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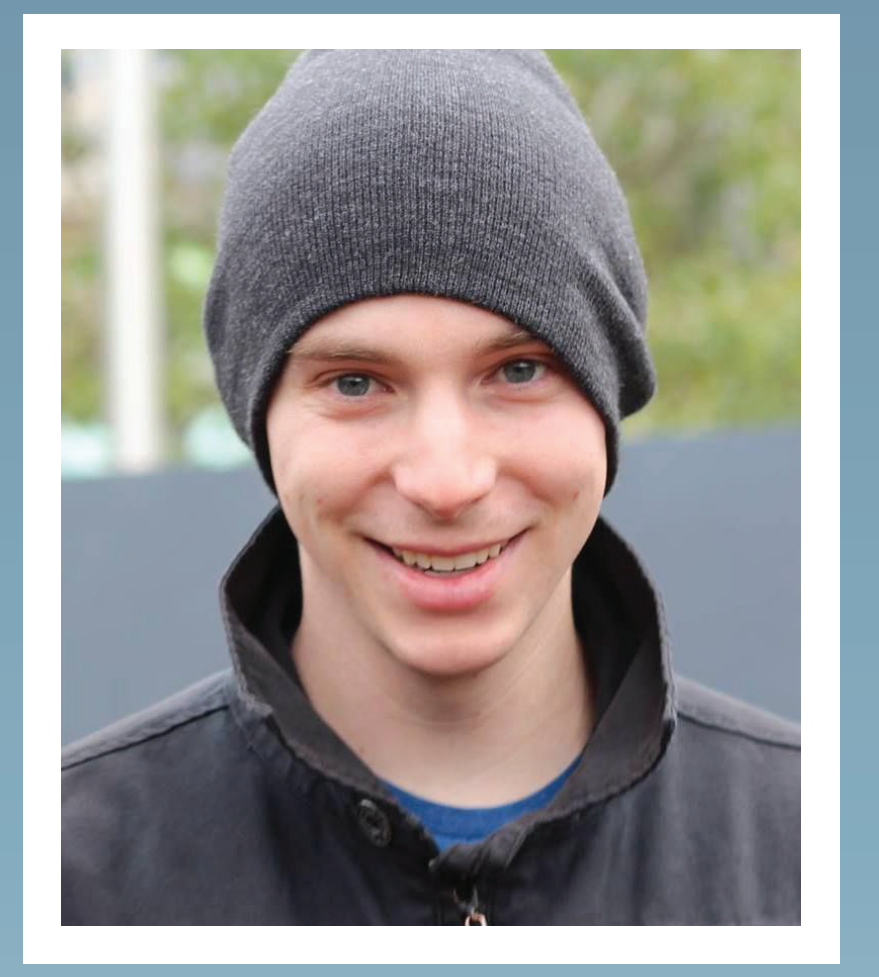

Comparison of EPA 1340 and CAB Method for Bioaccessible Lead and Arsenic in Soils

Brian Smith, Ariana Dionisio, Annie Carter
Brooks Applied Labs, 18804 North Creek Parkway, Suite 100, Bothell, WA 98011 USA

Department of Toxic Substances Control

The Ohio State University

Introduction

Contamination from trace metals, such as lead (Pb), in soils can lead to potential health risks from ingestion of the soil or vegetables grown in the soil. Typical risk assessment models apply an assumed number ranging from 60 - 100% of contaminant metals in a soil sample as bioavailable. These assumptions can result in incorrect health risk assessments or unnecessarily high remediation costs because actual bioavailability can range from 0 - 100%. Currently, *in vivo* animal studies are often used to determine bioavailability, which are expensive and time consuming. *In vivo* methods are giving way to new *in vitro* methods, such as EPA Method 1340 "*In Vitro* Bioaccessibility Assay for Lead in Soil". The California Department of Toxic Substances Control recently published recommended methodology for evaluating site-specific arsenic (As) bioaccessibility in soils. This method, the California Arsenic Bioaccessibility (CAB) Method, was developed to improve the accuracy of risk assessments and assist regulators in making responsible remediation decisions while still protecting human health.

Bioavailability refers to the concentration of metal that is readily absorbed when ingested and studied *in vivo*, while bioaccessibility refers to metals extracted by *in vitro* methods. *In vitro* bioaccessibility (IVBA) can be defined as the bioaccessible portion of total metal concentration in percentage, see equation below.

$$\text{IVBA} = (\text{concentration of bioaccessible})/(\text{total concentration})$$

Relative bioavailability (RBA) of Pb and As by in vivo testing has previously been shown to correlate to IVBA results by EPA 1340 and CAB, respectively. The ratio of RBA to IVBA can be defined by the following equations. Each equation is both metal and extraction specific. RBA Pb is calculated using the IVBA Pb recovery from EPA 1340, and RBA As is calculated using IVBA As recovery from CAB.[1, 2]

$$\text{Pb: RBA} = (0.878 \times \text{IVBA}) - 0.028$$

$$\text{As: RBA} = (0.81 \times \text{IVBA}) + 3.2$$

Note: both methods have an upper concentration limit. EPA 1340 is not recommended for total Pb concentrations >50,000 mg/kg, and CAB is not recommended for total As concentrations >1500 mg/kg.

It is well known that different molecular forms of Pb and As have different toxicological effects; however, the correlation between elemental species in the soil and the bioaccessibility of the metals from that soil has not been well established. For instance, inorganic As was used in pesticides and herbicides in US agriculture until 1993, and organic As was used up until 2013, while monosodium methanearsonate (MSMA), is still approved for use in the US as a herbicide in cotton agriculture.[3] All of these compounds could be present in contaminated soil and each has a different degree of bioaccessibility.

For this study, bioaccessible As and Pb concentrations in soil samples were determined using both EPA Method 1340 and the CAB Method, respectively, with sample analysis by inductively coupled plasma mass spectrometry utilizing collision and reaction cell technology (ICP-QQQ-MS). Both As and Pb were quantified in both extractions to evaluate if a streamlined method could be developed and applied to both elements. In addition, samples were analyzed for total recoverable As and Pb using EPA Method 3050B to assess the RBA & IVBA of each metal in the samples. In order to evaluate if there was a correlation between the bioaccessible metals concentrations and specific As compounds, direct quantification of different molecular forms of the metal (e.g., arsenite, arsenate, etc.) were performed on the soil samples.

Methods

Samples were extracted for As and Pb by CAB and EPA 1340, then extracts were analyzed by ICP-QQQ-MS on an Agilent 8800. Both extraction methods are designed to simulate human digestion. Soil samples were mixed with an extraction fluid, designed to simulate stomach acid, rotated and incubated at body temperature. Organic compounds in the extraction fluid vary based on the extraction method.

Soil samples were digested for total As and Pb by EPA 3050B, and analyzed by ICP-QQQ-MS on an Agilent 8800. EPA 3050B is a digestion of solid matrices for environmentally available metals. While this digestion will dissolve almost all elements that could become "environmentally available" it is not a true total digestion. Strongly bound analytes, for example silicate bound structures, are not solubilized by this method.

Arsenic species were extracted from the soil samples using two separate (acidic and basic) extractions. With this method, arsenite (As(III)), arsenate (As(V)), monomethyl arsenic acid (MMA), dimethyl arsenic acid (DMA) were directly measured and quantified utilizing ion chromatography inductively coupled plasma mass spectrometry (IC-ICP-MS) analysis on an Agilent 7700. Data from both extracts were evaluated to determine the most appropriate extraction conditions based on the chemistry inherent within each individual sample. Comparison of CAB and EPA 1340 is shown in Figure 1. CAB and EPA 1340 extractions were also performed on a known reference material, Montana Soil II NIST 2711a. Recoveries of the reference material were compared to known IVBA values to confirm proper extraction of the target metal.

Comparison of CAB and EPA 1340 Extraction Methods		
	EPA 1340	**CAB**
Initial Sample Mass	1.0 grams	1.0 grams
Extraction Solution Volume	**100 mL**	**150 mL**
Incubation Temp	37 ± 2 °C	37 ± 2 °C
Rotation Speed	30 ± 2 rpm	30 ± 2 rpm
Incubation Time	**60 minutes**	**120 minutes**
Extraction Time	**60-90 minutes**	**120-145 minutes**
Extraction Fluid	**Glycine** Hydrochloric Acid	**Pepsin** **NaCl** **Ascorbic Acid** Hydrochloric Acid
Extraction pH	1.5 ± **0.5** pH units	1.50 ± **0.01** pH units
Number of pH adjustments	**1**	**2**
Number of pH checks	**2**	**3**

Figure 1. Method comparison of CAB and EPA 1340 Extractions. Difference between methods are in bold.

EPA 1340

Dissolve 60.6 g of glycine into 1.5 L of DI H_2O

Heat solution to 37 ± 2 °C

pH adjust heated extraction solution with HCl to 1.5 ± 0.5 pH units

Bring to final volume of 2.0 L with DI H_2O

Add 100 mL of extraction solution to 1.0 g of each dried and sieved soil sample

Incubate samples at 37 ± 2 °C while rotating at 30 ± 2 rpm for 1 hour

Remove from incubator and allow sediment to settle

Filter solution through PES filter

Check pH of each final extraction; should be 1.5 ± 0.5 pH units

If within range analyze by ICP-QQQ-MS / If outside range re-extract sample

CAB Method

Dissolve 20 g of pepsin, 11.7 g of sodium chloride (NaCl) and 35.2 g of ascorbic acid into 1.5 L of DI H_2O

Heat solution to 37 ± 2 °C

pH adjust heated extraction solution with HCl to 1.5 ± 0.01 pH units

Bring to final volume of 2.0 L with DI H_2O

Add 150 mL of extraction solution to 1.0g of each dried and sieved soil sample

Incubate samples at 37 ± 2 °C while rotating at 30 ± 2 rpm for 1 hour

Remove samples from incubator and pH adjust samples to 1.5 ± 0.01 pH units

Incubate samples at 37 ± 2°C while rotating at 30 ± 2 rpm for 1 hour

Remove from incubator and allow sediment to settle

Filter solution through PES filter

Check pH of each final extraction; should be 1.5 ± 0.01 pH units

If within range analyze by ICP-QQQ-MS / If outside range re-extract sample

Results

Pb Recovery EPA 1340 vs CAB

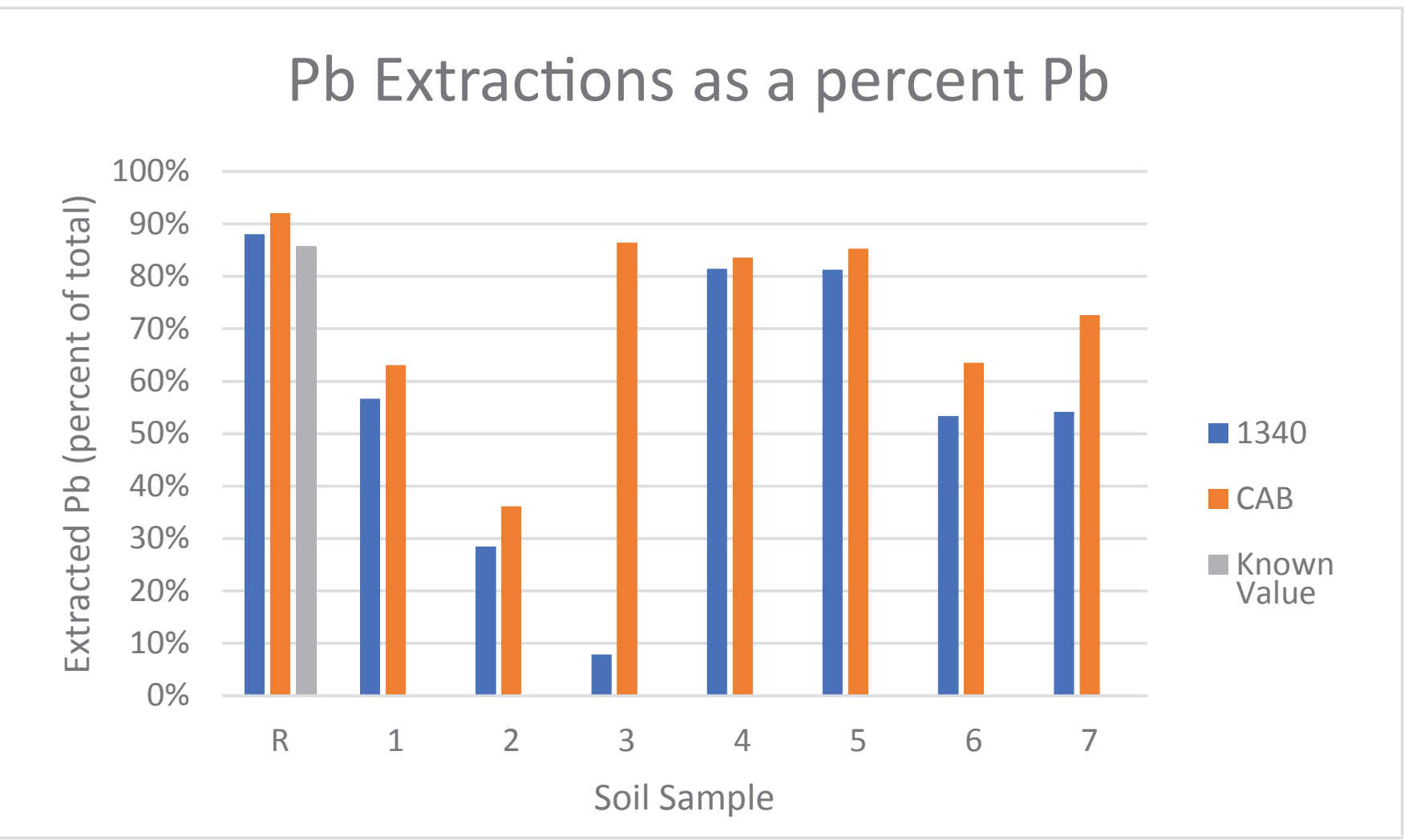

Figure 2. Pb extraction from soil samples was performed by EPA 1340 and CAB. Results show consistently higher concentrations of Pb are extracted when using the CAB method. However, the percent increase of Pb relative bioaccessibility by CAB extraction varied greatly between different soil matrices. While some soil samples only showed a slight increase in extracted Pb, sample #3 increased by 10-fold. Known value of reference material, Montana Soil II NIST2711a, confirms proper extraction of bioaccessible Pb by EPA 1340 and bias high recovery by CAB. *R designates a standard reference material, Montana Soil II NIST 2711a

As Recovery EPA 1340 vs. CAB vs. As(V)

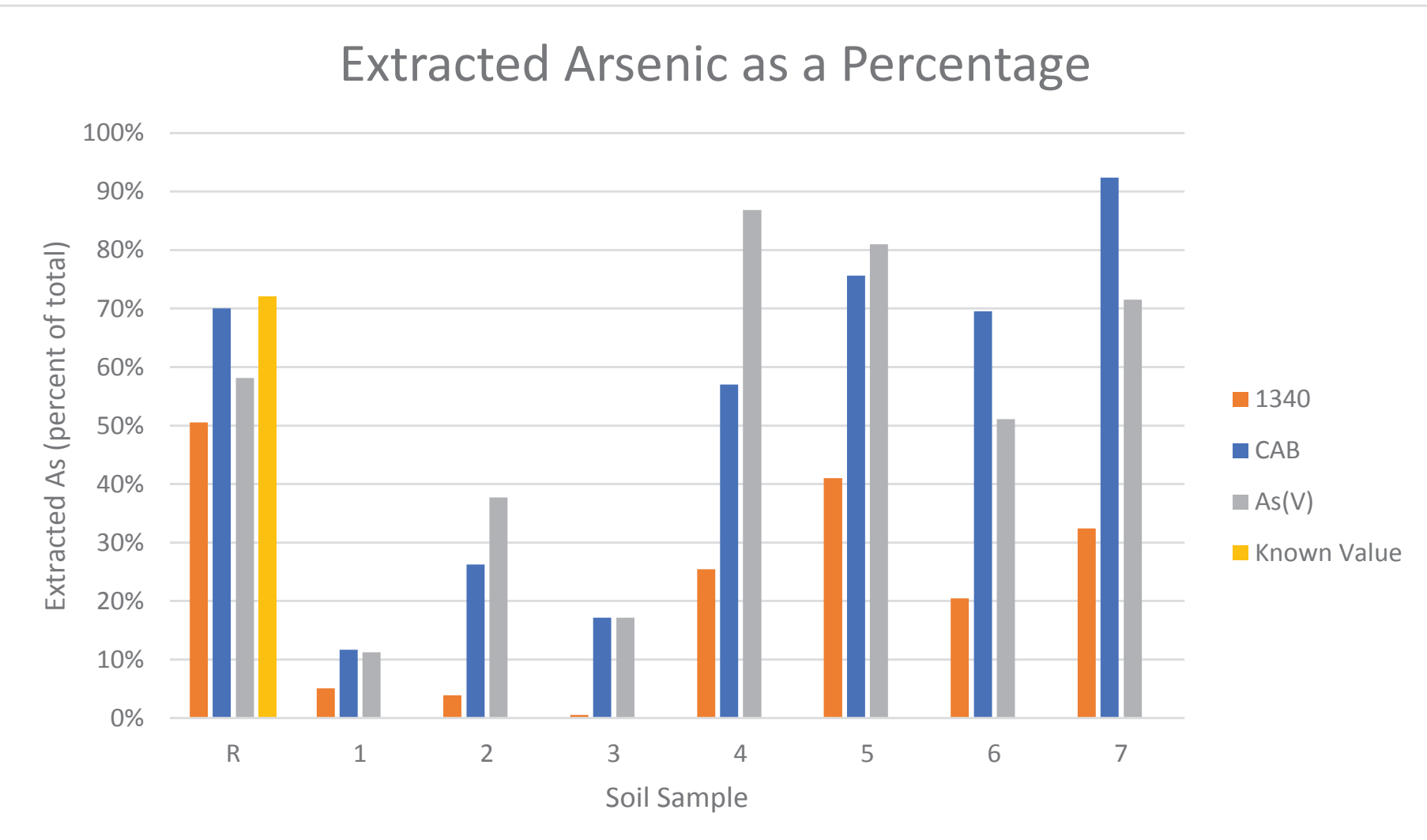

Figure 3. Arsenic extraction from soil samples was performed by EPA 1340 and CAB. Results consistently show significantly lower concentrations of As are extracted when using the EPA 1340 method. Known value of reference material confirms proper extraction of IVBA As by CAB and biased low results by EPA 1340.

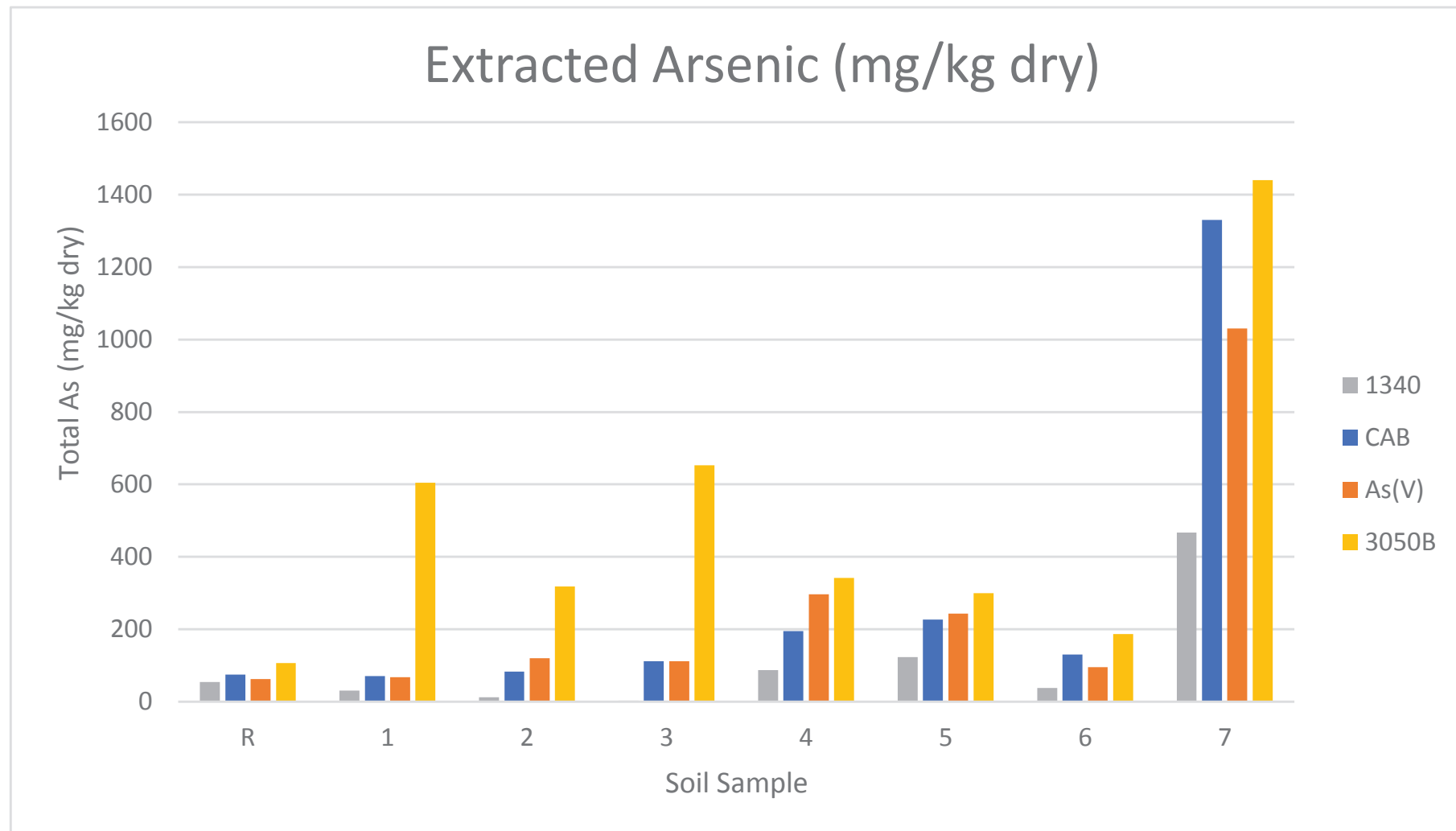

Figure 4. Total As concentrations ranged from 187 to 1440 mg/kg. Both bioaccessible As and As(V) concentrations showed minimal correlation to the total As concentration. Relative bioaccessible As can vary greatly regardless of the total As concentration.

As Speciation

Comparison of Arsenic, Bioaccessible Arsenic, and Arsenic Species (mg/kg)						
Sample	**3050B**	**CAB**	**As(III)**	**As(V)**	**DMA**	**MMA**
R	92.7	74.9	0.159	62.2	U	U
1	604	70.7	U	67.9	U	U
2	318	83.5	0.250	120	U	U
3	653	112	U	112	U	U
4	342	195	U	297	0.170	U
5	300	227	U	243	0.100	U
6	187	130	0.204	95.6	U	U
7	1440	1330	1.59	1030	0.950	U

Figure 5. The dominant As species was As(V) in every soil sample tested. Note: As speciation extraction is not a total recoverable method and the sum of the As species is typically less than the As concentration by EPA 3050B. Arsenic bound to some minerals is not recoverable by As speciation extraction however, a more rigorous digestion of mineral bound As is not possible because it will cause species conversion. *R designates known reference material, Montana Soil II NIST 2711a. ** U value in table designates concentration of analyte was under the detect limits.

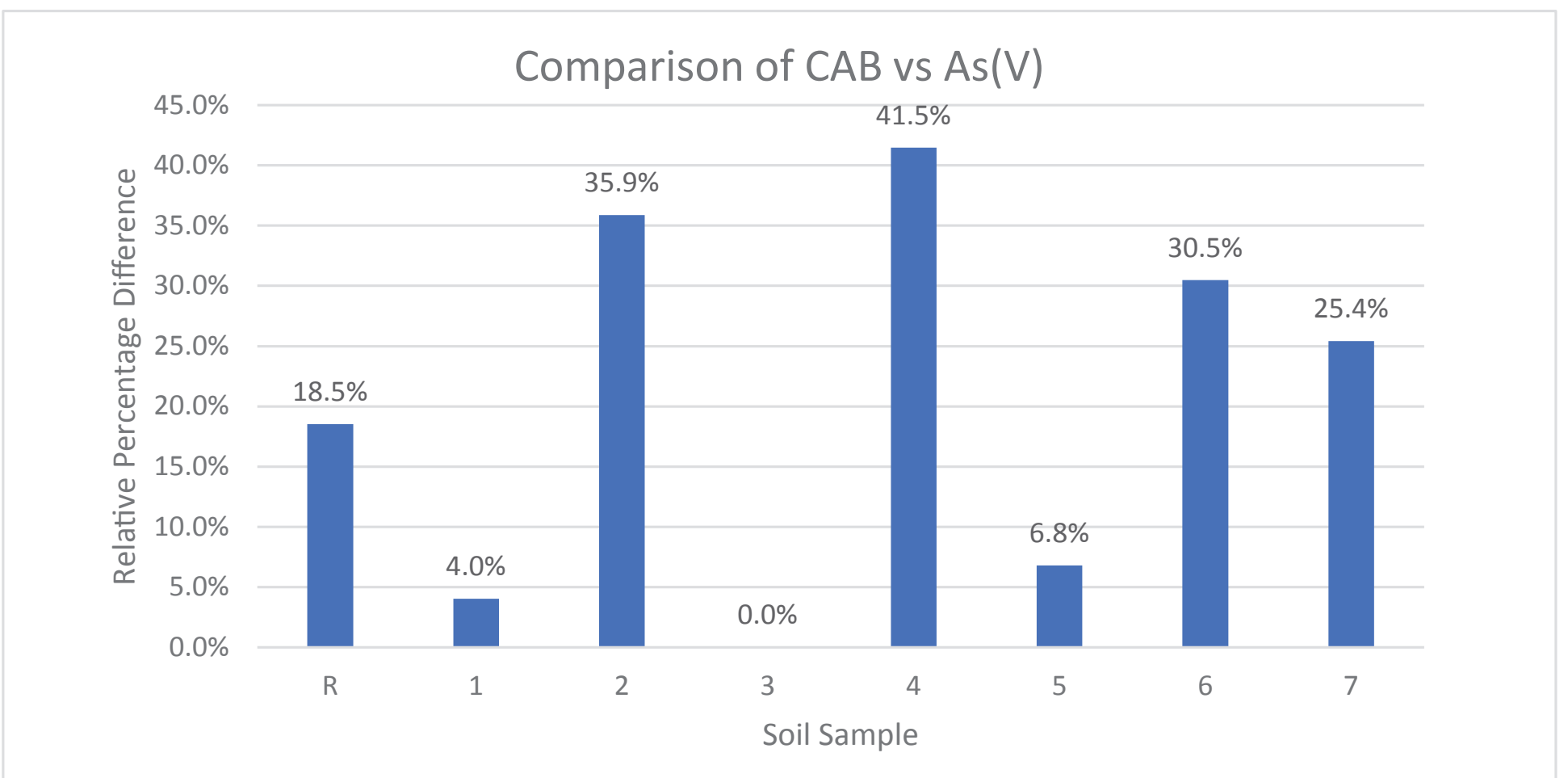

Figure 6. While the concentrations of As(V) and bioaccessible As in some samples were similar, in other samples bioaccessible As was significantly different than As(V) results. Relative percent difference (RPD) between bioaccessible As concentration and As(V) concentration ranged from 0% to 42%. It is suspected that bioaccessible As and As(V) correlations may be depend on the soil type and are not uniform across all soil matrices.

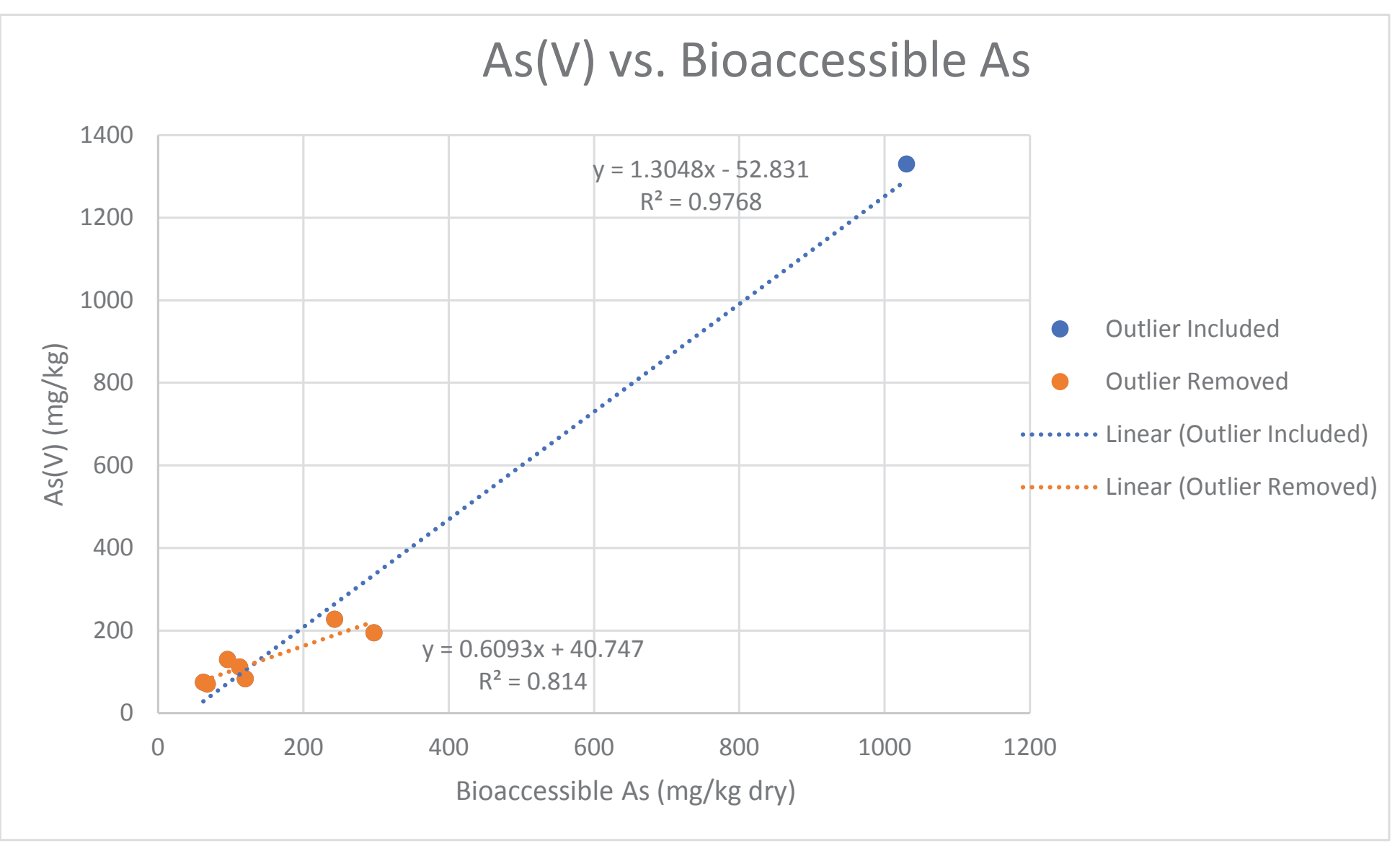

Figure 7. There is some evidence to suggest a correlation between As(V) and bioaccessible As however, it is not a strong correlation. R^2 values of 0.9768 with all points included, and 0.814 with a single outlier removed.

Relative Bioavailability of Pb and As

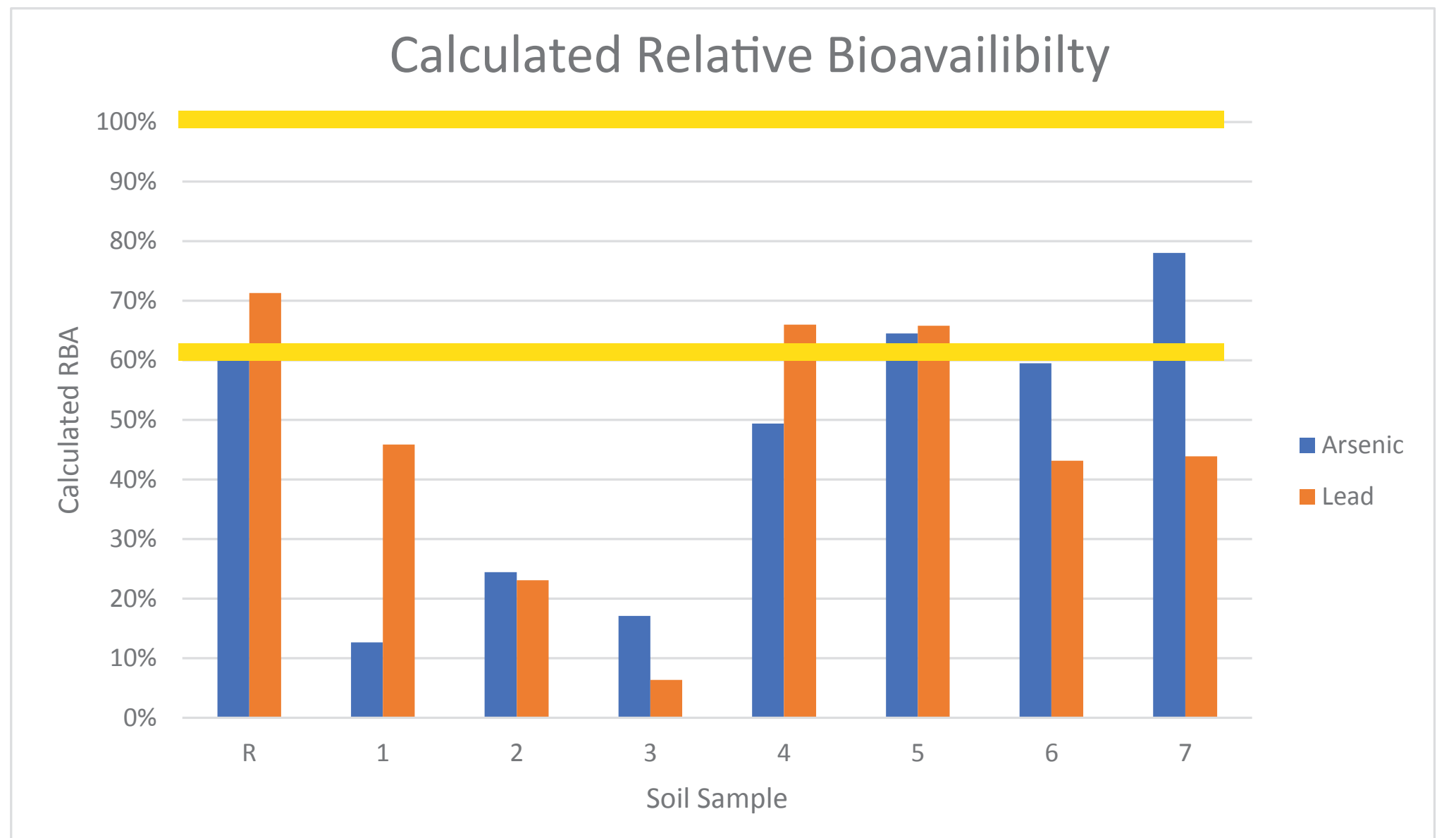

Figure 8. Relative Bioavailability (RBA) was calculated from IVBA for both As and Pb. RBA As was calculated using the CAB extraction recoveries, and RBA Pb was calculated using the 1340 extraction recoveries. Assumed RBA typically ranges from 60-100%. In most cases RBA concentrations are much lower than assumed concentration.

Quality Control

Quality control (QC) samples were prepared and analyzed with all extractions and digests. QC samples were analyzed in the same manner as sample.

Quality Control

CAB Extraction Quality Control Sample Recoveries	As (mg/kg)	Percent Recovery	Relative Percent Difference	Pb (mg/kg)	Percent Recovery	Relative Percent Difference
Method Blank 1	0.215			0.213		
Method Blank 2	0.214			0.111		
Method Blank 3	0.181			0.114		
Blank Spike	10.8	108%		1.06	106%	
Reference Material	74.9			1290	116%	
Source - Duplicate	227			1910		
Duplicate	234		3.00%	1850		3.00%
Source - Matrix Spikes	195			1580		
Matrix Spike**	200	46.0%**		1600	2190%**	
Matrix Spike Duplicate**	203	78.0%**	52.0%**	1610	2740%**	61.0%**

EPA 1340 Extraction Quality Control Sample Recoveries	As (mg/kg)	Percent Recovery	Relative Percent Difference	Pb (mg/kg)	Percent Recovery	Relative Percent Difference
Method Blank 1	0.002			0.01		
Method Blank 2	0.0004			0.004		
Method Blank 3	U*			0.007		
Blank Spike	9.96	100%		1.04	104%	
Reference Material	54.1	70.0%		1220	111%	
Source - Duplicate	123			1820		
Duplicate	121		1.00%	1760		3.00%
Source - Matrix Spikes	467			3230		
Matrix Spike**	475	79.0%**		3170	-6230%**	
Matrix Spike Duplicate**	472	57.0%**	33.0%**	3200	-3200%**	64.0%**

Figure 9. QC samples for CAB and EPA 1340 confirm samples were properly extracted for target analytes. Sample duplicates noted very low relative percent differences (RPD) in all cases, <4%. Blank spikes recovery very close to 100% in all instances. Reference material recoveries confirm EPA 1340 under recovers for As and CAB over recovers for Pb. *U designates results that were under the detection limits of the instrument. Matrix spikes (MS) and matrix spike duplicates (MSD) were spiked at the same level as blank spikes. This resulted in under-spiked MS and MSD, poor spiking recoveries are expected.**

Discussion

Two extraction methods for bioaccessible As and Pb were performed on various soil samples: EPA 1340 for bioaccessible Pb, and CAB for bioaccessible As. Samples were also analyzed by EPA 3050B for total recoverable metals and for various As species by IC-ICP-MS.

The CAB method showed higher concentrations of both As and Pb than EPA Method 1340. This indicates a higher extraction efficiency for the CAB method. This is likely due to the different reagents used in the extraction fluids and the increased incubation and extraction times in the CAB method. As a result, using the CAB method for Pb would provide results that are biased high for bioaccessible Pb for many samples. Inversely, using the EPA 1340 method for As would produce results that are biased low for bioaccessible As.

In all analyzed soil samples As(V) made up the vast majority of the detected As species. Some correlation was seen between IVBA As and As(V) however, relative percent differences ranged from 0-42%. IVBA As and As(V) correlation may be depend on each specific soil matrix.

Conclusion

- Varying extracted concentrations in each method illustrate the importance of using the appropriate extraction method for the desired target analyte.
- The newly developed CAB method gave consistent results for bioaccessible As but yields concentrations for bioaccessible Pb that are biased high compared to EPA 1340
- EPA 1340 yielded expected results for bioaccessible Pb but bias low results for bioaccessible As compared to CAB.
- As(V) speciation may give some indication of bioaccessible As but should not be used as a proxy for the CAB extraction method.
- Brooks Applied Labs, LLC plans to investigate applying these bioaccessibility extractions to other metals such as cadmium and selenium, due to their similar properties to lead and arsenic.

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