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REPLICATION OF AN IVG PROTOCOL TO ESTIMATE BIOACCESSIBLE ARSENIC IN MATERIALS FROM A GOLD MINING AREA IN BRAZIL[1]

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SUMMARY

Tests for bioaccessibility are useful in human health risk assessment. No research data with the objective of determining bioaccessible arsenic (As) in areas affected by gold mining and smelting activities have been published so far in Brazil. Samples were collected from four areas: a private natural land reserve of Cerrado; mine tailings; overburden; and refuse from gold smelting of a mining company in Paracatu, Minas Gerais. The total, bioaccessible and Mehlich-1-extractable As levels were determined. Based on the reproducibility and the accuracy/precision of the *in vitro* gastrointestinal (IVG) determination method of bioaccessible As in the reference material NIST 2710, it was concluded that this procedure is adequate to determine bioaccessible As in soil and tailing samples from gold mining areas in Brazil. All samples from the studied mining area contained low percentages of bioaccessible As.

Index terms: trace elements, *in vitro* tests, environmental pollution.

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RESUMO: *REPLICAÇÃO DO PROTOCOLO IVG PARA ESTIMAR ARSÊNIO BIOACESSÍVEL EM MATERIAIS DE ÁREA DE MINERAÇÃO DE OURO NO BRASIL*

Testes de bioacessibilidade são úteis na avaliação de risco à saúde humana. Dados de pesquisa com o objetivo de determinar o arsênio (As) bioacessível em áreas alteradas por atividades de mineração e beneficiamento de ouro são inéditos no Brasil. Em amostras coletadas em quatro áreas (RPPN, B2, estéril e rejeito) de uma mineradora em Paracatu, Minas Gerais, foram determinados os teores de As total, bioacessível e extraído por Mehlich-1. Com base na replicabilidade e na acurácia/precisão do protocolo in vitro gastrointestinal (IVG) para determinação de As bioacessível no material de referência NIST 2710, pode-se afirmar que o protocolo é adequado para a determinação de As bioacessível em amostras de solos/rejeitos de áreas de mineração de ouro situadas no Brasil. Todas as amostras da área de mineração estudada apresentaram baixos percentuais de As bioacessível.

Termos de indexação: elementos-traço, testes in vitro, poluição ambiental.

INTRODUCTION

Gold mining and smelting can be a significant source of environmental contamination by trace elements (*e.g.*, arsenic-As) arising from ore excavation, transportation, processing of the mineral, and disposal of large quantities of waste around the mines.

It is generally stated that gold mining areas are characterized by high levels of As (Borba et al., 2003; Lee et al., 2008; Choe et al., 2009). Health risks associated with the presence of toxic elements in the environment are influenced by the level of toxicity, bioavailability and total quantity of a toxic substance in the environment (Brown Jr. et al., 1999).

The main exposure route of human beings to As is by accidental soil ingestion by children, who frequently have contact with soil in regions with high levels of the element (Cohen et al., 1998; Rodriguez et al., 1999; Hemond & Solo-Gabriele, 2004; Kwon et al., 2004). Exposure to As by dermal absorption and inhalation is considered negligible compared to ingestion (Kwon et al., 2004; De Miguel et al., 2007). Considering the importance of this human exposure pathway (soil ingestion), and the restrictions associated to using human beings as test subjects, two types of tests (bioavailability and bioaccessibility) are used nowadays for the purpose of estimating exposure doses, an important step in human health risk assessment.

The term bioavailability is defined as the fraction of a contaminant (*e.g.*, As) that reaches the circulatory system from the gastrointestinal tract, while bioaccessibility is the fraction of a contaminant that is potentially soluble in the gastrointestinal tract and available for absorption (Ruby et al., 1996, 1999; Koch et al., 2007). Bioavailability tests (*in vivo*) using animals (*e.g.*, young pigs, rats, rabbits, and other animals) are costly and time-consuming. They also require specialized staff to deal with the animals and an adequate infrastructure (Hettiarachchi & Pierzynski, 2004). Bioaccessibility tests (*in vitro*), i.e., laboratory trials with solutions that simulate the conditions of the human gastrointestinal tract to evaluate the solubility of contaminating elements (Ruby et al., 1999; Oomen et al., 2002; Girouard & Zagury, 2009), have been developed as a reproducible and simple tool to evaluate human health risks, in cases of exposure to contaminated sites.

To calculate the tolerable daily As dose via soil ingestion, the total level of the element (*i.e.*, 100 % bioavailable) is generally considered to estimate the tolerable As input by this route in human health risk assessment. This approach can lead to an overestimation of the As input, since it assumes that the entire element content of the soil can enter the blood stream. It has been claimed that bioaccessibility of As in soil cannot arbitrarily be assumed to be 100 % (Rodriguez et al., 1999; Ruby et al., 1996; Yang et al., 2002; Sarkar et al., 2005; Juhasz et al., 2007; Girouard & Zagury, 2009;). Some equations have been proposed consider the bioaccessible fraction to calculate the daily ingested As dose in risk estimates (Pouschat & Zagury, 2006).

Increased As levels have been observed in soils worldwide and also in Brazil, especially due to mining activities (Bundschuh et al., 2012). However, no studies with the objective of determining bioaccessible As in soils and mine tailings to obtain relevant information on human health risks have been published so far in the State of Minas Gerais, as well as in the rest of Brazil. Such studies could support public policies by estimating the risk arising from the exposure to contamination of residents living near the mining areas.

This study was conducted with the objectives of: (i) replicate the *In Vitro* Gastrointestinal (IVG) bioaccessibility protocol in samples from a gold mining area in Brazil and also the reference material (NIST 2710) to allow comparisons of our results with other published data from *in vitro* experiments; and, (ii) compare bioaccessible with Mehlich-1-extracted As levels.

MATERIAL AND METHODS

Sampling procedure

The samples were collected in July 2008, on the grounds of a gold mine located in Paracatu, in the northwestern region of Minas Gerais, Brazil, from the following areas: 1) Private Natural Land Reserve (RPPN), which is an area of native vegetation (Cerrado), located within the mine area, with no commercial value for gold mining; 2) mined oxidized soil layer (B2), comprising a slightly weathered material from which gold had been extracted; 3) mine overburden dump from which no gold had been extracted, due to its low Au content; and, 4) mine tailings, i.e., refuse from gold smelting. Two composite samples of material were collected by hand from each of these four areas (0 - 0.20 m layer), while each composite sample contained three soil cores.

The samples were transported to the Departamento de Ciência do Solo (DCS) at the Universidade Federal de Lavras (UFLA), Brazil, air dried and then passed through a 2-mm diameter sieve. All analytical procedures were conducted at the DCS/UFLA.

As analysis

Microwave-assisted acid digestion. The USEPA Method 3051 A (USEPA, 1998) was used for acid digestion of the samples, in a MARS-5 microwave oven. For this process, 1 g (< 150 µm fraction) of the samples (in triplicate) plus 10 mL of concentrated HNO_3 were added to Teflon® tubes that were hermetically sealed. After digestion, 10 mL of bidistilled water were added and the solution filtered through Whatman Nº. 40 filter paper. The filtered extracts were refrigerated (4 °C) until analysis.

Extraction by Mehlich-1. For this determination, the same procedure as recommended for the routine analysis of phytoavailable phosphorus was used, due to similarities between phosphate and arsenate in soils. 10 g of air-dried solid material (< 2 mm) and 100 mL of a Mehlich-1 extracting solution (0.05 mol L^{-1} HCl + 0.0125 mol L^{-1} H_2SO_4) were filled in 125 mL Erlenmeyer flasks (Kuo, 1996). The solution was shaken for 5 min on a shaking table (at 150 rpm) and filtered through Whatman Nº. 40 filter paper. Filtered extracts were refrigerated (4 °C) until analysis.

Bioaccessibility. The IVG bioaccessibility test was chosen for this study because it was validated for the trace element As in parallel *in vivo* tests (with piglets) (Rodriguez et al., 1999). The test consisted in determining the level of soluble As in two sequential phases in the gastrointestinal tract: i) gastric phase, in which the pH of the medium is acidic; and ii) intestinal phase, in which the pH is alkaline.

The gastric phase consisted of a mixture of 1 g air-dried soil/tailing (< 150 µm fraction) with 150 mL of a gastric solution consisting of 1 % pepsin (Sigma-Aldrich, St. Louis, MO, Cat. No. P7000) in 0.15 mol L^{-1} NaCl (p.a., Merck, Darmstadt, Germany). Before the solid material was added, the gastric solution was acidified with concentrated HCl (37 %, Merck, Darmstadt, Germany) to pH = 1.80 ± 0.05. The Erlenmeyer flasks were then placed in a water bath (Dubnoff type, with adjustable digital temperature and agitation) at a temperature of 37 ± 0.5 °C (simulating human body temperature) under constant horizontal shaking (100 ± 2 rpm) for 1 hour.

The intestinal medium consisted of adjusting the pH of the gastric solution to 5.5 ± 0.1 with $NaHCO_3$ (p.a., Merck, Darmstadt, Germany). An amount of 0.525 g of a porcine bile extract (Sigma Aldrich, St. Louis, MO, Cat. No. B8631) and 0.053 g of pancreatin (Sigma-Aldrich, Cat. No. P1500) were added to the solution. The flask with the mixture was also subjected to horizontal shaking (100 ± 2 rpm) for 1 hour at 37 ± 0.5 °C.

At the end of each phase (gastric and intestinal), 10 mL of solution were collected and centrifuged at 10,000 rpm for 15 min. The supernatant was filtered through a 0.45 µm polyethersulfonic membrane (nylon) into 15-mL-capacity polypropylene centrifuge tubes. The filtered extracts were refrigerated (4 °C) until analysis.

The arsenic (total, Mehlich-1-extracted and bioaccessible) concentrations were determined by Atomic Absorption Spectrometry (AAS) in a Perkin Elmer AAnalyst 800® with a graphite furnace atomizer. Extracts with As levels > 1.0 mg kg^{-1} were analyzed by AAS with an air-acetylene flame atomizer.

Bioaccessible As was calculated using the following equation:

$$\text{Bioaccessible As (\%)} = \frac{\text{In vitro As}}{\text{Total As}} \times 100$$

Quality Assurance, Quality Control

Ultrapure water with 18.2 MΩ resistivity (Milli-Q System) was used to prepare the solutions. All of the glassware, flasks and materials were treated with a 10 % HNO_3 solution for 24 h, then rinsed three times with distilled water before use.

Samples were digested and analyzed in triplicate, with the exception of the total As content in certified NIST materials (duplicate). In each processed sample group, one blank sample was analyzed in parallel for quality control. Arsenic concentrations in blank samples were always lower than the detection limit (4 µg As L^{-1}) in the graphite furnace.

To evaluate reproducibility and accuracy/precision of the tested analytical procedures for As concentration and also to allow a comparison with other published results, certified reference materials NIST 2710 and NIST 2709 were used in analytical testing. The two reference materials were analyzed using USEPA 3051A method and only NIST 2710 was evaluated by the IVG protocol. NIST 2710 has been used in other As bioaccessibility studies (Hamel et al., 1998; Ellickson et al., 2001; Koch et al., 2005; Pouschat & Zagury, 2006; Koch et al., 2007; Girouard & Zagury, 2009).

RESULTS AND DISCUSSION

Recovery percentages of total As in the certified reference materials NIST 2710 (626 ± 38 mg kg^{-1}) and NIST 2709 (17.7 ± 0.8 mg kg^{-1}) were 89.86 ± 0.76 and 91.05 ± 2.03 %, respectively (Figure 1).

Bioaccessible As in NIST 2710 was estimated at 28.41 ± 2.13 % (gastric phase) and 27.41 ± 1.55 % (gastrointestinal) (Table 1). These values were very close to those reported in other studies (Koch et al., 2005; Pouschat & Zagury, 2006; Girouard & Zagury, 2009).

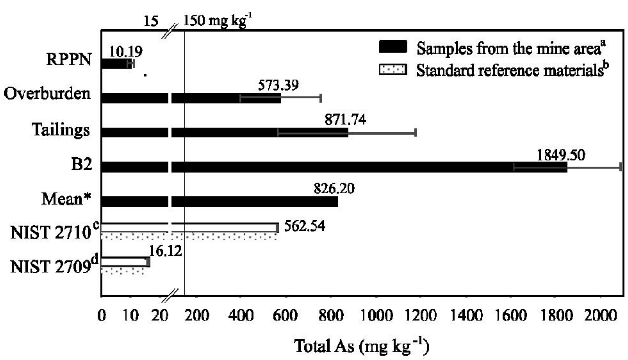

Figure 1. Total arsenic content in samples. -------- and ·······, industrial threshold and soil As prevention level, as established by CONAMA (2009), respectively. *mine refuse. [a,b]particle size < 150 µm and < 74 µm, respectively. [c,d]reference material for As content (626 ± 38 and 17.7 ± 0.8 mg kg^{-1}).

Table 1. Bioaccessible arsenic in samples from a gold mining area

Material[b]	Bioaccessible As (%)[a]	
	Gastric	Gastrointestinal
RPPN - Cerrado	< DL	< DL
Overburden	2.46 ± 1.04	3.52 ± 1.80
Smelting waste	2.68 ± 1.53	2.48 ± 0.64
B2 - tailings	0.88 ± 0.17	1.35 ± 0.26
Mean[c]	2.17	2.69
NIST 2710	28.41 ± 2.13	27.41 ± 1.55

<DL = below detection limit (4 µg L^{-1}). [a]mean ± standard deviation (n = 2, except for NIST, n = 3). [b]particle size < 150 µm, except for certified reference material (< 74 µm). [c]mine tailings, except in the private natural land reserve (RPPN, Cerrado).

Hamel et al. (1998), using the US Pharmacopoeia (USP) method, obtained a bioaccessible As value of 41 ± 18 % for NIST 2710 at a solution:solid ratio of 100:1. Considering the amplitude of the standard deviation (± 18 %) of this result, all average values of bioaccessible As cited above were within the range. *In vivo* experiments using Sprague Dawley rats as test animals reported the level of bioaccessible As as 37.8 % for the same material (Ellickson et al., 2001).

The materials from the mining area had a wide range of total As values, varying from 10.19 to 1849.50 mg kg^{-1} (Figure 1). In addition to the differences in As levels among the different materials, a great variability was recorded among samples of the same material. Relative standard deviations were 10.6 % (RPPN), 31.2 % (overburden), 34.95 % (tailings), and 12.79 % (B2). This shows that these materials are highly heterogeneous, especially those subjected to movement and constant disturbance (overburden, tailings, and B2).

Total As concentration in samples from the RPPN area is approximately 1.5 times lower than the As prevention level for soils (15 mg kg^{-1}) established by CONAMA (2009) (Figure 1). These samples also had the lowest average values of As extracted by Mehlich-1 (0.08 mg kg^{-1}) and bioaccessible As (<DL) (Figure 2).

The As concentrations of the materials evaluated in this study were compared with the industrial threshold for the element (150 mg kg^{-1}) established by CONAMA (2009), since reference values for trace elements in mine tailings and substrates were not available. Average As concentrations in mine overburden, tailings, and B2 were approximately 4, 6, and 12 times higher than the CONAMA

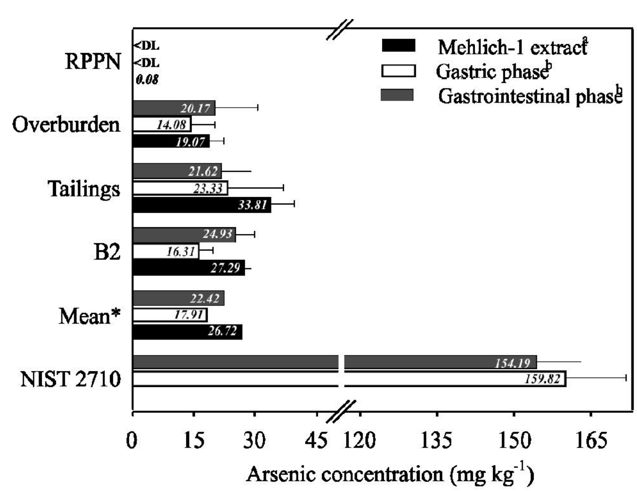

Figure 2. Mehlich-1 and bioaccessible arsenic in samples from the gold mining area. *gold mine tailings, except RPPN. < DL: below the detection limit (4 µg L^{-1}). [a,b]particle size < 2 mm and < 150 µm, except for certified reference material (< 74 µm), respectively.

industrial threshold, respectively (Figure 1). Thus, the final disposal of these materials requires adequate planning, in order to avoid As contamination of the surrounding areas. Despite the elevated total As concentrations in the materials from the gold mine study area, the risk of exposure is actually low, since the population does not have free access to these sites.

Table 1 shows that average contents of bioaccessible As (%) were low for both the gastric phase (<DL to 2.68 ±1.53 %), and the gastrointestinal phase (<DL to 3.52 ± 1.80 %) compared to those found in other bioaccessibility studies. Utilizing the IVG test for samples of material with high As concentrations, Pouschat & Zagury (2006) and Girouard & Zagury (2009) found bioaccessible As (%) (minimum and maximum values) in the gastrointestinal phase in the order of 25 ± 2.7 to 66.6 ± 2.3 (average 40.7 ±14.9) and 17 ± 0.4 to 46.9 ± 1.1 (average 30.5 ± 3.6), respectively.

Levels of bioaccessible As were similar among samples (overburden, tailings, and B2) (Figure 2), regardless of the great variations in total As concentrations (Figure 1). This is relevant since bioaccessible instead of total As should be considered when addressing human health risks associated with arsenic.

It is worth emphasizing that even though the gold mining areas in Brazil are numerous, and despite the proven high As concentrations in their surroundings (Matschullat et al., 2000; Deschamps et al., 2002; Borba et al., 2003; Andrade et al., 2008), studies on As bioavailability are still unprecedented in Brazil. Thus, the data from this study could not be compared with others carried out in the country.

The percentages of bioaccessible As in the gastrointestinal phase were slightly higher than in the gastric phase (Table 1). Studies carried out by Pouschat & Zagury (2006) and Girouard & Zagury (2009) showed the same trend. The opposite occurred with NIST 2710, for which bioaccessible As was slightly higher in the gastric phase (Table 1). Pouschat & Zagury (2006) and Girouard & Zagury (2009) found the same trend using NIST 2710.

Arsenic levels extracted from the materials collected in the gold mine area by Mehlich-1 (overburden, tailings and, B2) were similar to those obtained as bioaccessible As (Figure 2). Pearson parametric correlation analyses between bioaccessible and Mehlich-1-extracted As showed a significant positive correlation with the gastric phase ($r = 0.78$; $p < 0.05$) only. Therefore, since the Mehlich-1 method is a cheap routine analysis in comparison to *in vivo* and *in vitro* tests, it can be proposed as an alternative method to estimate bioaccessible As (gastric phase) in samples from studied mining areas, in substitution of the more complex and expensive, accredited *in vivo* or *in vitro* tests.

CONCLUSIONS

1. The IVG protocol has a high reproducibility and accuracy/precision to estimate bioaccessible As in gold mine materials.

2. In the RPPN (native cerrado area) in the gold mine, no risk in terms of As exposure to human health was detected, due to the low As concentrations. The total As levels in the other materials from the gold mine are above the industrial threshold, but the percentage of bioaccessible As was very low.

3. The existence of a significant and positive correlation between bioaccessible and Mehlich-1-extracted As was demonstrated. Despite the strong correlation between the methods, more research is needed involving a greater number of samples with large ranges of total As, Mehlich-1-extractable As and bioaccessible As, to increase the confidence in the application of the cheaper (Mehlich-1) routine method in other Brazilian gold mining areas.

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