Modification of an Existing In vitro Method to Predict Relative Bioavailable Arsenic in Soils

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Abbreviations: GI, Gastrointestinal; IVBA, In Vitro Bioaccessible; OSU-IVG, Ohio State University In Vitro Gastrointestinal Method; CAB, California Arsenic Bioaccessibility Method; RBA, relative bioavailability; HHRA, human health risk assessment

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ABSTRACT

The soil matrix can sequester arsenic (As) and reduces its exposure by soil ingestion. In vivo dosing studies and in vitro gastrointestinal (IVG) methods have been used to predict relative bioavailable (RBA) As. Originally, the Ohio State University (OSU-IVG) method predicted RBA As for soils exclusively from mining and smelting sites with a median of 5,636mg As kg⁻¹. The objectives of the current study were to (i) evaluate the ability of the OSU-IVG method to predict RBA As for As contaminated soils with a wider range of As content and As contaminant sources, and (ii) evaluate a modified extraction procedure's ability to improve prediction of RBA As. In vitro bioaccessible (IVBA) by OSU-IVG and California Bioaccessibility Method (CAB) methods, RBA As, speciation, and properties of 33 As contaminated soils were determined. Total As ranged from 162 to 12,483mg kg⁻¹ with a median of 73mg kg⁻¹. RBA As ranged from 1.30 to 60.0% and OSU-IVG IVBA As ranged from 0.80 to 52.3%. Arsenic speciation was predominantly As(V) adsorbed to hydrous ferric oxide (HFO) or iron (Fe), manganese (Mn), and aluminum (Al) oxides. The OSU-IVG often extracted significantly less As in vitro than in vivo RBA As, in particularly for soils from historical gold mining. The CAB method, which is a modified OSU-IVG method extracted more As than OSU-IVG for most soils, resulting in a more accurate predictor than OSU-IVG, especially for low to moderately contaminated soils (< 1,500mg As kg⁻¹) with $RBA\ As = 0.81\ IVBA\ As + 3.2, r^2 = 0.91$.

1. Introduction

Exposure risk associated with soils contaminated with arsenic (As) is assessed by human health risk assessment (HHRA). A critical component of HHRA is exposure assessment by various exposure pathways. In soils, often the most important pathway for As, the risk driver, associated with human exposure is incidental soil ingestion. However, use of total soil As often overestimates exposure because physiochemical properties of the soil matrix can sequester As and reduces its transmission through exposure pathways. Currently, the recommended default relative bioavailability (RBA) for soil As is 60% (U.S. EPA, 2010).

A more accurate site-specific HHRA accounts for measured RBA of As in a soil matrix as part of the exposure assessment. Many sites have soils with As bioavailability less than the default thus resulting in an over-estimation of risk. Applying site-specific bioavailability allows for a more accurate risk assessment and may reduce the need or extent of remediation.

Appropriate animal models, similar to human gastrointestinal (GI) physiology, are often used to determine bioavailability of As in contaminated soil. The most commonly used animal model for determining RBA As is the juvenile swine model (Rodriguez et al., 1999, Basta et al., 2007; Rees et al., 2009). In addition to juvenile swine, monkeys (Freeman et al., 1995; Roberts et al., 2002; Roberts et al., 2007), adult mouse (Bradham et al., 2011), and rabbit models (Freeman et al., 1993) have also been used. Use of animal models for determining RBA As has been reviewed by Basta and Juhasz (2014). Several disadvantages in conducting animal studies include expense, specialized facilities and personnel requirements, and time required to measure contaminant bioavailability.

In order to overcome some of the difficulties and expenses associated with animal dosing trials used to assess bioavailability of contaminants in soil, extensive research efforts have been directed toward development of in vitro methods that simulate the GI environment, to predict

RBA As. While there are multiple efforts to advance development and adoption of an in vitro bioaccessibility (IVBA) method, the two approaches in the United States employ pH buffered and unbuffered extraction gastric fluids; a 0.4 M glycine buffered gastric solution at pH 1.5 (Juhasz et al., 2006; Juhasz et al., 2007a, 2007b; Juhasz et al., 2008; Bradham et al., 2011; Brattin et al., 2013; Diamond et al., 2016), and the OSU-IVG which consists of an unbuffered gastric solution at pH 1.8 followed by an unbuffered intestinal solution at pH 6.5 (Basta et al., 2007; Nagar et al., 2009; Juhasz et al., 2009; Li et al., 2015). The experimental conditions of many of the bioaccessibility methods are similar. The scope of the current study was to investigate the effect of soil As content and source on the ability of bioaccessibility to predict RBA As. However, it is beyond the scope of this study to investigate all bioaccessibility methods and have selected the OSU IVG method for evaluation.

The OSU-IVG was the first in vitro method to be correlated with swine RBA As (Rodriguez et al., 1999). Juvenile swine RBA As were determined using soil in a dosing vehicle of wet feed. The OSU-IVG method of Rodriguez et al. (1999) incorporated this dosing vehicle in the OSU-IVG method to determine bioaccessible As. Use of the dosing vehicle in the in vitro method was problematic because (i) the IVG method should be developed for fasting conditions, and (ii) obtaining the exact type of dosing vehicle is problematic making it difficult to standardize the method for use by others. In Basta et al. (2007), bioaccessible As was determined by OSU-IVG with and without the dosing vehicle. Basta et al. (2007) data for 10 soils was combined with IVBA As values for 4 soils (Basta et al., 2001) that did not use dosing vehicle to obtain the following linear regression to predict RBA As.

$$\%RBA\ As = 0.883 (\%OSU-IVG\ GE) + 9.6, r^2 = 0.74 \quad [1]$$

The 14 soils used to produce Eqn. 1 were exclusively from mining and smelting sites with total As contents ranging from 405 to 17,500mg As kg⁻¹ with a median of 5,636mg As kg⁻¹. In many cases, these As concentrations are much higher than many soils for which bioavailability adjustments would be considered and also much higher than the swine in vivo vs. in vitro correlations (IVIVC) developed for the 0.4M glycine method reported by (Juhasz et al., 2009, median of 262mg As kg⁻¹) and Brattin et al. (2013, median of 385mg As kg⁻¹). While it has been demonstrated that IVBA As by the OSU-IVG is not related to total As content (Whitacre et al., 2013), it is unknown how well the OSU-IVG method will measure and/or predict RBA over a wider concentration range of soils As, particularly lower As concentrations, and from As sources outside those used to develop the regression. The objectives of the current study were to (i) evaluate the ability of the OSU-IVG method to predict RBA As for As contaminated soils with a wider range of As content and As contaminant sources, and (ii) evaluate a modified extraction procedure's ability to improve prediction of RBA As.

2. Materials and Methods

2.1 Study Soils

Thirty-three As containing soils that represent a wide variety of As sources were selected for this study. Soil samples were homogenized and sieved to <250 µm. Total content of As and other elements were determined using microwave assisted acid digestion using a CEM Corporation Mars Express Microwave (U.S. EPA Method 3051a, U.S. EPA, 2007a) with subsequent analysis by inductively coupled plasma optical emission spectroscopy (ICP-OES) using an Agilent 720 (U.S. EPA, 2007b). Reactive Fe was determined by the acid ammonium oxalate method of McKeague and Day (1966).

Other additional soil and solid waste analyses including soil pH, organic carbon, electrical conductivity, and soil texture have been reported previously (Basta et al., 2016). Arsenic speciation was determined from As X-ray absorption near-edge structure (XANES) spectra and the extended x-ray absorption fine structure (EXAFS). Linear combination, least-squares fitting provided quantitative determination of species relative abundance in samples containing multiple As or Fe species. Detailed procedures for arsenic speciation are described in Basta et al. (2016) and California DTSC (2015).

2.2 Determination of Relative Bioavailable Arsenic

Juvenile swine bioassays were used to determine RBA As for the study soils according to (Brattin and Casteel, 2013). In short, soils were administered to a dose group at a known As levels for 15 days. Additionally, the study included a non-treated group to serve as a control for determining background arsenic levels as well as a sodium arsenate reference group. All doses were administered orally and As excreted in urine was used to determine RBA As for each soil. Further details of the bioassay and RBA derivation are described in Basta (2016) and California DTSC (2015).

2.3 Determination of Bioaccessible Arsenic

In vitro laboratory methods were used to determine IVBA As. The two IVBA methods investigated were the OSU-IVG (Basta et al., 2007) and the California Arsenic Bioaccessibility Method (CAB, California DTSC, 2015). Bioaccessible As was determined from the gastric phase of both methods.

Bioaccessible As was determined by the OSU-IVG method as follows. The extraction solution consisted of 0.10 M NaCl and 1% porcine pepsin. The solution was heated in an open extraction vessel, in a 37° C hot water bath. When the solution reached 37° C, soil (1 g, < 250

1 μm) was added. The sample was thoroughly mixed with the solution using a paddle stirrer to
2 maintain a homogenous suspension, and the pH is adjusted drop wise to 1.8 using 6M trace metal
3 grade HCl. The solution pH is continuously monitored and adjusted to 1.8 ± 0.1 . After 1 h, 10
4 mL of gastric solution was removed for analysis. The extract was immediately centrifuged
5 (11,160 g for 15 min) and then filtered (0.45 μm). The extracted As is expressed as IVBA as
6 shown in eqn. 2.

$$7 \text{ IVBA As (\%)} = [\text{bioaccessible As (mg kg}^{-1}\text{)}] / [3051\text{a As (mg kg}^{-1}\text{)}] * 100 \quad [2]$$

8 Bioaccessible As was determined using the CAB method as follows. The extraction
9 solution consisted of 0.10 M NaCl, 1% porcine pepsin, and 100mM L-ascorbic acid at pH 1.5.
10 The extraction was conducted by the addition of 1.0 g soil (<250 μm) to a 175 mL HDPE bottle
11 containing 150 mL of gastric solution (preheated to 37°C) and placed into an end over end
12 rotator shaker located in a 37°C incubator. Soil samples were rotated at 40 ± 2 rpm for 2 h.
13 Gastric solution pH was maintained at 1.5 ± 0.1 using drop wise 50% NaOH (sodium hydroxide)
14 and/or 6M trace metal HCl solution. After 2 h, 10 mL aliquot of suspension was collected with a
15 syringe and filtered (0.45 μm) for ICP-OES analysis. As extracted in the OSU-IVG and CAB
16 methods was determined by ICP-OES according to USEPA method 6010C using an Agilent 720
17 (USEPA 2007b).

18 *2.4 Prediction of RBA As from IVBA As*

19 The ability of the OSU-IVG method to predict RBA As for soils and solid wastes with
20 range in of soil As content as well as contaminant sources (objective 1) was evaluated as follows.
21 The predictive regressive (Eqn. 1) was used to calculate RBA As from IVBA As of the study
22 soils and regressed against the measure RBA As. In addition, analysis of Covariance
23 (ANCOVA) and simple linear regression (RBA As vs. IVBA As) were utilized evaluate the

ability of the OSU-IVG and CAB methods to compare the predictive relationships of the two methods.

3.0 Results and Discussion

3.1 Study Soils

Study soils had a wide range in As, reactive Fe content, and other key soil properties required for a robust method evaluation (Table 1). Total As ranged from 162 to 12,483mg kg⁻¹ with a median of 731mg kg⁻¹, reactive Fe ranged from 0.56 to 120g kg⁻¹ with a median of 6.13g kg⁻¹. There was also a wide range in both RBA and bioaccessible As; RBA As ranged from 1.30 to 60.0% and OSU-IVG IVBA As ranged from 0.80 to 52.3% (Table 1) due to a wide range in key soil properties known to affect As solubility and bioaccessibility, including; organic C content from 2.7 to 81.2 g kg⁻¹, soil pH from 2.14 to 9.28 and clay content from 10 to 111g kg⁻¹ (California DTSC, 2015; Basta et al., 2016).

Specific soil properties that have been demonstrated to have an influence on As solubility and bioaccessibility are iron oxides and pH. Iron oxides adsorb As by ligand exchange with hydroxyl groups at the oxide surface (Grossl and Sparks, 1995; Grossl et al., 1997). The amount of As adsorbed to the oxide depends on the amount of oxide and therefore the number of available coordination sites for arsenate. Maximum As adsorption densities of 0.25mol of sites per mol of Fe or on hydrous iron oxides have been reported (Dombak and Morel, 1990). Yang et al. (2002) demonstrated a relationship between bioaccessible As in a physiologically based extraction test (PBET) using a multiple regression equation with Fe measured by citrate bicarbonate dithionite and soil pH as variables. Similarly, Whitacre et al. (2013) indicate that the majority of non-bioaccessible As was associated with reactive Fe in soil. Solution pH affects oxide surface charge and arsenate protonation, thereby affecting the adsorption of As. Arsenic

acid (H^3AsO^4) dissociates with the following pKa values; pKa 1 = 2.2, pKa2 = 6.97, and pKa 3 = 11.53 (Harris, 2003). As pH increases, the surface charge of iron oxide becomes more negative as well as the charge on arsenate ($\text{H}_n\text{AsO}_3^{-n}$). The negative repulsion results in a decrease in As absorption above pH 7, which corresponds to an inflection point at pKa 2 of the sorption envelope for As (Goldberg, 2002; Raven et al., 1998).

In addition to soil properties, results of XANES and EXAFS arsenic speciation are also presented in Table 1. Arsenic speciation was closely tied to Fe chemistry in the soils evidenced by the most abundant As species being As(V) adsorbed to hydrous ferric oxide (HFO) or Fe, Mn, and Al oxides (Table 1). However, lead (Pb) arsenate was a significant As species in orchards treated with lead arsenate pesticide. All study soils were aerobic topsoil, where oxidation of As(III) to As(V) had mostly occurred, and the predominance of As adsorption to Fe mineral surfaces is consistent with previous studies (Deschamps et al., 2003; Manning, 2005). The geochemistry of As is commonly linked to Fe because of its strong specific bonding to arsenic as well as the abundance of Fe in most soils (Bowell et al., 2014). Due to the affinity of As oxyanions for surfaces of Fe oxides (Schwertmann and Taylor 1989; Carlson et al., 2002; Kneebone et al., 2002) co-precipitation of As and Fe (sulfides) can also occur, resulting in strong correlation coefficients between Fe and As (Smith et al., 2013). In mining-contaminated soil, arsenopyrite is the most common mineral identified in association with sulfide minerals.

Chemical speciation using spectroscopic methods, including XANES and EXAFS, is directly related to As bioaccessibility (Meunier et al., 2010, Table 2). Soils with As predominantly as arsenopyrite or scorodite will likely have low bioaccessibility and bioavailability. On the contrary, soils with As predominantly as Ca-Fe arsenate minerals (i.e., yukonite) will likely have high bioaccessibility and bioavailability. While mixed mineral content

in soil can make bioaccessibility and bioavailability difficult to predict, soil 30 in the current study demonstrates the trend presented in Table 2 with As primarily present as arsenopyrite (52%), which corresponds to very low RBA As (4%).

3.2 Prediction of RBA As from IVBA As

Predicted RBA from OSU-IVG IVBA As using Eqn. 1 is compared to the measured RBA for the current study soils (Fig. 1). The slope close to one indicates that on average, the OSU-IVG could be predictive of the RBA. However, the low correlation coefficient ($r^2 = 0.57$) indicates that there can be large prediction error for individual soils, ranging from under-prediction up 56% (Soil 19) and over-prediction up to 879% (Soil 13). Under predictions generally occurs when the RBA As are high (>50%) and the OSU-IVG IVBA As is considerably less as in than RBA As. In these cases, the y-intercept (9.6) of the regression is not enough to compensate for under-extraction of As, resulting in a predicted RBA As lower than the actual RBA As. On the other hand, over-prediction occurs when RBA As is low (<10%) and the high y-intercept forces the predicted RBA As values above the measured. In both of these cases, the As source was different than the mining wastes used to develop Eqn. 1. Also, the soil As concentrations for these study soils were well below the median As of soils and solid wastes used to develop Eqn. 1. The limited ability of the OSU-IVG to predict RBA in soils outside of mining soils, especially low to moderate As concentrations, is in agreement with (Juhasz et al., 2009) in which the OSU-IVG was applied to railway (pesticide), cattle dip, mining, and gossans soils ranging from 42 to 1,118mg As kg⁻¹.

Further assessment of initial bioaccessibility testing indicated that the OSU-IVG extracted significantly less As in vitro than in vivo RBA As, in particularly for soils contaminated with waste rock and tailings from historical gold mining (Table 1, Fig. 2), on

average extracting as little as 13% of RBA As (Soil 4). Soils in order of increasing As content (Fig. 2), clearly show RBA As or IVBA As were not dependent on total As content. Linear regression of RBA As vs. Total As produced an $r^2 = 0.07$ which shows total As is not predictive of RBA As. Previous studies have concluded that in vitro bioaccessible method extraction pH is the parameter that most strongly influences IVBA As (Juhasz et al., 2009). However, we found lowering the OSU-IVG pH from 1.8 to 1.5 alone did not increase IVBA As enough to make it an accurate predictor of RBA As. A comprehensive review of the GI physiology literature revealed a potentially key gastric constituent lacking from the OSU-IVG (Basta et al., 2007) is ascorbic acid (AA). Ascorbic acid can lower the redox potential of gastric solution, thereby potential increasing the solubility As, and is naturally secreted in the stomach (Maggi et al., 1957; Muto et al., 1997; Muto et al., 1998; Tuo et al., 2000).

The addition of AA significantly impacted Eh, IVBA As, and dissolved Fe in the GI extraction solution. Ascorbic acid decreased Eh (Fig. 3A) and increased IVBA As GE up to 100 mM AA (Fig. 3B), after which, increasing AA concentration had little additional effect (Fig. 3B). The dissolution of As with AA was significantly correlated ($P < 0.01$) with Fe dissolution for the six soils (Fig. 4).

The ability of the OSU-IVG and the AA modified method, the California Bioaccessibility Method (CAB), to predict RBA As for all 33 study soils was determined by in vivo – in vitro correlation (IVIVC) and presented in Figs. 5A and 5B respectively. Simple linear regression (Fig. 5) produced almost identical results as weighted least squares analysis of covariance (ANCOVA). The relationship between RBA As and OSU-IVG IVBA As was significant ($r^2 = 0.57$, $P < 0.01$) (Fig. 5A), but weaker than the previous linear regression using soils exclusively from mining and smelting with higher total As contents (405 to 17,500 mg As kg⁻¹) (Basta et al.,

2007, eq. 1). However, the modifications to the OSU-IVG to produce CAB, greatly improved the relationship between RBA As and CAB IVBA As. The CAB method extracted significantly more IVBA As than OSU-IVG for most soils, which more accurately mimics the RBA As from the swine model. This resulted in an $r^2 = 0.72$ ($P < 0.01$) (Fig. 5B). In addition, the two methods perform differently with increasing total soil As concentration. Analysis of 1:1 plot residuals (IVBA As – RBA As) shows OSU-IVG IVBA < RBA As at low total soil As concentrations but IVBA As $\approx$ RBA As at higher soil As concentrations (Fig. 6A). The CAB method IVBA As $\approx$ RBA As at low total soil As concentrations but IVBA > RBA As at total soil As concentrations > 1,500mg As kg⁻¹ (Fig. 6B). The dependence on total As concentration on the 1:1 plot residuals could be due to in vitro extraction or to in vivo absorption processes. High concentrations of As in soil can result in saturation of in vitro solution. Because IVBA As is a ratio of bioaccessible As/total As, IVBA As would decrease with increasing total As if saturation occurred. In vivo GI chemistry and/or physiology could also affect RBA As. Arsenic saturation of GI solution could increase with total soil As limiting the mass of As available for absorption and the RBA As. The increased positive plot residuals observed in our study is more likely due to in vivo GI chemistry than in vitro chemistry. Plot residuals distributed around 0 (Fig. 6B) are consistent with less likelihood of saturation of As in solution at lower Total As.

While the CAB method is less likely to underestimate RBA As for low to moderately contaminated soils, it is more likely to overestimate RBA As for highly contaminated soils. The plot residuals were positively biased for IVBA for > 1,500mg As kg⁻¹. However, the plot residuals were not biased for IVBA for < 1,500mg As kg⁻¹. Based on the lack of bias observed in Fig. 6B at < 1,500mg As kg⁻¹, IVIVCs for the OSU-IVG and CAB for soils with < 1,500mg As kg⁻¹ were also compared (Fig.7). The OSU-IVG regression (Fig. 7A) showed little difference

($r^2 = 0.53$) when total soil As concentration was restricted to $1,500\text{mg As kg}^{-1}$. However, the CAB method regression (Fig. 7B) further improved ($r^2 = 0.91$, $m = 0.81$). The improvement of the CAB regression for soils with $< 1,500\text{mg As kg}^{-1}$ is due primarily the increased As solubility due to the AA that was added to the CAB method but absent in the OSU-IVG. AA lowered the redox potential of gastric solution thereby potential increasing the solubility As. Ascorbic acid is naturally secreted in the human stomach (Maggi et al., 1957; Muto et al., 1997; Muto et al., 1998; Tuo et al., 2000) and likely in the swine stomach. Inclusion of AA in the gastric fluid improved simulated in vivo conditions and likely improved the ability of IVBA to predict RBA As.

The CAB IVIVC for soils $< 1,500\text{mg As kg}^{-1}$ meets the suggested criteria for an acceptable IVIVC, $r^2 > 0.6$, and a slope between 0.8 and 1.2 (Wragg et al., 2011; Denys et al., 2012). In addition, the CAB method meets the suggested criteria of a y-intercept that does not deviate significantly from zero (Juhasz et al., 2014) with a y-intercept of 3.2. A large non-zero y-intercept in the in vitro in IVIVC is due to IVBA As results significantly less than RBA as IVBA approaches zero in the data set used to generate the predictive equations and produces prediction results. This was the case with the OSU-IVG in the current study (y-intercept = 11.2) as well as Juhasz et al. (2009) for which the OSU-IVG had a y-intercept of 14.3.

4. Conclusions

The OSU-IVG extracted significantly less As in vitro than in vivo RBA As, in particularly for soils contaminated with waste rock and tailings from historical gold mining. The CAB method, which is a modified OSU-IVG method that additionally contains ascorbic acid, extracted more As than OSU-IVG for most soils. This resulted in a more accurate prediction of RBA using the CAB method, particularly for low to moderately contaminated soils ($< 1,500\text{mg}$

As kg⁻¹) with RBA As = 0.81 IVBA As + 3.2, r² = 0.91. Accurate prediction at the low to moderate soil arsenic contamination is pertinent to risk assessment because the low to moderate range is where bioavailability adjustments are most applicable. Further, the narrow confidence bands produced by the CAB method reduce the uncertainty associated with predicted RBA As. Extending the application of the CAB method above 1,500mg As kg⁻¹ results in increased uncertainty in the prediction. However, CAB IVBA As can still be utilized as a conservative estimate of RBA As for soils above 1,500mg As kg⁻¹.

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Figures

Figure 1. Measured RBA As in current study soils vs. predicted RBA As using the OSU-IVG and eqn. 1.

Figure 2. Comparison of bioaccessible arsenic extracted by OSU-IVG with relative bioavailable (RBA) As in order of increasing total soil As concentration.

Figure 3. Effect of ascorbic acid addition to gastric solution on redox potential (A) and arsenic dissolution (B) for six study soils.

Figure 4. The relationship between arsenic dissolution and dissolved iron in gastric solution with increasing ascorbic acid additions (0, 50, 100, and 250mM) for six study soils.

Figure 5. Comparison of regressions; RBA As vs. IVBA As form the OSU-IVG (A) and CAB (B) methods. Dashed lines represent

Figure 6. Comparison of relative bioavailable (RBA) As and in vitro bioaccessible (IVBA) As with total soil As concentration using 1:1 plot residuals (IVBA As – RBA As) for the OSU-IVG (A) and CAB (B) methods. Vertical line in plot B is placed at 1,500 mg As/kg.

Figure 7. Comparison of regressions; RBA As vs. IVBA As from the OSU-IVG (A) and CAB (B) methods for soils with less than 1,500 mg As/kg.

Tables

Table 1
Characterization of study soils by source, primary and secondary arsenic phases, total arsenic, reactive iron, relative bioavailable arsenic and in vitro bioaccessible As by OSU-IVG and CAB methods.

Soil	Source	Primary As Phase* (%)	Secondary As Phase (%)	Total As (mg kg ⁻¹)	Reactive Fe (g kg ⁻¹)	RBA (%)	OSU-IVG (%)	CAB (%)
1	Copper Smelting Residential Area Near Gold	G (47)	B (32)	162	4.99	54.9	28.4	59.5
2	Mining	A (74)	D (14)	203	2.11	11.8	1.92	5.10
3	Silver Mining Residential Area Near Iron	^b NA	^b NA	205	0.560	8.2	25.5	21.2
4	Mining	C (51)	A (38)	249	34.8	60.0	35.2	73.6
5	Lead Arsenate Pesticide	B (66)	E (25)	283	2.37	31.0	24.6	39.0
6	Gold Mining	A (72)	D (10)	302	6.30	23.7	9.36	25.1
7	Lead Arsenate Pesticide	B (71)	E (16)	332	4.59	52.3	35.0	67.3
8	Lead Arsenate Pesticide	B (71)	E (23)	353	5.73	41.0	23.0	40.7
9	Gold Mining	A (73)	B (20)	370	2.33	11.7	1.52	6.07
10	Lead Arsenate Pesticide	B (71)	E (23)	375	7.23	53.0	29.1	50.1
11	Lead Arsenate Pesticide	B (86)	E (15)	391	5.97	49.0	21.1	41.4
12	Tailings from Mine Site	C (56)	A (44)	521	0.770	14.0	10.8	20.5
13	Gold Mining Residential Area Near Gold	NA	NA	603	1.43	1.30	3.54	2.86
14	Mining	A (65)	D (19)	610	4.36	12.4	3.0	16.1
15	Gold Mining	A (53)	B (24)	633	18.5	19.2	4.6	12.4
16	Gold Mining	^b NA	^b NA	641	4.39	9.2	12.8	11.0
17	Iron, silver, gold Mining	^b NA	^b NA	731	10.8	5.90	3.88	3.95
18	Gold Mining	^b NA	^b NA	753	14.5	37.6	52.3	46.8
19	Gold Mining	B (74)	A (14)	839	5.01	41.7	9.7	45.8
20	Slag from Copper Mining	C (78)	B (13)	1,236	54.5	39.7	29.7	48.0

Table 1 Continued

Characterization of study soils by source, primary and secondary arsenic phases, total arsenic, reactive iron, relative bioavailable arsenic and in vitro bioaccessible As by OSU-IVG and CAB methods.

Soil	Source	Primary As Phase ^a (%)	Secondary As Phase (%)	Total As (mg kg ⁻¹)	Reactive Fe (g kg ⁻¹)	RBA (%)	OSU-IVG (%)	CAB (%)
21	Gold Mining	A (84)	H (8)	1,237	1.15	12.5	2.22	6.22
22	SRM 2710A	C (82)	A (10)	1,540	12.7	41.8	25.2	83.0
23	Gold Mining	A (70)	D (20)	1,906	17.3	15.30	3.66	21.8
24	Gold Mining	A (70)	B (19)	2,541	23.1	15.30	3.70	22.7
25	Tailing from Mine or Smelter Residential Area Near Glass	C (57)	A (33)	3,913	36.2	19.0	5.4	47.1
26	Manufacturing	A (100)		3,996	3.70	26.0	21.9	39.1
27	Glass Manufacturing	A (100)		4,553	4.97	48.0	49.1	76.7
28	Gold Mining	A (66)	B (29)	5,647	25.1	22.7	8.05	46.51
29	Gold Mining	^b NA	^b NA	6,681	8.99	14.2	20.1	19.9
30	Gold Mining	F (52)	A (21)	10,482	26.0	4.0	1.8	12.0
31	Gold Mining	A (69)	D (8)	12,041	32.5	23.0	9.68	50.10
32	Gold Mining	A (66)	B (17)	12,095	36.3	19.7	3.50	44.38
33	Calcine Material from Copper Mining	B (67)	A (33)	12,483	120	7.9	0.8	26.5
			Min	162	0.560	1.300	0.802	2.861
			Mean	2,694	16.3	25.7	15.8	34.3
			Median	731	6.302	19.700	9.722	39.023
			Max	12,483	119.540	60.000	52.315	82.969

^aAs(V) ads Hydrous Ferric Oxide - A

^aAs(V) ads Al/Fe/Mn Oxides - B

^aHydrous Ferric Arsenate - C

^aArsenatian Jarosite - D

^aLead Arsenate - E

^aArsenopyrite - F

^aAs(III) ads Fe/Al Oxides or Aluminosilicate - G

^aCa and Ca-Fe Arsenates - H

^bData not available

Table 2

Arsenic mineralogy and bioaccessibility ranking (Adapted from Meunier et al. 2010)
from most bioaccessible (1) to least bioaccessible (8).

General Phase	Specific Phase	Bioaccessibility Ranking
Calcium-Iron Arsenates	Yukonite, Asiderite, Calcium Arsenate Amorphus	1
Clay Minerals	Undifferentiated	2
Sulfates	Tooeleite, Jarosite, Schwertmannite	3
As bearing Aluminum (oxy) Hydroxides	Gibbsite, AlOH ₃ , As(III) sorbed to Kaolin	4
Roaster Iron Oxides	Hematite, Maghemite	5
Arsenic bearing Iron(oxy) Hydroxides	Goethite, Lepidocrocite, Akaganeite, Ferrihydrite	6
Iron Arsenates	Scorodite, Kankite, Pharmacosiderate, Amorphous	7
Sulfides	Arsenopyrite, Realgar, Pyrite, Orpiment	8