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Modifying Effect of Soil Properties on Bio-Accessibility of As and Pb from Human Ingestion of Contaminated Soil

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Abstract: Exposure to soils contaminated with heavy metals can pose human health risk to children through ingestion of contaminated soil. Soil properties such as soil pH, reactive Fe and Al oxide content, clay content, soil organic matter (SOM), and cation exchange capacity (CEC) can reduce contaminant bio-accessibility and exposure. In vitro bio-accessibility (%IVBA) of As and Pb in 19 soils was determined using U.S. EPA Method 1340. Soil properties reduced the bio-accessibility of As by 17–96.5% and 1.3–38.9% for Pb. For both As and Pb, bio-accessibility decreased with increasing Al and Fe oxide content. Al oxides were found to be the primary driver of As and Pb bio-accessibility. Multiple regressions with AlOx, soil pH, %clay and/or FeOx predicted %IVBA As ($p < 0.001$). The multiple regression including log (FeOx + AlOx) and %clay explained 63% of the variability in %IVBA Pb ($p < 0.01$). Fe and Al oxides were found to be important drivers of As and Pb bio-accessibility, regardless of in vitro method. These findings suggested soil pH should be used in addition to reactive oxides to predict bio-accessible As. Risk-based adjustments using soil properties for exposure via incidental ingestion should be considered for soils contaminated with As and/or Pb.

Keywords: lead; arsenic; bio-accessibility; contaminated soils; soil properties; spiked soils; physico-chemical properties; risk-assessment

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1. Introduction

Human ingestion of toxic heavy metals can have a variety of effects on health including cancer, decreased lung function, central nervous system disorders, decreased IQ, and a weakening of the skeletal system [1]. Heavy metal contamination of soil is commonly associated with anthropogenic activities such as mining, battery production, leaded gasoline, and land application of fertilizers, coal combustion residues, and sewage sludge [2]. These contaminants include lead (Pb) and arsenic (As) [3]. Arsenic and Pb, commonly found in contaminated urban soil, are ranked 1 and 2 on the Agency for Toxic Substances and Disease Registry Substance Priority list [4]. The United States Environmental Protection Agency (USEPA) child residential soil screening levels (SSL) for As and Pb are 0.39 $mg \cdot kg^{-1}$ and 400 $mg \cdot kg^{-1}$, respectively [5].

Heavy metal exposure in children is often associated with ingestion of soils through hand-to-mouth activity [6]. Therefore, models that predict the human health risk of exposure are often dependent on the amount absorbed from the gastrointestinal tract. The fraction of the ingested contaminant absorbed into the body is termed the bio-accessible fraction. The bioavailable fraction is the ingested amount that reaches systemic circulation. The bio-accessibility of a contaminant is dependent on its solubility, speciation, and the presence of modifying soil properties that can result in the production of insoluble solid phase compounds [7]. Consequently, risk assessment based on total content will overestimate the human health risk from soil contamination [8–11]. In the case of Arsenic (As), prior to

2013, the USEPA had a recommended relative bioavailability (RBA) of 100%. The USEPA conducted a comprehensive literature review of contaminated soils and recommended 60% RBA be used as a default value in human health risk assessment [12].

Properties that can have modifying effects on the speciation and mobilization of contaminants in soil include soil pH, Fe/Al oxide content, clay content, presence of soil organic matter (SOM), and cation exchange capacity (CEC). Soil pH is considered one of the most important soil properties dictating the availability of heavy metals directly or indirectly by influencing other soil properties [3,13,14]. Several studies have reported the effect of soil properties on bio-accessibility of Pb and As associated with incidental ingestion [15–19]. These studies incorporated total Pb or As a variable in the multiple regression used to predict bio-accessibility. The strong relationship between total Pb or As and their respective bio-accessibility can mask and/or overwhelm the effects of soil properties on As or Pb bio-accessibility For example, a study with very high total metal content, i.e., mining sites, will only require total metal content to predict total bio-accessibility. Conversely, a study with low metal contamination will likely show soil properties are more important than total metal content to predict metal bio-accessibility. Thus, it is critical to distinguish between studies with high concentrations of As or Pb in contaminated soil and studies with lower spike concentration of clean soils. In this study, one spike concentration of As or Pb was used to remove the effect of total metal as a source of variability. This allowed for a clear investigation of only key soil properties on Pb or As bio-accessibility. The objectives of this research are to (i) determine the effect of soil physicochemical properties on As and Pb bio-accessibility and (ii) identify soil properties that reduce human exposure associated with soil ingestion.

2. Materials and Methods

2.1. Soil Selection

Nineteen soils were evaluated in this study which provided a wide range in key soil properties including pH, organic carbon (OC), %clay, reactive Fe and Al oxide, and effective cation exchange capacity (eCEC). Soil pH ranged from 4.0 to 8.0 control soils, organic carbon (OC) from 0.50 to 3.0, effective cation exchange capacity (eCEC) from 3.0 to 32.4 cmolc·kg^{-1}, clay content from 5.0 to 71.3%, reactive Fe oxide from 1.8 to 142 mmol·kg^{-1} and Al oxide from 5.5 to 53.4 mmol·kg^{-1} (Table S1). Background metal concentration were determined by acid digestion using microwave (CEM MDS 2100, CEM Corporation, Matthews, NC, USA) according to the USEPA Method 3051 to provide a baseline of contaminant levels prior to analysis of chemical and physical properties [20]. Background Pb ranged from <2.5 to 14.4 mg·kg^{-1} and background As ranged from <2.5 to 15.0 mg kg^{-1}. Soils were found to be within the Pb and As concentration range for typical uncontaminated soil [2,21].

2.2. Soil Chemical and Physical Properties

Analysis of soil chemical and physical properties was performed on <2 mm sieved, soils in duplicate. Soil pH was determined using a 1:1 soil:water slurry measured with a combination pH electrode [14]. Soil pH was measured on spiked and unspiked (control) soils, as metal salts can cause acidification due to metal hydrolysis [22]. The spiked soil pH was used for all statistical analyses. Soil organic carbon (SOC) was determined via oxidation of organic C by acid dichromate reduction [23]. Soil texture was determined by the hydrometer method, after being pretreated with hydrogen peroxide to remove organic matter [24]. The clay content of the soils was determined using the pipette method [25]. The effective cation exchange capacity was determined using the unbuffered salt ($BaCl_2$) extraction method [26] and the iron and aluminum oxides using acid ammonium oxalate extraction on <250 µm sieved soils [27].

2.3. Preparation of Contaminated Soil

Soils were sieved to <2 mm and were individually spiked with reagent grade $Pb(NO_3)_2$ at 2000 mg·kg^{-1} Pb, and H_2NaAsO_4•$7H_2O$ at 250 mg·kg^{-1} As. Soils were spiked with only one metal to avoid competitive adsorption effects [22]. One liter of spiking solution was prepared using deionized water and the metal salts. Soil (5 kg) was mixed with a liter of the spiking solution and additional DI water to form a saturated paste. The spiked soils then underwent three wet-dry cycles at 105 °C for 24 h followed by periodic wetting and drying for a total of 90 days to minimize the salt effect by increasing the reaction between the soil matrix and the metals [28].

2.4. Determination of Bio-Accessibility

Bio-accessibility of As and Pb in the spiked soils were evaluated using the USEPA method 1340 at pH 1.5. Soil (1.0 ± 0.01 g, <250 µm sieved) was weighed into a 125 mL high density polyethylene bottle (HDPE) with 100 mL of gastric solution (0.4 M glycine, at 37 °C and pH 1.5) added. The samples were then individually adjusted to 1.5 pH with dropwise additions of trace metal grade 50% HCl to account for soil buffering. Soil samples were rotated at 30 ± 2 rpm and maintained at 37 °C for 1 h with frequent checks to adjust sample pH to 1.5 ± 0.05 using dropwise addition of 50% NaOH and/or 50% trace metal HCl. After an hour, an aliquot of the suspension was collected and syringe filtered (0.45 µm). Resulting samples were analyzed using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-OES) following USEPA Method 6010C [29].The in vitro bio-accessible Pb (IVBA Pb) were determined and expressed as a percentage of total Pb as follows:

$$\%\text{IVBA Pb} = (\text{IVBA extractable Pb } [\text{mg}\cdot\text{kg}^{-1}])/(\text{total soil Pb } [\text{mg}\cdot\text{kg}^{-1}]) \times 100\% \quad (1)$$

The %IVBA As concentrations were similarly calculated.

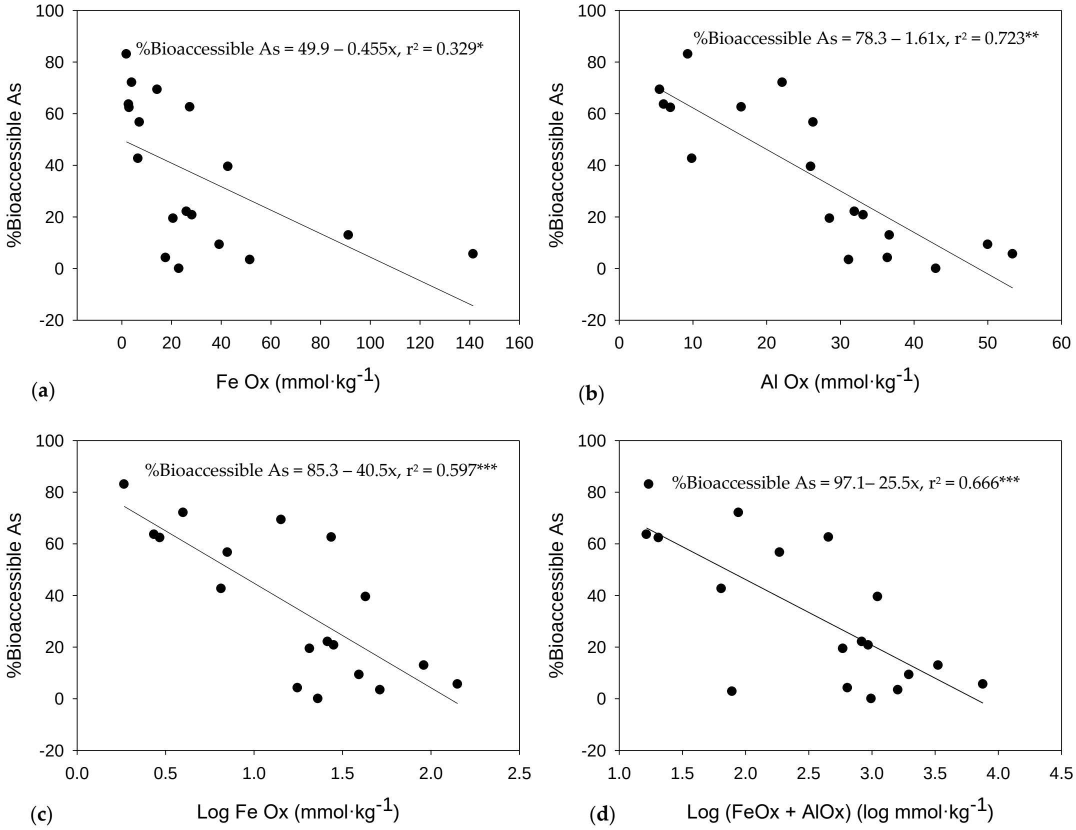
2.5. Statistical Analysis

Multiple regressions were performed to determine the relationships of soil properties with bioaccessible As or Pb. Forward stepwise regression was conducted using Proc Reg in SAS 9.4, for the independent variables %IVBA Pb and %IVBA As. Seven variables (FeOx, AlOx, FeOx + AlOx, $\log_{10}$ FeOx, $\log_{10}$ (FeOx + AlOx), %clay and eCEC) (n = 19) were regressed with %IVBA Pb; and six variables (FeOx, AlOx, FeOx + AlOx, $\log_{10}$ FeOx, %clay and pH) (n = 18) were regressed with %IVBA As. For both metals, the best multiple regression model was determined as the one having the lowest residual mean square, and the condition that variables met at least the 0.5 significance level for entry into the model. The residuals for the final model were inspected for independent distribution using residual vs. predicted plots and were tested for normality using the Shapiro-Wilks test in Proc Univariate of SAS (Version 9.4.)

3. Results

3.1. As Bio-Accessibility

Soil properties had notable effects on bio-accessible As. Bio-accessible As, %IVBA As, ranged from 0% (Dennis B) to 83% (Mansic B) (Figure S1). Mean and median IVBA As were 36.1% and 30.8%, respectively. Several significant linear relationships between %IVBA As and the various soil properties were found (Table S2). Both reactive Fe and Al oxide content of the soils greatly reduced %IVBA As compared to the other soil properties (Figure 1a,b). In general, less Al oxide was needed to sequester As and reduce %IVBA As by Al oxide than Fe oxide (Figure 1a,b; Table S2). The relationship between IVBA As and Fe oxide was an exponential relationship (Figure 1c). The log (FeOx + AlOx) relationship with %IVBA As (Figure 1d) was also significant ($p < 0.001$) as was AlOx. (Table S2). This suggests that FeOx and AlOx may work in tandem to immobilize As but AlOx is the more dominant As immobilization mechanism.



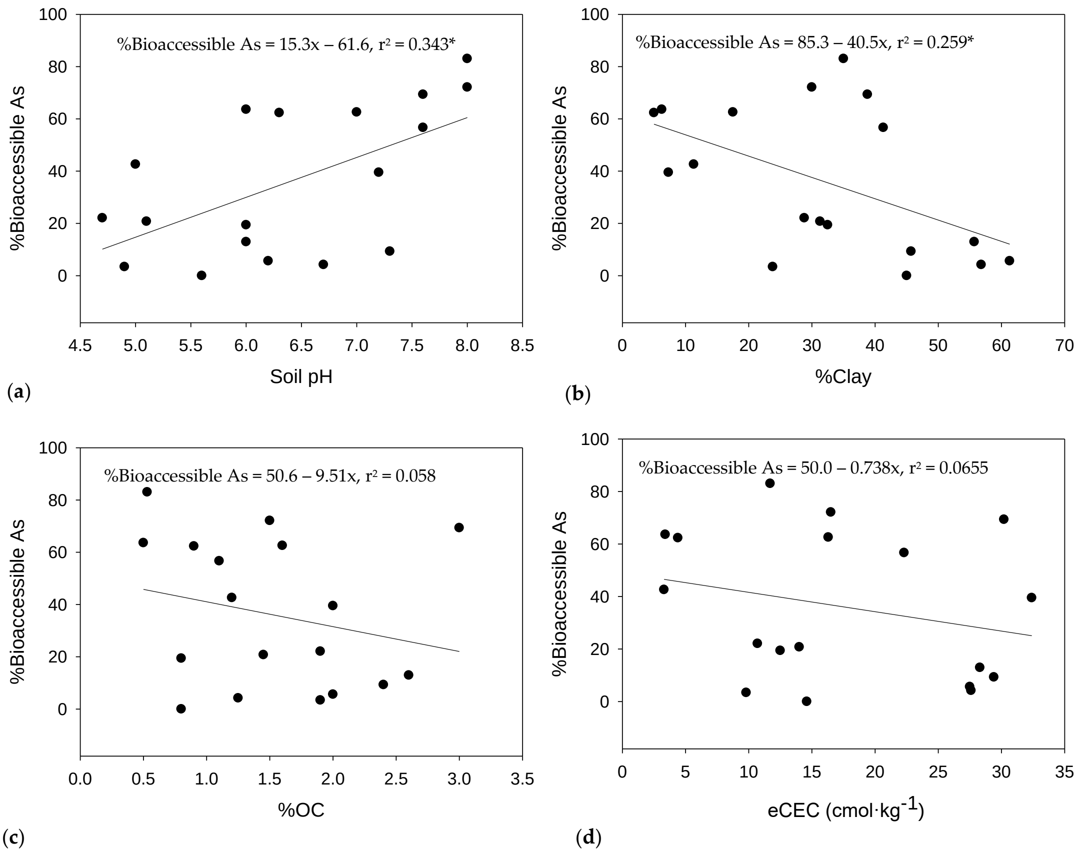
–Significance level: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 1. Effect of hydrous oxides on %bio-accessible (IVBA) As. (**a**) FeOx (mmol·kg^{-1}) effect on %bio-accessible As (**b**) Al Ox (mmol·kg^{-1}) effect on %bio-accessible As (**c**) Log FeOx (mmol·kg^{-1}) effect on %bio-accessible As (**d**) Log (FeOx + AlOx (log mmol·kg^{-1}) effect on %bio-accessible.

Simple linear regression analysis showed the relationship between other soil properties, pH, %clay, %OC, or eCEC and IVBA As (Figure 2) were weaker than those found for FeOx and AlOx (Figure 1). Bio-accessible %IVBA As had a weak but significant relationship with pH (r = 0.585, $p < 0.05$) (Table S2). There was also a weak association between IVBA As and % clay (r = −0.509, $p < 0.05$), likely to be the result of Fe and Al oxides being associated with the clay fraction of soil (r = 0.62) (Table S3). The relationship between %IVBA As with %OC or eCEC was not significant (Table S2).

Multiple regressions were then performed to determine if multiple soil properties better explained the variance in the IVBA As data. The influence of correlations for these soil properties were examined before multiple regression analysis [30]. Strong significant Pearson intercorrelations between influential soil properties were excluded from regression analysis. Excessive multicollinearity can result in a singular matrix which provides erroneous multiple regression results [31]. It is difficult to determine exact criteria to identify excessive multicollinearity. We used a functional criteria of correlation coefficient r > 0.7 and $p < 0.05$ between two soil property variables.

All the r values were below 0.7, showing soil properties were not highly intercorrelated and multiple regression analysis was possible using these soil properties. When evaluated for logFeOx, logAlOx, soil pH, and %clay, the multiple regression was able to explain 85.1% of the variance ($R^2 = 0.851$, $p < 0.001$), a significant improvement over the %IVBA As vs. log (FeOx + AlOx) simple regression.



–Significance level: * $p < 0.05$.

Figure 2. Effect of soil properties on %bio-accessible (IVBA) As. (**a**) Effect of soil spike pH on %bio-accessible As (**b**) Effect of soil %clay on %bio-accessible As (**c**) Effect of soil %OC on %bio-accessible As (**d**) Effect of soil effective CEC ($\text{cmol}\cdot\text{kg}^{-1}$) on %bio-accessible As.

$$\text{IVBA As (\%)} = 29 + 12.9\ \text{pH} - 0.402\%\text{Clay} - 38.9 \log(\text{FeOx} + \text{AlOx}) \quad (2)$$

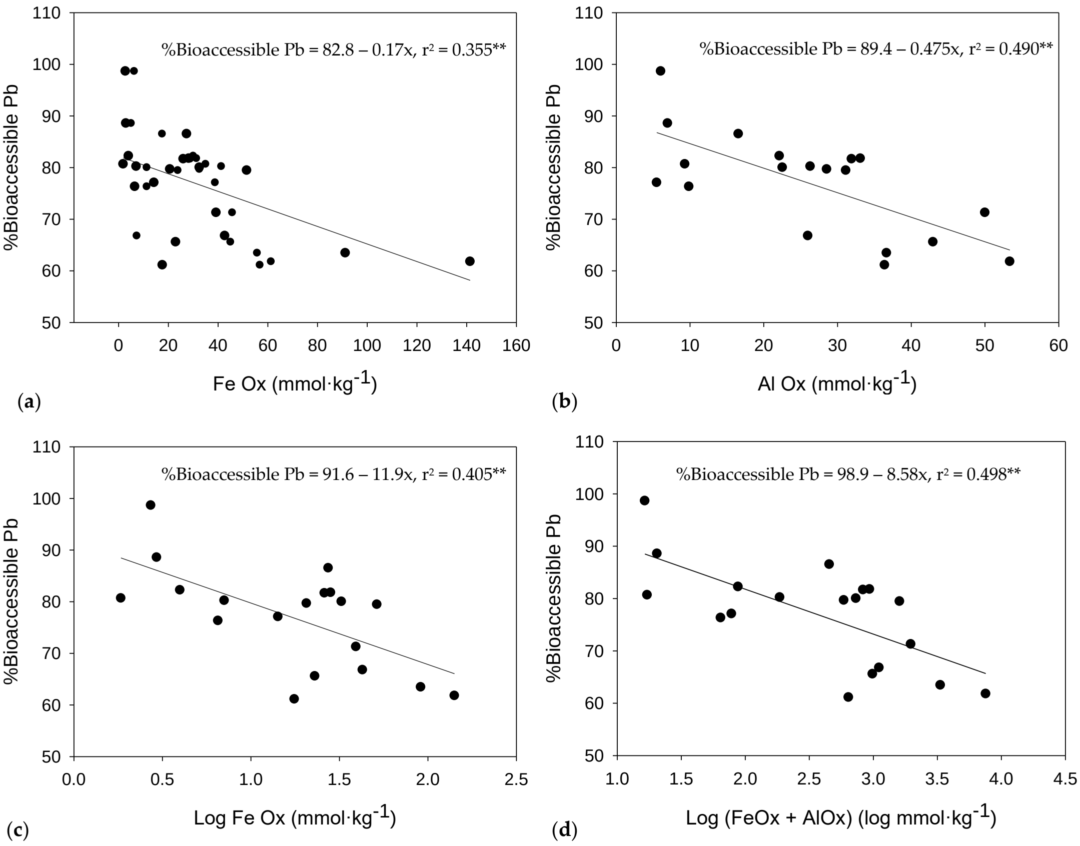
Multiple regression analysis also found significant variables were soil pH, Al oxide, and %clay without FeOx and resulted in an R^2 of 0.901 and $p < 0.001$ (Equation (3)).

$$\text{IVBA As (\%)} = -1.66 + 12\ \text{pH} - 0.145\%\text{Clay} - 1.30\ \text{AlOx} \quad (3)$$

The residuals for both these models were normally and independently distributed.

3.2. Pb Bio-Accessibility

Soil properties had notable effects on bio-accessible Pb. Bio-accessible Pb was reduced from 1.3–38.9% from original spike concentrations based on the soil type (Figure S2). The mean %IVBA Pb was 77.0% and the median %IVBA Pb was 79.7%. Bio-accessible Pb had an inverse relationship with the Fe and Al oxide content of the soils (Figure 3a, b; Table S2). A stronger relationship between %IVBA Pb and log Fe oxide content (Figure 2c) than with FeOx (Figure 3a) suggests %IVBA Pb decreased exponentially with FeOx. When expressed as log AlOx, the %IVBA Pb had a slightly weaker correlation with the r decreasing from −0.700 ($p < 0.01$) to −0.652 ($p < 0.01$) (Supplemental Table S2). This would suggest that the %IVBA Pb relationship with AlOx is more linear than with FeOx. However, AlOx was found to be the stronger sorbent of Pb as, even when evaluated as log AlOx, it resulted in less %IVBA Pb compared to log FeOx (Table S2).There was an improvement in %IVBA Pb prediction when regressed against log (FeOx + AlOx). (Figure 3d). This result suggests that FeOx and AlOx work in tandem to immobilize Pb.



–Significance level: ** $p < 0.01$.

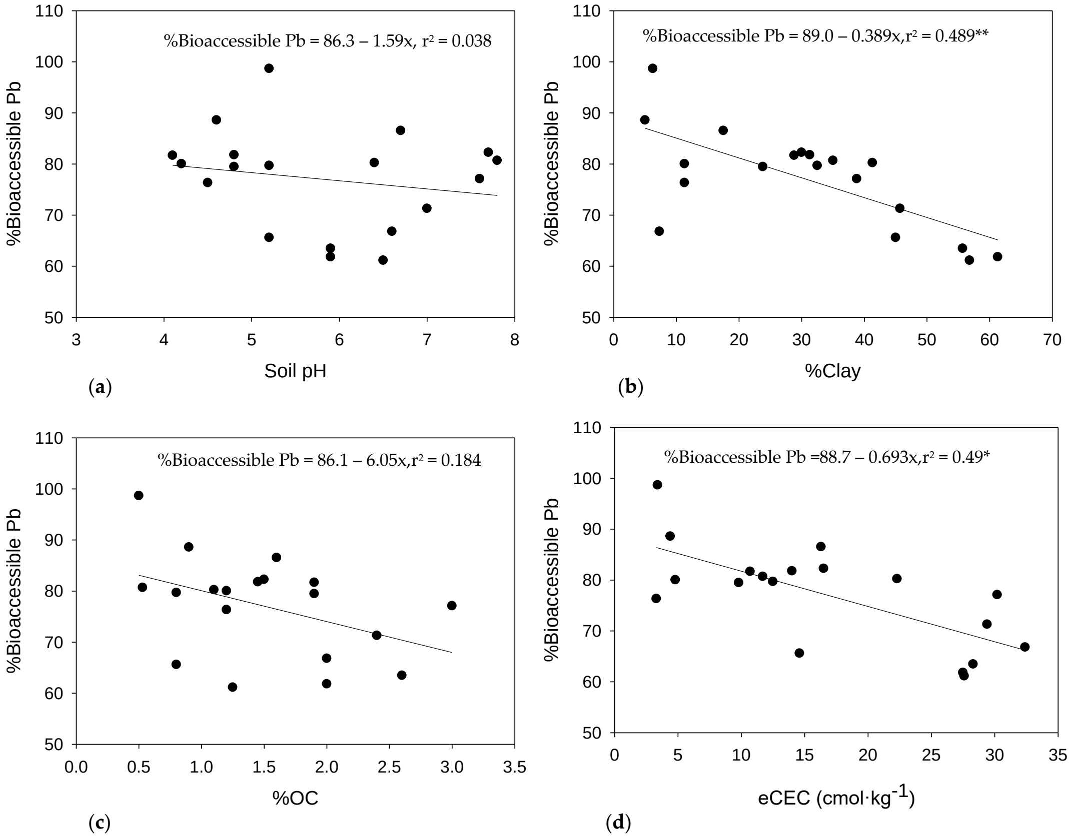
Figure 3. Effect of hydrous oxides on %bio-accessible (IVBA) Pb. (**a**) FeOx (mmol·kg^{-1}) effect on %bio-accessible Pb (**b**) AlOx (mmol·kg^{-1}) effect on %bio-accessible Pb (**c**) Log FeOx (mmol·kg^{-1}) effect on %bio-accessible Pb (**d**) Log (FeOx + AlOx) (log mmol·kg^{-1}) effect on %bio-accessible Pb.

Simple regression analysis showed an inverse relationship between %clay ($r = -0.699$, $p < 0.01$)) and eCEC ($r = -0.700$, $p < 0.01$) (Figure 4; Table S2). The relationships between %IVBA Pb with soil pH or %OC were not significant (Figure 4; Supplemental Table S2).

Stepwise multiple regressions were performed to determine if evaluating multiple soil properties better explained the variance in the %IVBA Pb data. All the r values were below 0.7 showing that soil properties were not highly intercorrelated and multiple regression analysis was possible using these soil properties (Table S4). The multiple equation was able to explain 68.6% of the variance ($R^2 = 0.686$, $p < 0.01$) using log FeOx, log AlOx, and %clay.

$$\%\text{IVBA Pb} = 103.8 - 0.245\ \text{Clay} - 11.9\ \log(\text{FeOx} + \text{AlOx}) \tag{4}$$

Including clay in the multiple regression (Equation (4)) explained significantly more variability in %IVBA Pb than the simple linear log (FeOx + AlOx) without clay (Figure 3d). The residuals for this model were normally and independently distributed.



–Significance level: * $p < 0.05$, ** $p < 0.01$.

Figure 4. Effect of soil properties on %bio-accessible (IVBA) Pb. (**a**) Effect of soil spike pH on %bio-accessible Pb (**b**) Effect of soil %clay on %bio-accessible Pb (**c**) Effect of soil %OC on %bio-accessible Pb (**d**) Effect of soil average effective CEC (cmol·kg^{-1}) on %bio-accessible Pb.

4. Discussion

4.1. As Bio-Accessibility

The relationship between soil properties and %IVBA As has been reported from several studies (Table 1). However, these studies varied on the soil type (e.g., waste contaminated vs. spiked soils), bio-accessible methods, and robustness of the range of soil properties. Few studies have been performed using a wide range of soil properties and spiked soils. Selected studies on the relationship between soil properties and %IVBA As are summarized in Table 1.

In general, a significant inverse relationship between Fe and/or Al oxide content and %IVBA As were reported. Smith et al. (2008) and Bradham et al. (2011) used Method 1340 (also known as the Solubility/Bioavailability Research Consortium Method (SBRC) and the Simplified Bioaccessibility Extraction Test (SBET)) and polluted soils in their studies (Table 1) [32,33]. The contaminant source was often mining waste containing As minerals. The high concentration of As in these minerals could mask the effect of soil properties on IVBA As. Bradham et al. (2011) reported the combination of reactive Fe and Al best predicted IVBA As using the following equation:

$$\text{IVBA As (\%)} = 50.1 - 67.5 \log \text{FeAl} \quad (5)$$

In our study using the same bio-accessible method, we found the AlOx relationship with %IVBA was linear but produced the following equation when log (FeOx + AlOx) was accounted for:

$$\text{IVBA As (\%)} = 97.1 - 25.5 \log(\text{FeOx} + \text{AlOx}) \quad (6)$$

Table 1. Studies on the Effect of Soil Properties on Bio-accessible As.

Title of Paper	Authors	Number of Soils Studied	Properties Studied	IVBA Method [a]	Soil Type	Significant Properties on IVBA
Adsorption, Sequestration, and Bio-accessibility of As (V) in Soils	Yang et al., 2002	36	soil pH, cation exchange capacity (CEC), total inorganic carbon TOC, particle size, and Fe and Mn oxides	PBET	Spiked soils	Inverse relationship: FeOx IVBA As (%) = 11.3 pH − 30.5 log Fe Positive relationship: soil pH
Adsorption, Oxidation, and Bio-accessibility of As(III) in Soils	Yang et al., 2005	36	soil pH, CEC, total inorganic carbon, TOC, particle size, and Fe and Mn oxides	PBET	Spiked soils	Inverse relationship: FeOx Positive relationship: soil pH
Bio-accessible and non-bio-accessible fractions of soil As	Whitacre et al., 2013	19	determination of sorptive phases in IVBA and non-IVBA As soil fractions	OSU-IVG	Spiked soils	Inverse relationship: FeOx and Al Ox Reduction in IVBA As greater for FeOx than AlOx
Bio-accessibility of arsenic and cadmium assessed for in vitro bio-accessibility in spiked soils and their interaction during the Unified BARGE Method (UBM) extraction	Xia et al., 2016	7	TOC, CEC, reactive Fe, Mn, and Al oxides, soil pH, particle size	Unified BARGE Method (UBM)	Spiked soils	Inverse relationship: FeOx, AlOx, %TOC Positive relationship: soil pH
Modifying Effect of Soil Properties on Bioaccessibility of As and Pb from Human Ingestion of Contaminated Soil	Lake et al., 2021		soil pH, eCEC, Fe and Al oxides, clay content, organic carbon content	USEPA Method 1340	Spiked soils	Inverse relationship: FeOx, AlOx, %clay Positive relationship: soil pH
Bio-accessibility of arsenic in soils developed over Jurassic ironstones in eastern England	Palumbo-Roe et al., 2005	73	arsenic fractionation in soil, source of IVBA arsenic in soil	PBET	Naturally contaminated	Inverse relationship: less reactive iron oxide phases Positive relationship: carbonate and iron aluminosilicate/oxyhydroxide phases
The impact of sequestration on the bio-accessibility of arsenic in long-term contaminated soils	Smith et al., 2008	12	sequential fractionation of soils, soil pH, total and Free fe, total as, total, Al, total P	SBET/USEPA Method 1340	Polluted soils	Inverse: FeOx and AlOx but varies with crystallinity of the fractions
Relative bioavailability and bio-accessibility and speciation of arsenic in contaminated soils	Bradham et al., 2011	9	Soil pH, total As, Al, Fe, and Mn	SBRC in vitro assay/USEPA Method 1340	Polluted soils	Inverse relationship: Fe and Al concentration IVBA As (%) = 50.1–67.5 logFeAl
Assessment of bio-accessibility and exposure risk of arsenic and lead in urban soils of Guangzhou City, China	Lu et al., 2011	25	GI tract phases, soil pH, OM, particle size, soil metal content (Pb, As, Mn, Fe)	OSU-IVG	Polluted Soils	Stomach phase positive relationship: organic matter content inverse relationship: silt and clay Intestinal phase positive relationship: organic matter and total As content inverse relationship: silt and cla

[a] IVBA, in vitro bioaccessible; PBET, physiologically based extraction test; OSU-IVG, Ohio State University in vitro gastrointestinal; SBET, solubility based extraction test; SBRC, solubility based research consortium.

Similar variability was explained by the linear regressions from the Bradham et al. (2011) study and our own, 62% and 66.6%, respectively (Figure 2f; Table S2). Smith et al. (2008) also used USEPA Method 1340 to evaluate %IVBA As in polluted soils with a variable As content. Their results support the conclusion that Fe and Al oxides are the principal drivers of As bio-accessibility with oxide crystallinity being the main factor. Despite the wide range of arsenic concentration in polluted soils used in Bradham et al. (2011) and Smith et al. (2008), their results agree with our study, which only used one spike concentration of As, where reactive oxides were the most important soil properties that decreased % IVBA As. Palumbo-Roe et al. (2005) used the Physiologically Based Extraction Test (PBET) method on naturally contaminated soils and similarly found an inverse relationship between FeOx and %IVBA As [16].

Whitacre et al. (2013) used spiked soils and measured bio-accessible As by the Ohio State University (OSU)-IVG method, and also found that Fe oxides played a larger role in As sequestration compared to Al oxides [34]. Another study using the OSU-IVG method and polluted soils also supported our finding that clay content had an effect on %IVBA As, though this may be confounded with FeOx and AlOx content [15].

There was also a consensus between many studies that there was a positive relationship between soil pH and %IVBA As, using the Unified BARGE Method (UBM), the PBET method, or the USEPA Method 1340 on spiked and polluted soils to arrive at the same conclusion (Table 1). Yang et al. (2002) found, using the PBET method, that including soil pH in a multiple regression with log Fe better explained the %IVBA As variability using the following equation:

$$\text{IVBA As (\%)} = 11.3\ \text{pH} - 30.5\ \log \text{Fe} \tag{7}$$

They were able to predict As bio-accessibility with an R^2 of 0.743, $p < 0.001$ [18].

Multiple regression analysis in our study found significant variables were soil pH, Al oxide, %clay and/or FeOx. and resulted in an R^2 of 0.901 and $p < 0.001$ (Equations (2) and (3)).

Including soil pH and %clay significantly improved the variability explained compared to the simple regression of %IVBA As vs. log (FeOx + AlOx). The contribution of clay is likely to have improved the regression because Fe and Al oxides only account for a small percentage of the reactive surfaces found in clays. Clay manganese oxides and micropores could also play a role in reducing %IVBA As. Although many of the studies reported that FeOx and AlOx were needed to explain variability in %IVBA As, our study showed FeOx was not needed to predict %IVBA As (Equation 3). However, the coefficient of determination of multiple regressions with or without FeOx were highly significant ($p < 0.001$) (Equations (2) and (3)). Whitacre et al. (2013) used spiked soils and measured bio-accessible As by the OSU-IVG method, at a pH similar to Method 1340, and also found that Fe oxides played a larger role in As sequestration compared to Al oxides [34]. Results from Table 1 suggest the bio-accessible As method does not significantly affect the ability to predict the dominant properties of FeOx and/or AlOx, and/or clay content that affect %IVBA As. Our data with spiked soil and similar soils show the effect of AlOx > FeOx. It may be prudent to use a predictive equation that includes both FeOx and AlOx for a different set of soils.

4.2. Pb Bio-Accessibility

Selected studies on the relationship between soil properties and %IVBA Pb are summarized in Table 2. Many studies included either clay content or reactive Fe and/or Al oxides in their evaluations.

ShunAn et al. (2013) used Method 1340 to measure bio-accessible Pb and reported clay content to have an inverse relationship with %IVBA Pb but they did not evaluate the effect of reactive oxides [35]. Using Method 1340, Liu et al. (2015) found that Mn oxyhydroxides and amorphous Fe and Al oxyhydroxides soil content contributed to less bio-accessible Pb [36].

Table 2. Studies on the Effect of Soil Properties on Bio-accessible Pb.

Title of Paper	Authors	Number of Soils Studied	Properties Studied	IVBA Method [a]	Soil Type	Significant Properties on IVBA
Metals pollution and human bio-accessibility of topsoils in Grugliasco (Italy)	Poggio et al., 2009	66	pH, organic matter content, cation exchange capacity, and particle size distribution	PBET	Polluted soils	Inverse relationship: silt and clay contents Positive relationship: SOM, total Pb content, and sand content
Cd, Pb, and Zn oral bio-accessibility of urban soils contaminated in the past by atmospheric emissions from two lead and zinc smelters	Roussel et al., 2010	27	total metal trace element content, total nitrogen, total carbonates, clay contents, soil pH, particle size distribution, organic matter (OM), cation exchange capacity (CEC), assimilated P, free Mn, Al, and Fe	UBM	Polluted soils	Inverse relationship: Fe and Mn oxides; carbonate and iron content (gastric phase); total N and pH (gastrointestinal phase) Gastric IVBA Pb (%) = 171.7 − 1.09 $CaCO_3$ − 225.9 Fe + 0.68 total Pb Gastrointestinal (GI) IVBA Pb (%) = 1020.6 − 32.6 N_{tot} − 131.1 + 0.39 total Pb Positive relationship: total metal concentration
Assessment of bio-accessibility and exposure risk of arsenic and lead in urban soils of Guangzhou City, China	Lu et al., 2011	25	GI tract phases, soil pH, OM, particle size, soil metal content (Pb, As, Mn, Fe)	OSU-IVG	Polluted soils	Intestinal phase No observed relationships Gastric phase Inverse relationship: soil Fe and Mn content Positive relationship: soil organic matter and total Pb content
Bio-accessibility of trace elements as affected by soil parameters in smelter-contaminated agricultural soils: A statistical modeling approach	Pelfrêne et al., 2012	280 to build the model and 110 to test (390 total)	particle size distribution, soil pH, OM, total carbonate, assimilated P, and free Mn, Fe and Al oxides	UBM	Polluted soils	Negative relationship: total carbonate, OM, and pseudo-total Al and Pb contents Positive relationship: assimilated P
Application of in vitro digestion approach for estimating lead bio-accessibility in contaminated soils: influence of soil properties	ShunAn et al., 2013	22	in vitro extraction methods, soil pH	SBET/US EPA Method 1340 and PBET	Polluted soils	SBET results in greater IVBA Pb than PBET Inverse relationship: clay content and soil pH
Lead bio-accessibility in 12 contaminated soils from China: Correlation to lead relative bioavailability and lead in different fractions	Li et al., 2015	12	relationship between IVBA Pb and Pb sorbent pools	UBM, SBRC, OSU-IVG, PBET	Polluted soils	Exchangeable and carbonate soil fractions contributed most to IVBA Pb
Investigating the relationship between lead speciation and bio-accessibility of mining impacted soils and dusts	Liu et al., 2017	36 (18 top soils and 18 house dusts)	relationship between IVBA Pb and speciation of sorbed Pb	USEPA Method 1340	Polluted soils	Inverse: Mn oxyhydroxides and amorphous Fe and Al oxyhydroxides Positive: carbonates
The source of lead determines the relationship between soil properties and lead bio-accessibility	Yan et al., 2019	31	distribution of soil properties based on size fractions, CEC, TOC, soil pH, total metal content, particle size	USEPA Method 1340	Polluted Soils	Inverse relationship: EC IVBA Pb (%) = 1.79 CEC − 4.165 EC + 1.666 Clay + 0.007Total Pb + 38.71 Positive relationship: CEC, total Pb content
Modifying Effect of Soil Properties on Bio-accessibility of As and Pb from Human Ingestion of Contaminated Soil	Lake et al., 2021		soil pH, eCEC, Fe and Al oxides, clay content, organic carbon content,	USEPA Method 1340	Spiked soils	Inverse relationship: FeOx, AlOx, %clay

[a] IVBA, in vitro bioaccessible; PBET, physiologically based extraction test; UBM, unified bioaccessibility method; OSU-IVG, Ohio State University in vitro gastrointestinal; SBET, solubility based extraction test; SBRC, solubility based research consortium.

Our study evaluated the effect of log FeOx, log AlOx, and %clay in the multiple regression, and that regression accounted for 63% of the %IVBA Pb (Equation (4), $p < 0.01$). The inclusion of the clay parameter resulted in a 13% better explanation of the variability compared to our simple regression with log (FeOx and AlOx) (Table S2, Figure 3d). The clay parameter is likely to have accounted for the reactive surfaces present in clay other than Fe and Al oxides. From our results, it can be assumed that, while reactive oxides are the primary drivers of Pb sequestration, the inclusion of a clay content parameter improves the fit of the %IVBA Pb model.

Yan et al. (2019) used USEPA Method 1340 (also know as the Relative Bioaccessibility Leaching Procedure (RBALP)) and reported 86% of the IVBA Pb variability for highly contaminated soils (mining soils) was explained by the following multiple regression equation [19].

$$\text{IVBA Pb (\%)} = 1.79\ \text{CEC} - 4.165\ \text{EC} + 1.666\ \text{Clay} + 0.007\ \text{Total Pb} + 38.71 \tag{8}$$

It is unclear why the relationship between %IVBA Pb and clay content or CEC were positive. Clays are common sinks for Pb and often associated with Fe and Al oxides which can be strong sorbents. Clay is also a major source of CEC. It is likely that the Pb source and the Pb concentration may have been more important than soil properties in affecting bio-accessible Pb. When they expanded their study to include Pb contaminated soils from a variety of sources rather than just mining soils, their multiple regression included CEC and EC and only accounted for 31% of the variability in %IVBA Pb. These results underscore the importance of using a single spike level in determining the clear effect of soil properties on bio-accessible Pb. It is likely that the properties in question were overshadowed by the variable Pb content in the study soils.

Poggio et al. (2009) used the PBET method to determine bio-accessible Pb on their polluted soils and found clay to be a strong driver of IVBA Pb as did our study [37]. Roussel et al. (2010) found, using UBM, that for gastric %IVBA Pb, carbonates, total Fe content, and total Pb content best explained the variability (97%) [17]:

$$\text{IVBA Pb (\%)} = 171.7 - 1.09\ \text{CaCO}_3 - 225.9\ \text{Fe} + 0.68\ \text{Total Pb} \tag{9}$$

Formation of Pb precipitates with carbonates would reduce soluble Pb; however, when subjected to the low pH of the in vitro, most of the Pb carbonate would become bio-accessible. It is unclear why an inverse relationship between CaCO_3 content and %IVBA Pb was reported. Formation of Pb precipitates, especially at higher pH, would reduce Pb available to become tightly bound to oxide soil surfaces further increasing the %IVBA Pb [38–40]. Li et al. (2015), Liu et al. (2017), and Yan et al. (2019) found that carbonate minerals contribute significantly to increased IVBA Pb compared to reactive oxides and these studies included a variety of methods, including EPA Method 1340 [19,36,41]. Pelfrêne et al. (2012) used the UBM method and also reported an inverse relationship with carbonates [42].

The only consensus across all IVBA methods is the impact of clay content on bio-accessible Pb, though many of the studies did not also evaluate reactive oxides. However, it can be concluded that lead content in polluted soils can affect the study of the relationship between %IVBA Pb and soil properties.

4.3. Effect of Fe and Al Oxides on Bio-Accessible As and Pb

Our results show Fe and Al oxides best reduced bio-accessible As and Pb. Past studies have shown that Fe and Al oxides are strong sinks for heavy metals. Iron (oxy-hydr)oxides has also been shown to be important in controlling the mobility and speciation of soil As via adsorption or coprecipitation possibly due to its high specific surface area [43].

In particular, Fe and Al oxide surfaces have a high preference for sorption of polyvalent metals such as (V) which may explain the higher sequestration of As compared to Pb [40]. Bonding with Fe and Al oxides is specific and the metal may become part of the soil surface, decreasing its availability [9]. Consequently, these metals are more tightly bound and less accessible, even at the low pH of the GI tract. Arsenic is more likely to be specifically absorbed to surfaces such as Fe and Al oxides, organic matter, or clay surfaces [9,40]. Arsenic is covalently bonded to the soil surface resulting in less desorbable As and, therefore, less bio-accessible As, even at the low pH found in the GI tract (pH 1.5) [44]. Iron oxides reduce both bio-accessible As and Pb in spiked and polluted soils (Tables 1 and 2).

Whitacre et al. (2013) found that soil Fe appears to be stable in the acidic conditions of the gastric extraction, and therefore As sorbed to Fe oxides was not likely to have contributed significantly to %IVBA As measured by the OSU-IVG method [34]. However, the gastric extraction dissolves a significant fraction of soil Al, particularly amorphous Al, and therefore As sorbed to Al oxides is likely to have contributed to %IVBA As extraction.

4.4. Effect of Soil pH on Bio-Accessible As and Pb

Soil pH was not the dominant soil property affecting bio-accessible As or Pb. It was found to have a slightly significant effect on %IVBA As, with IVBA As increasing as soil pH increases. This is consistent with increased pH decreasing As sorption to soil. However, it had no significant effect on %IVBA Pb.

Soil pH has an effect on many other soil properties including the charge of Al and Fe oxides and the exchange capacity of clays. Additionally, it affects the solubility of the metals themselves, with increasing cation (e.g., Pb) solubility at lower pH [9,40]. Bio-accessible As was shown to increase with pH due to the loss of positively charged binding sites [18]. For Pb, pH may have an effect on bio-accessibility in the soil environment but not in the stomach gastric environment. At pH > 7.5, carbonates are prevalent and may bind with Pb to form Pb carbonates. These carbonates result in the Pb becoming less plant available, but they are readily dissolved at stomach pH resulting in the Pb increasing in human bio-accessibility [9,38,45]. Consequently, soil pH may have a greater effect on As than Pb bio-accessibility.

4.5. Effect of eCEC and Clay Content on Bio-Accessible As and Pb

Clay content may have a significant effect on %IVBA As and Pb due to its surface area and strong association with Al and Fe oxides. For this same reason, the sandiest soils (Pratt A and B) had the greatest As and Pb bio-accessibility (Table S1).

Metal cations will adsorb to the soil effective cation exchange capacity (eCEC) and can potentially decrease bio-accessibility. Heavy metal cations adsorbed to the eCEC sites are less likely to be leached from rainfall from the soil profile but are weakly adsorbed and plant available [9]. Given that these metals are still plant available, they would also be bio-accessible at the low pH (pH < 2) of the stomach. Thus, Pb adsorbed to the eCEC would be bio-accessible. However, the strong relationship between eCEC and IVBA Pb observed appears to contradict the above discussion. The relationship between IVBA Pb and %clay was almost identical to the relationship between IVBA Pb and eCEC ($r = 0.699$, $p < 0.01$ and $r = 0.700$, $p < 0.01$ respectively). Thus, the association between eCEC and %IVBA Pb is likely to be confounded by soil clay content. Clay content can reduce bio-accessible Pb through sorption by FeOx, AlOx, MnOx, and other reactive clays. It is likely that clay, not eCEC, affected bio-accessible Pb and it was therefore excluded from the multiple regression.

Arsenic existed as anionic arsenate in our study and would not be adsorbed to a negatively charged eCEC. This would explain the lack of correlation of eCEC with %IVBA of As.

5. Conclusions

Soil properties had a large effect on reducing bio-accessible As and Pb. The most important observed soil property were the soil Fe and Al oxide content. For both As and Pb, bio-accessibility decreased exponentially with increasing FeOx content and linearly with AlOx content. AlOx had a stronger impact on As and Pb bio-accessibility than FeOx.

Other soil properties (soil pH, %clay, %OM, and average eCEC) had less or little effect on bio-accessible As and Pb. Soil pH increased bio-accessible As, however, no significant relationship was found between soil pH and bio-accessible Pb. Soil clay content also had a weakly inverse relationship with %IVBA As and Pb.

Similar results were reported from several studies using different bio-accessibility methods to measure bio-accessible As. Reactive Fe and/or Al oxide decreased %IVBA As while soil pH increased %IVBA As.

The effect of soil properties from several studies using different bio-accessibility methods on bio-accessible Pb differed. The disparities were likely due to different Pb sources and high Pb concentrations in these polluted soils. The results of this study further supported the importance of using spiked soils rather than using polluted soils. Including a variable range of the total metal content can mask the effect of other soil properties and therefore the possible conclusions to be drawn.

Soils that have a large natural abundance of reactive Fe and/or Al will greatly reduce the bio-accessibility and exposure of As and Pb via soil ingestion. Soil pH should be used in addition to reactive oxides to predict bio-accessible As and the inclusion of clay content can further improve predictor models. Risk-based adjustments using soil properties should be considered for soils contaminated with As and/or Pb.

Supplementary Materials: The following are available online at https://www.mdpi.com/2076-3263/11/3/126/s1, Table S1: pH of metal spiked soils and soil chemical and physical properties related to metal bio-accessibility, Table S2: Simple linear correlation coefficients (r) and regression equations for heavy metal bio-accessibility vs. soil properties, Table S3: As soil properties correlation analysis, Table S4: Pb soil properties correlation analysis, Figure S1: Effect of soil type on %bio-accessible As, Figure S2: Effect of soil type on %bio-accessible Pb.

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