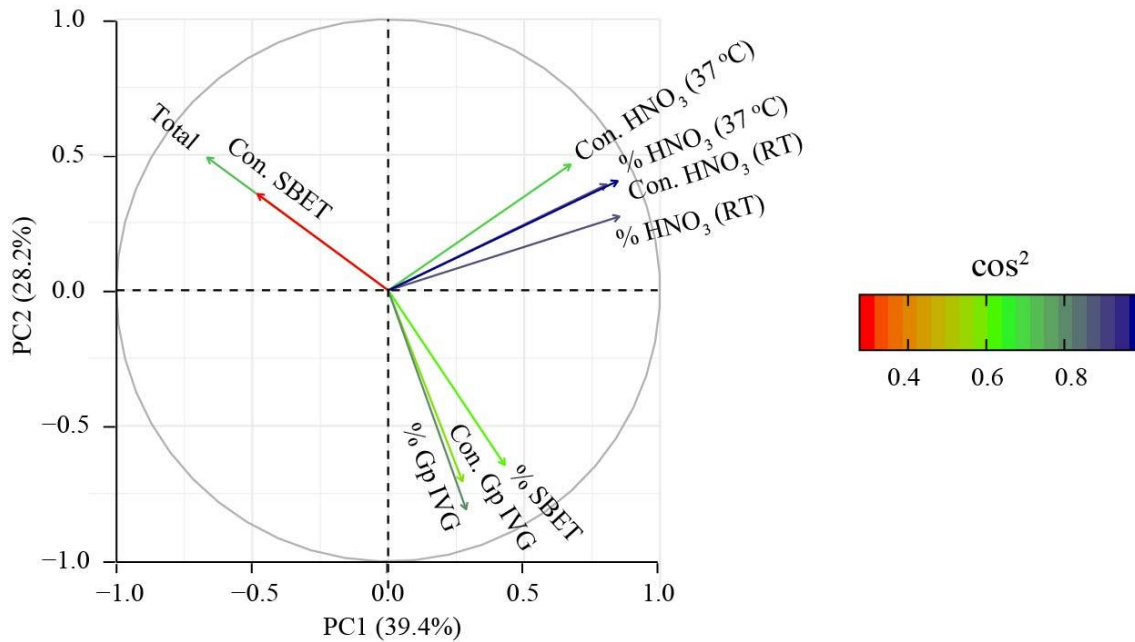


Figure 4: Principal component analysis for the bioaccessibility tests conducted in soil samples contaminated from Descoberto. Total: Total contents of Hg in soils; Con. HNO₃ (RT): Bioaccessible contents of Hg estimated by dilute nitric acid at room temperature; % HNO₃ (RT) : Bioaccessible contents percentage of Hg estimated by dilute nitric acid at room temperature; Con. HNO₃ (37 °C): Bioaccessible contents of Hg estimated by dilute nitric acid at body temperature; %HNO₃ (37 °C): Bioaccessible contents percentage of Hg estimated by dilute nitric acid at body temperature; Con.SBET: Bioaccessible contents of Hg estimated by the SBET method; % SBET: Bioaccessible contents percentage of Hg estimated by the SBET method Conc.Gp IVG: Bioaccessible contents of Hg estimated by the gastric phase of the IVG protocol; % GP IVG: Bioaccessible contents percentage of Hg estimated by the gastric phase of the IVG protocol.

4. Conclusion

Spiking soil samples and performing bioaccessibility tests have shown that soil properties as clay contents, organic matter contents and pH influenced in several different ways the estimation of these contents, depending on the methodology used. In addition, bioaccessibility methods that use enzymes or aminoacids, such as the SBET and IVG protocol allow more realistic estimation of bioaccessible contents of Hg in contaminated soils.

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CONSIDERAÇÕES FINAIS

Este trabalho evidenciou a importância de se monitorar áreas com contaminações de Hg a curto prazo, pois as mais significativas frações nesse caso são a bioacessível ao estômago humano e Hg(0), que é passível de volatilização. Em solos com valores de background de Hg as formas de Hg mais significativas são as que estão associadas a ocorrência natural de Hg e a deposição atmosférica desse elemento (Hg 0). A estimativa de algumas frações de Hg que originalmente são estabelecidas por extrações sequenciais podem ser realizadas a partir de extrações individuais, tornando o fracionamento geoquímico mais eficiente. Apesar das extrações sequenciais e discretas mostrarem esses resultados, evidências de falta de especificidade do procedimento exigem mais estudos a respeito das interações entre Hg e solos tropicais. Portanto, mesmo esse procedimento sendo utilizado por vários outros autores, falhas nas extrações podem levar a erros de avaliação nos solos contaminados.

A avaliação de biodisponibilidade por vários métodos mostrou que métodos que utilizam soluções extratoras com compostos orgânicos (enzimas e /ou aminoácidos) podem ser mais realísticos na avaliação de Hg em solos. Os teores bioacessíveis em solos contaminados com Hg divergem muito dos teores totais, o que evidencia a necessidade de incluir esse procedimento na avaliação de solos contaminados e na determinação de valores orientadores para solos.